



Australian Government

Department of Health

Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for DIR 062/2005

**Commercial release of herbicide tolerant Liberty
Link[®] Cotton for use in the Australian cropping
system**

Applicant: Bayer CropScience Pty Ltd

August 2006

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the intentional release (DIR) of herbicide tolerant genetically modified (GM) cotton into the Australian environment, in respect of application DIR 062/2005 from Bayer CropScience Pty Ltd (Bayer).

The DIR 062/2005 licence permits the commercial release of the GM cotton on an unrestricted basis in all areas of Australia. It should be noted that cultivation of this GMO may require additional approvals under State or Territory legislation that restrict the commercial release of GM crops on marketing grounds.

The *Gene Technology Act 2000* (the Act) and the Gene Technology Regulations 2001 (the Regulations) govern the process undertaken by the Regulator before a decision is made on the whether or not to issue a licence. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in consultation with a wide range of experts, agencies and authorities and the public.

More information on the process required for the comprehensive 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).

The application

Bayer applied for a licence to release a herbicide tolerant GM cotton, Liberty Link[®] Cotton, into the environment. Bayer is seeking approval for unrestricted, commercial scale planting of the GM cotton in all current cotton growing areas and potential future areas with environmental conditions suitable for cotton cultivation in Australia.

The GM cotton has only one introduced gene, the herbicide tolerance gene (*bar*), isolated from a common soil bacterium, *Streptomyces hygroscopicus*. The *bar* gene expresses a protein that provides tolerance to glufosinate ammonium, the active ingredient in the herbicide Liberty[®], and enables the herbicide to be applied for weed control in the GM cotton crop. Otherwise, the GM cotton has the same water and climatic requirements as non-GM and commercially released GM cotton lines, and provides an alternative in crop weed control method to GM cottons that are tolerant to the herbicide glyphosate.

The GM cotton proposed for release has been approved previously (described as Liberty[®] or LLCotton25) for limited and controlled releases under DIR licences 015/2002, 038/2003 and 056/2004.

The applicant requests approval for commercial scale cultivation without containment measures, and the use of the GM cotton plants and their by-products in the same manner as non-GM or other commercially approved GM cotton. This would include conventional breeding with elite non-GM cotton cultivars to produce seed optimised for use under Australian conditions, sale of seed for commercial planting, use in human food and stockfeed, sale of lint, export of seed and unrestricted transport. Bayer has developed a training package and technical manual that will form part of the company's agreement with retailers and growers to purchase and handle Liberty Link[®] Cotton.

Under Australia's integrated framework for the regulation of genetically modified organisms, regulatory decisions are coordinated as far as possible. Bayer has received approval from Food Standards Australia New Zealand for the use of oil and linters derived from the Liberty

Link[®] Cotton in food (FSANZ report A533). In addition, the Australian Pesticides and Veterinary Medicines Authority is currently assessing an application from Bayer to register Liberty[®] 150 Herbicide for the control of various weeds in Liberty Link[®] Cotton.

Risk assessment

Background

The risk assessment first considered what harm to the health and safety of people or the environment could arise as a result of gene technology, and how it could happen, during the proposed release of the GM cotton into the environment (**hazard identification** refer to Chapter 2 for more information).

The risks to people and the environment from the proposed commercial release were assessed in comparison to non-GM cotton and GM cotton lines previously approved for commercial release by the Regulator, in the context of the intended agronomic management practices, and the environmental conditions in the regions proposed for the release.

Hazards are particular sets of circumstances (**events**) that might give rise to adverse outcomes (ie cause harm). When an event was considered to have some chance of causing harm, it was identified as posing a risk that required further assessment.

Each event associated with an **identified risk** was then assessed to determine the seriousness of harm (**consequence** - ranging from marginal to major) and the chance of harm (**likelihood** - ranging from highly unlikely to highly likely). The level of risk (ranging from negligible to high) was then estimated using a Risk Estimate Matrix (refer to Chapter 2 for more information).

Hazard identification

Of the 28 events compiled during the hazard identification process, three were selected for further assessment. The potential adverse outcome to the environment associated with these events was weediness. The remaining 25 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 weediness

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

- Expression of the herbicide tolerance gene (*bar*) increasing spread and persistence of the GM cotton plants through tolerance to glufosinate ammonium (event 1)
- Expression of the herbicide tolerance gene (*bar*) in non-GM *Gossypium hirsutum* or *G. barbadense* cotton plants increasing spread and persistence through providing glufosinate ammonium tolerance (event 2)
- Expression of the herbicide tolerance gene (*bar*) with introduced genes in other commercially approved GM cotton lines increasing spread and persistence through providing glufosinate ammonium tolerance as well as glyphosate tolerance and/or reduced insect attack on the plants (event 3).

The risk assessment considered the consequence and likelihood of harm that might result from each of the above events. The estimate of risk for all three events is **negligible**.

Risk management

The level of risk to health and safety of people or the environment for the three events that were assessed was estimated as **negligible**. The *Risk Analysis Framework* defines negligible

risks as insubstantial, with no present need to invoke actions for their mitigation. Therefore, no risk treatment measures are imposed.

The licence, detailed in Chapter 5 of the RARMP, 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 Liberty Link[®] Cotton poses **negligible** risks to the health and safety of people and the environment as a result of gene technology.

The risk management plan concludes that the negligible risks do not require specific risk treatment measures. Licence conditions that have been imposed relate to ongoing licence holder suitability; auditing and monitoring provisions; reporting requirements, including a compliance plan, annual report and other relevant information; and a suitable detection methodology.

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Abbreviations

% dm	Percentage dry mass
ACCRC	Australian Cotton Cooperative Research Council
ANZFA	Australia New Zealand Food Authority (now FSANZ)
APHIS	Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine Inspection Service
<i>bar</i>	Gene encoding bialophos resistance isolated from <i>Streptomyces hygroscopicus</i>
Bt	<i>Bacillus thuringiensis</i>
<i>cp4 epsps</i>	Gene encoding 5-enolpyruvylshikimate-3-phosphate synthase from <i>Agrobacterium</i> sp. strain CP4
<i>cry</i>	Gene encoding a crystal insecticidal protein isolated from <i>Bacillus thuringiensis</i> protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAD	DNA databank of Japan Amino acid sequence Database
DDBJ	DNA Databank of Japan
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
EMBL	European Molecular Biology Lab
FAO	Food and Agriculture Organisation of the United States
FDA	Food and Drug Administration of the United States
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
g	Gram
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
His-tag	Histidine tagged
HRAC	Herbicide Resistance Action Committee
IgE	Immunoglobulin E
kD	KiloDaltons
kg	Kilogram
km	Kilometre
LD ₅₀	Amount of a substance given in a single dose that causes death in 50% of a test population of an organism
MAFF	UK Ministry of Agriculture, Fisheries and Food (now called DEFRA)
mg	Milligram
MPB	4-methylphosphonico-butanoic acid
MPP	3-methyl phosphinico-propionic acid (3-hydroxy methyl phosphinoyl propionic acid)
mRNA	Messenger ribonucleic acid
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
<i>nos</i>	Gene encoding nopaline synthase
<i>nptII</i>	Gene encoding neomycin phosphotransferase type II protein from <i>E. coli</i>
OECD	Organisation for Economic Cooperation and Development

OGTR	Office of the Gene Technology Regulator
<i>pat</i>	Gene encoding phosphinothricin acetyltransferase protein from <i>Streptomyces viridochromogenes</i>
PAT	Phosphinothricin acetyltransferase
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
pg	picogram
PIR	Protein Information Resource
ppm	Parts per million
PPO	4-methylphosphonico-2-oxo-butanoic acid
PPT	phosphinothricin
RARMP	Risk Assessment and Risk Management Plan
RNA	Ribonucleic acid
SD	Standard Deviation
T-DNA	Transfer deoxyribonucleic acid
TEP	Total extractable protein
TGA	Therapeutic Goods Administration
Ti	Tumor inducing
EMBL	European Molecular Biology Laboratory
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
USDA	United States Department of Agriculture
WHO	World Health Organisation
µg	Microgram

Technical summary

Introduction

The Gene Technology Regulator (the Regulator) has decided to issue a licence (DIR 062/2005) to Bayer CropScience Pty Ltd (Bayer) for dealings involving the intentional, commercial scale release of herbicide tolerant genetically modified (GM) cotton into the Australian environment.

The DIR 062/2005 licence permits the commercial release of the GM cotton on an unrestricted basis in all areas of Australia. It should be noted that cultivation of this GMO may require additional approvals under State or Territory legislation that restrict the commercial release of GM crops on marketing grounds.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 (the Regulations) and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether or not to issue a licence to deal with a GMO.

The Regulator's *Risk Analysis Framework* explains the approach used to evaluate licence applications and to develop the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions¹.

The RARMP for DIR 062/2005 has been finalised in accordance with the gene technology legislation. Matters raised in the consultation process regarding risks to the health and safety of people or the environment from the dealings proposed by the applicant were taken into account by the Regulator in deciding to issue a licence and the conditions that have been imposed.

Consistent with Australia's integrated regulatory framework for gene technology, the Regulator has also liaised closely with other regulatory agencies that have been considering applications relating to this release, namely Food Standard Australia New Zealand (FSANZ) and the Australian Pesticides and Veterinary Medicines Authority (APVMA), to avoid duplication and enable coordinated decision making.

¹ More information on the assessment of licence applications and copies of the [Risk Analysis Framework](#) are available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030

Section 1 – Application

Title:	Commercial release of herbicide tolerant Liberty Link® Cotton for use in the Australian cropping system*
Applicant:	Bayer CropScience Pty Ltd
Common name of the parent organism:	Cotton
Scientific name of the parent organism:	<i>Gossypium hirsutum</i> L.
Modified trait(s):	Herbicide tolerance
Identity of the gene(s) responsible for the modified trait(s):	<i>bar</i> gene from the bacterium <i>Streptomyces hygroscopicus</i>
Proposed location(s):	Unrestricted planting in current and potential cotton growing areas
Proposed release size:	Phased introduction over 3 years to commercial scale planting, as well as transport and stockfeed use anywhere in Australia.
Proposed time of release:	Ongoing from August 2006

*The title of the licence application submitted by Bayer is *Commercial release of the herbicide tolerant cotton event LLCotton25 (Gossypium hirsutum) for use in the Australian cropping system*

Bayer applied for a licence to release a herbicide tolerant GM cotton, Liberty Link® Cotton, into the environment. Bayer is seeking approval for unrestricted, commercial scale planting of the GM cotton in all current cotton growing areas and potential future areas with environmental conditions suitable for cotton cultivation in Australia.

The GM cotton has only one introduced gene, the herbicide tolerance gene (*bar*), isolated from a common soil bacterium, *Streptomyces hygroscopicus*. The gene encodes the phosphinothricin acetyltransferase (PAT) protein. The PAT protein provides tolerance to glufosinate ammonium, the active ingredient in the herbicide Liberty®. This enables the herbicide to be applied for weed control in the GM cotton crop and provides an additional option for incorporation into integrated weed management strategies.

More detailed information on the GMOs, the introduced genes and their products is provided in Chapter 1.

The GM cotton proposed for release has previously been approved (described as Liberty® or LLCotton25) for limited and controlled releases under DIR licences 015/2002, 038/2003 and 056/2004.

The applicant requests approval for commercial scale cultivation without containment measures, and the use of the GM cotton plants and their by-products in the same manner as non-GM or other commercially approved GM cotton. This would include conventional breeding with elite, non-GM cotton to produce varieties suitable for use under Australian conditions, sale of seed for commercial planting, use in human food and stockfeed, sale of lint, export of seed and unrestricted transport anywhere in Australia.

The company anticipates a phased introduction over three years, involving large scale grower evaluations and seed increases, and the development of additional lines adapted for particular regional conditions. The rate of uptake will be determined by market acceptance, and seed and variety availability. Bayer expects the most substantial adoption of the GM cotton to occur initially in the existing cotton growing regions of New South Wales (NSW) and Queensland (QLD), followed by uptake in other areas where environmental conditions are suitable for cotton cultivation. Potential future cotton growing regions, as identified by the

applicant, include additional parts of NSW and QLD, some areas of the Northern Territory (NT) and northern Western Australia (WA), and in South Australia (SA) and Victoria (VIC) close to the NSW border (discussed further in Chapter 1). Small scale use for demonstrations and educational purposes is also proposed outside these areas.

Bayer has developed a Reseller and Agronomist Training and Accreditation package and a Technical and Crop Management Plan to optimise and maintain the use of its technology. These will form part of the company’s agreement with retailers and growers to purchase and handle Liberty Link® Cotton.

Section 2 Risk assessment

The risk assessment considered information contained in the application, previous GM cotton assessments, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received during consultation with a wide range of prescribed experts, agencies and authorities on the application, including all local government authorities in Australia (summarised in Appendix B), and on the RARMP (see Appendix D).

Similarly, advice received from the public on the application and from consultation on the RARMP and how it was considered is summarised in Appendices C and E, respectively.

The risk assessment first considered what harm to the health and safety of people or the environment could arise due to gene technology, and how it could happen during this release of GMOs into the environment (hazard identification), in comparison to non-GM and commercially released GM cotton and in the context of the proposed release area.

A hazard (source of potential harm) may be an event, substance or organism (OGTR 2005). The hazard identification process resulted in the compilation of a list of 28 events that describe sets of circumstances (events) by which the proposed release could potentially give rise to adverse outcomes.

A risk is identified when a hazard is considered to have some chance of causing harm to people and/or the environment. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance 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			

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.

Three of the 28 events characterised in the hazard identification process for the proposed release were identified as requiring further assessment. The potential adverse outcome associated with these events was increased spread and persistence (weediness). This identified risk was assessed in comparison to the parent organism and other GM cotton lines previously approved for commercial release, in the context of the intended agronomic management practices, and the environmental conditions in the regions where the proposed release might occur.

The consequence and likelihood assessments used to derive risk estimates for these three events are summarised in Table 1 (the detailed risk assessments are in Chapter 3). More information on the remaining 25 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

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
Event 1 Expression of the <i>bar</i> gene increasing spread and persistence of the GM cotton plants through tolerance to glufosinate ammonium	Marginal <ul style="list-style-type: none"> • Glufosinate ammonium is not effective for the control of established cotton volunteers. • In the presence of glufosinate ammonium, the small competitive advantage of the GM cotton is offset by abiotic and biotic factors (such as water availability, temperature, soil type and nutrients) that limit the spread and persistence of all cotton in Australia. 	Highly unlikely <ul style="list-style-type: none"> • Glufosinate ammonium is not a widely used herbicide for the control of cotton volunteers as other methods are more commonly used, such as 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 (eg transportation, use as stockfeed, via animals or flooding) finding suitable ecological niches and establishing as weeds would be no greater than for non-GM and commercially approved GM cotton lines. 	Negligible	No
Event 2 Expression of the <i>bar</i> gene in other <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants (not including commercially released GM cotton lines) providing glufosinate ammonium tolerance	Marginal <ul style="list-style-type: none"> • Glufosinate ammonium is not effective for the control of established cotton volunteers. • In the presence of glufosinate ammonium, the small competitive advantage of the GM cotton is offset by abiotic and biotic factors (such as water availability, temperature, soil type and nutrients) that limit the spread and persistence of all cotton in Australia. 	Highly unlikely <ul style="list-style-type: none"> • Cotton is primarily self-pollinating and gene transfer to other cotton plants is only expected to occur in close proximity and at low frequencies. • The requirement to apply insecticides to herbicide tolerant GM cotton will further reduce the chance of gene transfer via insects. • Glufosinate ammonium is not a widely used herbicide for the control of cotton volunteers as other methods are more commonly used, such as mechanical means or, if still at the seedling stage, alternative herbicides. 	Negligible	No

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
Event 3 Expression of the <i>bar</i> gene in combination with <i>cp4 epsps gene</i> and/or <i>cry1Ac</i> and <i>cry2Ab</i> genes providing dual herbicide tolerance and reducing lepidopteran herbivory	Minor <ul style="list-style-type: none"> • Glufosinate ammonium and glyphosate is not effective for the control of established cotton volunteers. • In the presence of glufosinate ammonium, and glyphosate and/or lepidopteran herbivory, the small competitive advantage of any stacked GM cotton is offset by abiotic and biotic factors (such as water availability, temperature, soil type and nutrients) that limit the spread and persistence of all cotton in Australia. • The <i>bar</i> gene operates independently of the herbicide tolerant and insecticidal genes present in other GM cotton lines and there is no evidence of any interactions. 	Highly unlikely <ul style="list-style-type: none"> • The current commercially approved GM cotton lines are only authorised for unrestricted release in southern areas of Australia. Stacking is not expected to occur in northern areas of Australia as field trials with GM cotton in northern Australia are required to be conducted under limited and controlled conditions. • Cotton is primarily self-pollinating and gene transfer to other cotton plants is expected to occur in close proximity and at low frequencies. • The requirement to apply insecticides to herbicide tolerant GM cotton will further reduce the chance of gene transfer via insects. • Glufosinate ammonium and glyphosate are not used to control established cotton volunteers as other methods are more commonly used, such as mechanical means or, if still at the seedling stage, alternative herbicides. 	Negligible	No

Section 3 Risk management

A risk management plan builds upon the risk assessment to consider whether any action is required to mitigate the identified risks, and what can be done to protect the health and safety of people and the environment.

The risk assessment considered three events that might lead to a risk to the environment. The risk estimates for the adverse outcome associated with all three events are **negligible** (ie insubstantial with no present need to invoke actions for their mitigation). Therefore, no risk treatment measures for identified risks were imposed.

The licence, detailed in Chapter 5 of the RARMP, 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.

3.2 Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework (OGTR 2005). Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration, National Industrial Chemicals Notification and Assessment Scheme, National Health and Medical Research Council and Australian Quarantine Inspection Service. Dealings conducted under any licence issued by the Regulator may also be subject to regulation by one or more of these agencies².

² More information on Australia's integrated regulatory framework for gene technology is contained in the [Risk Analysis Framework](#) available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030.

FSANZ is responsible for human food safety assessment, including GM food. FSANZ has approved the use of food (oil and linters) derived from Liberty Link[®] Cotton (FSANZ report A533).

The use of herbicides containing glufosinate ammonium on the Liberty Link[®] Cotton is subject to regulation by the APVMA. Bayer has a research permit for use of glufosinate ammonium in current cotton trials involving this GMO, and the APVMA is currently assessing an application from Bayer to register Liberty[®] 150 Herbicide for the control of various weeds in Liberty Link[®] Cotton.

Section 4 Conclusions of the RARMP

The risk assessment concludes that this commercial release of herbicide tolerant GM cotton poses **negligible** risks to the health and safety of people and the environment as a result of gene technology.

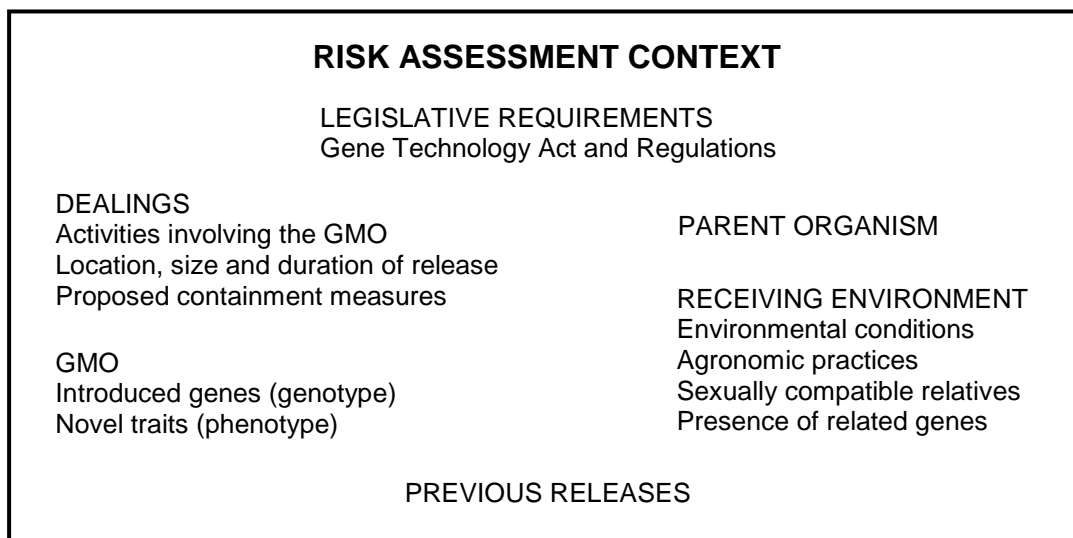
The risk management plan concludes that the negligible risks do not require specific risk treatment measures. Licence conditions that have been imposed relate to ongoing licence holder suitability; auditing and monitoring provisions; reporting requirements, including a compliance plan, annual report and other relevant information; and a suitable detection methodology.

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 and the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation³, details of the intended dealings, the GMO(s) and parent organism(s), previous approvals and releases of the same or similar GMOs in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.1.

Figure 1.1 Components of the risk context considered during the preparation of the Risk Assessment



2. Sections 49 to 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Regulator must take into account, and who she must consult with, in preparing the RARMPs that form the basis of her decision on licence applications.

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

- the proposed size, duration and regions requested by the applicant
- characteristics of the parent organism and other commercially released and widely grown GM cotton lines (OGTR 2002)
- the nature and effect of the genetic modification
- the environmental conditions in the regions where the release would occur
- presence of the same or similar gene and its product in the environment
- presence of the same or other GM cotton lines relevant to this application in the environment
- relevant agricultural practices (however, it does not assume compliance with Bayer's Technical and Crop Management Plan, refer to Section 2.1 of this Chapter)
- previous approvals for release of the GMO in Australia and overseas.

³ More information on the assessment of licence applications and copies of the [Risk Analysis Framework](#) are available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030

4. Initial consideration of the application under section 49 of the Act determined that public consultation was not required for the preparation of the consultation version of the RARMP. Even though public comment was not sought on the preparation of the consultation version of the RARMP, one submission from the public was received (summarised in Appendix C).

5. In accordance with section 50 of the Act, the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government agencies, the Minister for Environment and Heritage and all local councils in Australia were consulted on matters relevant to the preparation of the RARMP. This advice, and where it was taken into account in the RARMP, is summarised in Appendix B.

6. In accordance with section 52 of the Act, the Regulator notified the public that a RARMP had been prepared and invited written submissions. Advice on the RARMP was also sought from the same experts, agencies and authorities as mentioned above. The issues raised and how they were addressed in the RARMP are summarised in Appendices D and E, respectively.

Section 2 The GMO and proposed dealings

2.1 The proposed dealings

7. Bayer proposes to release genetically modified (GM) herbicide tolerant Liberty Link[®] Cotton into the environment.

8. Bayer is seeking approval for unrestricted commercial scale planting of the GM cotton in current and potential cotton growing regions of Australia. The company anticipates a phased introduction over three years, involving large scale grower evaluations and seed increases, and the development of additional lines adapted for particular regional conditions. The rate of uptake will be determined by market acceptance, and seed and variety availability. Bayer expects the most substantial adoption of the GM cotton to occur initially in the existing cotton growing regions of New South Wales (NSW) and Queensland (QLD), followed by uptake in other areas where environmental conditions are suitable for cotton cultivation (refer to Section 3.3.2 of this Chapter). Potential future cotton growing regions as identified by the applicant include additional parts of NSW and QLD, some areas of the Northern Territory (NT) and northern Western Australia (WA), and in South Australia (SA) and Victoria (VIC) close to the NSW border. Small scale use for demonstrations and educational purposes is also proposed outside these areas.

9. Bayer intends that the GM cotton plants and their products would be used in the same manner as conventional and other commercially approved GM cotton lines. Hence, the dealings would include:

- conventional crossing with elite non-GM cotton varieties to produce lines suitable for use under Australian conditions
- use of oil and linters from the GM cotton in human food
- sale of seed for commercial planting
- sale of lint
- export of seed

and would involve transportation, storage and use of cotton seed as stockfeed in all areas of Australia.

10. Cotton (including this GM cotton if approved) is used as both a fibre and food/feed crop. The cotton lint (long cellulose fibres) removed from seed cotton during ginning are used to produce cotton fabrics for clothing, upholstery, towels and other household products. De-linted cotton seed is processed into four major products: oil, meal, hulls and linters (a type of

short cellulose fibre) (Cherry & Leffler 1984). Whole cotton seed, meal and hulls are used in stockfeed. The oil is used in a variety of food products (including frying oil, salad dressing and margarine) and the linters are used as a cellulose base for several consumer food and hygiene products.

11. Food Standards Australia New Zealand (FSANZ) has approved the use of food (oil and linters) derived from Liberty Link[®] Cotton (known as LL25 in their assessment)(FSANZ report A533).

12. Bayer does not propose to use any containment measures. However, Bayer proposes to implement a program that involves the education of all parties dealing with the GM cotton. A Reseller and Agronomist Training and Accreditation package is proposed to be used and is designed to provide a working knowledge of the Liberty Link[®] Cotton system and the registered glufosinate ammonium herbicide use recommendations, as well as strategies for responding to various issues such as cotton volunteer management. Any ongoing regulatory obligations impacting on Liberty Link[®] Cotton and the registered glufosinate ammonium herbicide will be included in the training and accreditation package. Bayer will require retailers and growers of Liberty Link[®] Cotton to be accredited and to have signed an agreement with the company before they can purchase and handle Liberty Link[®] Cotton.

13. As part of the grower and reseller education, Bayer proposes that any partial bags of Liberty Link[®] Cotton seeds (not planted) are disposed of in a manner consistent with reducing the unintended spread of the GM cotton or mixing with other cotton seed before sowing. Bayer also intends that growers should take care not to distribute cotton trash which may contain viable seed to other areas, paddocks or off-site. This information will be included in a technical manual for Liberty Link[®] Cotton titled 'Bayer CropScience Liberty Link[®] Cotton Technical Manual and Crop Management Plan' and this will be provided to growers.

14. In the technical manual, Bayer recommends a number of strategies to minimise dissemination of seed including:

- seed drilling equipment is cleaned in the field after use and before leaving the field to prevent any spills of seeds into unplanned areas
- spillage of seed is minimised when travelling to and from the field and on field boundaries (by securing loads)
- if seed spillage does occur, volunteers are controlled by appropriate cultivation and/or herbicides in subsequent crops according to Good Agricultural Practice
- maintenance of good records.

15. The waste produced from growing and harvesting the GM cotton plants will be treated in the same way as waste from non-GM cotton without segregation.

2.2 The parent organism

16. The parent organism is cultivated cotton (*Gossypium hirsutum L.*), which is exotic to Australia and is grown as an agricultural crop in NSW, southern and central QLD. More detailed information on cotton can be found in the document, [The Biology and Ecology of Cotton \(*Gossypium hirsutum*\) in Australia \(OGTR 2002\)](#), which was produced to inform the risk assessment process for licence applications involving GM cotton plants.

2.3 The GMO

17. The GM cotton contains a single copy of a gene (*bar*), derived from the common soil bacterium *Streptomyces hygroscopicus*. The protein encoded by the *bar* gene is the enzyme⁴

⁴ Enzymes are proteins which catalyse specific biochemical reactions.

phosphinothricin acetyltransferase (PAT) that confers tolerance to glufosinate ammonium (the active constituent in herbicides such as Basta[®] and Liberty[®]). The PAT protein converts glufosinate ammonium into an inactive form and thus renders the plant tolerant to the herbicide.

18. The *bar* gene confers tolerance to the herbicide glufosinate ammonium in both laboratory cultures during the initial stage of selection of GM plants and when applied to whole plants in the field. No other selectable marker gene was used. The GM plants are tolerant to the application of the herbicide at all developmental stages.

19. Short regulatory sequences that control expression of the gene are also present in the GM cotton. These are derived from Cauliflower mosaic virus and the common soil bacterium *Agrobacterium tumefaciens*. Although both organisms are plant pathogens, the regulatory sequences comprise only a small part of their total genomes, and are not capable of causing disease.

2.4 The introduced gene and its product

2.4.1 The herbicide tolerance gene (*bar*)

20. The *bar* gene was isolated from *Streptomyces hygroscopicus*, strain ATCC21705 (Murakami et al. 1986), a common saprophytic, soil-borne microorganism that is not considered to be a pathogen of plants, humans, or other animals (OECD 1999a). The *bar* gene encodes the PAT protein, which confers tolerance to glufosinate ammonium, the active component in a number of herbicides.

21. The active constituent of glufosinate ammonium is the amino acid phosphinothricin, or PPT (an analogue of glutamate). PPT is found naturally as a component of bialaphos and phosalacine, tripeptide antibiotics produced by members of the bacterial genera *Streptomyces* and *Kitasatosporia* (Omura et al. 1984; Wehrmann et al. 1996). Bialaphos (phosphinothricyl-L-alanyl-L-alanine) consists of PPT and two L-alanine residues, while phosalacine (phosphinothricyl-L-alanyl-L-leucine) consists of PPT, an alanine residue and a leucine residue.

22. Glufosinate ammonium is widely used as a broad-spectrum herbicide and is registered for use in many countries. However, in Australia it is not a widely used herbicide for reasons including its higher cost than some other commonly used herbicides as well as it is not registered by the APVMA for use in broadacre crops apart from GM canola (which is not currently grown commercially). Proprietary names include Basta[®], Finale[®] and Liberty[®]. Glufosinate ammonium is an inhibitor of the enzyme glutamine synthetase, a key enzyme in the nitrogen metabolism of plants. Inhibition of glutamine synthetase by glufosinate ammonium causes rapid accumulation of ammonia, as well as inhibition of photosynthesis, leading to cell death (Droge-Laser et al. 1994). In GM plants containing the *bar* gene, the addition of an acetyl group to phosphinothricin by the PAT protein prevents this inhibition of glutamine synthetase and as a result detoxifies the herbicide.

23. To enable correct expression of the protein in plants and to facilitate integration of the DNA into the cotton genome, the N-terminal two codons of the wild type *bar* gene coding region have been substituted for the codons ATG and GAC, respectively. Accordingly, the serine residue in position 2 of the *bar* gene was substituted for an aspartic acid and therefore the PAT protein expressed in Liberty Link[®] Cotton is slightly different from the wild-type PAT protein (Berghman 2003; De Beuckeleer 2003b). However, the modified protein is functionally equivalent to the wild-type PAT protein.

24. Expression of the *bar* gene in Liberty Link[®] Cotton plants is controlled by the 35S promoter from Cauliflower mosaic virus (Odell et al. 1985), a DNA virus that infects members of the *Cruciferae* (eg cabbages, cauliflowers, mustard). In the natural situation, the 35S promoter drives high level expression of RNA transcripts that are required for viral replication in infected plant cells (Guilley et al. 1982).

25. The 35S promoter is the most common promoter used to control the expression of introduced genes in GM plants because it is a constitutive promoter (Potenza et al. 2004). This means that genes that are linked to this promoter are generally expressed at relatively high levels throughout the growing season and in most tissues of the plant. More information on the 35S promoter can be found in the RARMP for DIR 049/2004, available on the [OGTR website](#). Information on the expression pattern and levels of the *bar* gene and encoded PAT protein in Liberty Link[®] Cotton is provided in Section 2.4.3 of this Chapter.

26. The termination and polyadenylation signals that are also responsible for controlling *bar* gene expression are derived from the 3' end of the nopaline synthase (*nos*) gene of *Agrobacterium tumefaciens* (Depicker et al. 1982).

27. Although the regulatory sequences used to express the *bar* gene in Liberty Link[®] Cotton are derived from two plant pathogens, these sequences are not capable of causing disease.

28. The different genetic elements introduced into Liberty Link[®] Cotton are described in Table 1.1.

Table 1.1: Description of the genetic elements introduced into Liberty Link[®] Cotton

Genetic element	Function	Size (base pairs)	Source	Reference
Right border repeat	cis-acting element for T-DNA transfer	25	<i>Agrobacterium tumefaciens</i>	(Gielen et al. 1984)
Polylinker sequence	for cloning purposes	28	Synthetic	
35S promoter	regulates constitutive gene expression	1385	Cauliflower mosaic virus	(Odell et al. 1985)
<i>bar</i> gene	herbicide tolerance and selectable marker	552	<i>Streptomyces hygroscopicus</i>	(Thompson et al. 1987)
Polylinker sequence	for cloning purposes	19	Synthetic	
3' <i>nos</i> terminator	stop signal	261	<i>Agrobacterium tumefaciens</i>	(Depicker et al. 1982)
Polylinker sequence	for cloning purposes	51	Synthetic	
Left border repeat	cis-acting element for T-DNA transfer	25	<i>Agrobacterium tumefaciens</i>	(Gielen et al. 1984)

29. Liberty Link[®] Cotton plants do not contain any antibiotic resistance or other selectable marker genes. The *bar* gene confers tolerance to glufosinate ammonium both in laboratory cultures during the initial stage of selection of GM plants and when applied to whole plants in the field.

30. Both the *bar* and *pat* genes have been widely used in producing GM plants with tolerance to glufosinate ammonium-based herbicides (Wehrmann et al. 1996).

2.4.2 Characterisation of the inserted genetic material and stability of the genetic modification

31. Southern blot and PCR analysis was used to demonstrate that a single intact copy of the *bar* gene construct (see Table 1.1 of this Chapter) has been inserted into the cotton genome in

Liberty Link[®] Cotton and that this copy is not rearranged (Berghman & De Beuckeleer 2002; De Beuckeleer 2004). The sequence of the inserted DNA is identical to the corresponding sequence in the plasmid vector used to introduce the *bar* gene construct.

32. Polymerase Chain Reaction (PCR) and Southern blot analysis have further demonstrated that no unnecessary vector sequences are present in Liberty Link[®] Cotton and that the sequences flanking the right and left border of the *bar* gene construct insertion are derived from *Gossypium hirsutum* (Aerts & De Beuckeleer 2003). Analysis of the plant DNA sequences flanking the insertion site (using the Basic Local Alignment Search Tool algorithm SYNERGY) did not detect any meaningful sequence similarity to sequences published within the GenBank, EMBK, DDBJ and PDB databases. There was no evidence to support the possibility that a novel transcript might arise at either junction of the insert (De Beuckeleer 2003c).

33. The insert was shown (by Southern blot analysis) to be stably inherited in individual Liberty Link[®] Cotton plants of multiple backgrounds and generations grown under greenhouse conditions in Europe (Aerts & De Beuckeleer 2002a), and in individual plants grown in 11 different environments in the USA (Aerts & De Beuckeleer 2002b). Bayer states that CSIRO has been working with cotton containing the LLCotton25 transformation event in Australia over a number of seasons and have not found any instability with the transformation event in terms of tolerance to glufosinate ammonium or the expected segregation of the trait in breeding populations.

34. Under DIR 015/2002, field assessments were carried out on the F₂ homozygous lines produced from backcrosses of cotton containing the LLCotton25 transformation event into elite Australian cultivars. Assessments involved spraying plants with discriminating doses of glufosinate ammonium herbicide. A percentage of sprayed GM cotton plants were tolerant to the herbicide, showing that the *bar* gene was functional and segregating as a single locus. At each generation in the backcross, the number of herbicide tolerant plants recovered was the expected 50%. When these F₁ plants were selfed, the glufosinate ammonium tolerant plants segregated in the expected 3:1 ratio amongst the 300-400 plants tested.

2.4.3 Expression of the PAT protein in the GM cotton

35. Levels of the PAT protein produced from the expression of the *bar* gene in Liberty Link[®] Cotton have been assessed in various plant tissues, either untreated or treated with glufosinate ammonium based herbicide. PAT protein expression was measured by detection of the protein with enzyme linked immunosorbent assays (ELISA). Northern blot analysis was used to measure mRNA expression levels of the *bar* gene.

36. PAT protein content in leaves, stems, roots and pollen of Liberty Link[®] Cotton plants grown under greenhouse conditions in the USA during 2001/2002 were measured by ELISA (Table 1.2). No glufosinate ammonium was applied. Samples were taken from five different plants at the 2- to 4-leaf stage of growth. PAT protein content detected in these tissues was low. As a percent of total crude protein, PAT protein content of leaves and stems was similar, and about two to three times higher than the amount in roots. This is consistent with a report which suggests that, in rice, the *35S* promoter does not function as well in roots compared to leaves and stems (Battraw & Hall 1990). Comparable values for pollen could not be determined because not enough pollen was available for the crude protein analysis (Currier 2002).

37. Instead, comparisons were made on PAT protein content as a percent of total extractable protein (TEP) (Table 1.2) (Currier 2002). Although some samples of fresh pollen contained the highest level of PAT protein, the average PAT protein contents as a percent of TEP for

roots, stems and leaves were about 20 to 40 times higher than the average value for fresh pollen. The range in PAT protein content was greatest in fresh pollen from individual flowers, followed by frozen pollen from composite samples of five flowers.

38. No PAT protein was detected in the parental non-GM cotton variety.

Table 1.2: PAT protein levels in different tissues of Liberty Link[®] Cotton plants

Tissue	PAT Protein content (µg per g fresh weight)		PAT content (as % of crude protein)	Average TEP ^b (mg/g fresh weight ± SD)	Average PAT content as % of TEP
	Range	Average			
Roots	5.63 - 10.1	7.97 ± 1.86	0.08	2.26 ± 0.22	0.35
Stems	34.3 - 45.5	36.8 ± 6.7	0.23	4.99 ± 0.92	0.74
Leaves	45.1 - 57.3	52.9 ± 6.0	0.19	7.13 ± 0.79	0.74
Pollen - frozen	4.44 - 13.0	8.23 ± 3.20	NA ^a	146 ± 8	0.006
Pollen - fresh	0.11 - 170	19.3 ± 39.2	NA	107 ± 21	0.018

^aNA: not applicable as crude protein determinations could not be made on these samples.

^bTEP: total extractable protein

39. PAT protein content was measured in Liberty Link[®] Cotton leaf samples from the four vegetative plant growth stages that are critical for expression of the herbicide-tolerant phenotype (Scott & Currier 2002). These four stages are:

- 2- to 4-leaf stage (less than one week before first spray)
- 4- to 6-leaf stage (approximately two weeks after first spray)
- beginning of bloom
- full bloom.

40. Liberty Link[®] Cotton plants were grown under greenhouse conditions in the USA during 2001/2002 and received different herbicide treatments (Table 1.3). The average PAT protein measured at the four growth stages and the different herbicide treatments ranged from 57.7µg/g to 98.3µg/g fresh weight in Liberty Link[®] Cotton leaf samples, which is equivalent to 0.21 to 0.35% of the total crude protein. PAT as percent of the TEP increases until flowering and then declines (Scott & Currier 2002). PAT protein content in Liberty Link[®] Cotton leaves was found to have an upper limit of about 130µg/g fresh weight. No PAT protein was detected in the non-GM Coker 312 control.

Table 1.3: PAT protein content in leaves of non-GM cotton and Liberty Link[®] Cotton at different stages of plant growth (with or without application of Liberty[®] herbicide)

Sample	PAT protein content at specified growth stages (µg/g fresh weight ± SD)			
	2- to 4-leaf	4- to 6-leaf	early bloom	full bloom
non-GM Coker 312 (control)	ND ^a	ND	ND	ND
Liberty Link [®] Cotton sprayed once	NA ^b	85.0 ± 15.6	98.3 ± 16.8	92.6 ± 15.1
Liberty Link [®] Cotton sprayed twice	NA	NA	NA	92.6 ± 20.3
Liberty Link [®] Cotton unsprayed	57.7 ± 5.3	74.0 ± 12.3	90.2 ± 14.4	75.1 ± 25.6

^aND: not detected; ^bNA: not applicable

41. PAT protein content in cotton raw agricultural commodities (RACs) of Liberty Link[®] Cotton was determined by ELISA for three fractions: cleaned seed, lint coat (hull with linters) and lint (Table 1.4) (Kowitz & Currier 2001). The Liberty Link[®] Cotton plants used were grown under typical production conditions, which included two herbicide (Liberty[®]) applications, on four locations in the USA in 2000. Due to difficulties in sample processing, fuzzy seed was not analysed directly but separated into two fractions, cleaned seed and lint coat (containing linters and the associated seed coat). PAT content of each fraction was determined and from these the amount of PAT protein in the fuzzy seed was calculated. More than 98.5% of the PAT protein in the boll was found in the cleaned seed fraction and therefore would also be present in the fuzzy seed fraction (cleaned seed plus lint coat). The lint coat and lint fractions contained less than 1.5% of the PAT protein. PAT protein was not found in any of the non-GM controls.

Table 1.4: PAT protein content in RACs of Liberty Link[®] Cotton

Sample	Average PAT Protein Content (as µg per g fresh weight) ± SD		Average PAT Protein Content (as % of crude protein)	
	Liberty [®] sprayed	No Liberty [®]	Liberty [®] sprayed	No Liberty [®]
Cleaned seed	127 ± 18	113 ± 24	NA ^a	NA
Lint coat	1.15 ± 0.45	0.92 ± 0.50	NA	NA
Fuzzy seed ^b	69.9 ± 6.0	63.0 ± 10.3	0.030	0.027
Lint	0.78 ± 0.63	0.50 ± 0.42	0.003	0.003

^aNA: not applicable as crude protein determinations were not made on these samples.

^b values are the sum of *cleaned seed* and *lint coat* data

42. Expression of the *bar* gene was also investigated by Northern blot analysis, with *bar* gene mRNA being detected in leaves, stems, roots and seed of Liberty Link[®] Cotton (De Beuckeleer 2003c). Expression levels of the *bar* gene ranged from 4 to 8 pg/µg total RNA in leaf and stem samples, 2 to 4 pg/µg total RNA in root samples and 0.1 to 0.2 pg/µg total RNA in seeds. No *bar* gene mRNA product was detected in the non-GM controls.

43. The possibility that the insertion resulted in cryptic gene expression was also investigated using Northern blot analysis. Northern blot experiments failed to detect any such cryptic RNA expression in the tested leaf, stem, root or seed samples (De Beuckeleer 2003a; De Beuckeleer 2003c).

2.5 Method of genetic modification

44. Liberty Link[®] Cotton was produced by *Agrobacterium*-mediated transformation (Zambryski 1992) of the Coker 312 cotton variety (LLCotton25 transformation event). Coker cotton varieties are US cultivars that are widely used in producing GM cottons because they can be readily cultured and regenerated in the laboratory.

45. *Agrobacterium tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. Plants can be genetically modified by the transfer of DNA (transfer-DNA or T-DNA, a specific segment located between specific right and left border sequences on a resident plasmid) from *A. tumefaciens* through the mediation of the genes from the *vir* (virulence) region of *Ti* (*tumour inducing*) plasmids (Christie 1997; Zupan et al. 2000). Normally when using *Agrobacterium* vectors, only the T-DNA is transferred and integrated into the plant genome (Chilton et al. 1977; Zupan et al. 2000), although flanking vector sequences can also be transferred. It is generally accepted that T-DNA transfer into

plant cells by *Agrobacterium* is irreversible (Huttner et al. 1992) and cannot be re-mobilised to transfer elsewhere in the genome or to other organisms.

46. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains do not contain the genes responsible for the overproduction of auxin and cytokinin (*iaaM*, *iaaH* and *ipt*), which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). *Agrobacterium* plasmid vectors used to transfer T-DNAs contain well characterised DNA segments required for their replication and selection in bacteria, and for transfer of T-DNA from *Agrobacterium* and its integration into the plant cell genome (Bevan 1984; Wang et al. 1984). *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing genes into plants without causing any biosafety concerns or any adverse reactions.

47. In this instance, the binary plasmid vector pGSV71 (Berghman 2003) was used to introduce the gene construct containing the *bar* gene into the Coker 312 variety. This plasmid is a derivative of plasmid pGSC1700 (Cornelissen & Vandewiele 1989). The genetic elements of pGSV71 that were transferred into the plant genome are described in Table 1.1 of this Chapter. The pGSV71 plasmid also carried a copy of the *aadA* selectable marker gene from the bacterial transposon Tn7 (from *Escherichia coli*), conferring resistance to the antibiotics streptomycin and spectinomycin, for propagation and selection of the plasmid in bacteria and *A. tumefaciens*. However, this marker gene was not transferred into the Liberty Link[®] Cotton plant genome.

48. The plasmid vector pGSV71 was maintained in *Escherichia coli*, and transferred to a suitable *Agrobacterium tumefaciens* strain prior to plant transformation. Following co-cultivation with *A. tumefaciens*, cotton cells were cultured in the presence of phosphinothricin (an analogue of glufosinate ammonium), to select for those containing the inserted *bar* gene (since the *bar* gene confers tolerance to phosphinothricin). Subsequently, GM cotton plants containing the *bar* gene were regenerated from these cells and tested for herbicide tolerance. Of the many different transformation events originally produced, the LLCotton25 transformation event was selected. This line contained a single copy of the *bar* gene, and showed consistent, efficient herbicide tolerance both in the laboratory as well as during field trials in the USA and in Australia. It was therefore selected as an elite transformation event for conventional breeding into commercially useful cotton varieties.

49. The GM cotton varieties proposed for release (known as Liberty Link[®] Cotton) are backcross progeny of conventional crosses between GM cotton containing the LLCotton25 transformation event and a number of elite Australian cotton cultivars that are suitable for Australian cotton production areas. Further crosses into additional cultivars will also be conducted as part of the release.

Section 3 The receiving environment

50. The receiving environment forms part of the context in which the risks associated with dealings involving the GMO are assessed. This includes the size, duration and regions of the dealings, any relevant biotic/abiotic properties of the regions where the release would occur; intended agronomic 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 2005).

3.1 Size and duration of the proposed release

51. The size and duration of the proposed release are outlined in Section 2.1. The proposed release is to occur in the current cotton growing areas in NSW, and southern and central QLD, and other areas with environmental conditions suitable for cotton cultivation. The GM cotton may also be planted on a small scale in different regions for demonstrations and educational purposes. The applicant intends for the GM cotton to be transported and used as stockfeed anywhere in Australia.

3.2 Major cotton growing regions of Australia

52. Table 1.5 lists the major cotton growing regions in Australia. Detailed information about cotton production and the individual valleys where cotton was grown commercially in the 2004–05 season is available from Cotton Australia (Cotton Australia 2005). A [map showing the local government areas in which cotton was grown](#) in 2001 is available on OGTR website.

Table 1.5 Major cotton growing regions as of 2003^a

State	Cotton growing region	LGAs	Towns
QLD	Central Highlands	Emerald, Peak Downs	Emerald
QLD	Dawson - Callide	Banana	Theodore, Biloela, Moura
QLD	St George - Dirranbandi	Balonne	St George, Dirranbandi
QLD	Darling Downs	Wambo, Dalby, Jondaryan, Chinchilla, Pittsworth, Milmerran	Dalby, Chinchilla, Oakey, Pittsworth, Milmerran, Toowoomba
QLD/NSW	Macintyre Valley	Waggamba (QLD), Moree Plains (NSW)	Goondiwindi, Mungindi, Bogabilla
NSW	Gwydir Valley	Moree Plains, Walgett	Moree, Collarenebri
NSW	Upper Namoi	Gunnedah	Gunnedah, Boggabri, Curlewis
NSW	Lower Namoi	Narrabri, Warren	Narrabri, Wee Waa, Walgett
NSW	Macquarie Valley	Narromine, Warren	Narromine, Warren, Trangie, Dubbo
NSW	Bourke	Bourke	Bourke
NSW	Lachlan - Murrumbidgee	Carrathool, Lachlan	Hillston, Lake Cargellico, Griffith

^a Source: modified from (Reeve et al. 2003)

3.3 Environmental conditions suitable for growing cotton

3.3.1 Areas currently growing cotton in Australia

53. Climates with long, warm summers are typical for summer cotton growing regions in Australia. The areas where cotton can be grown in southern Australia are mainly limited by the amount of irrigation water available (for irrigated cotton) and the length of the summer season. Climatic data for some of the current cotton growing regions are given in Table 1.6.

54. Temperature is the dominant environmental factor affecting cotton development and yield (Constable & Shaw 1988; Australian Cotton Cooperative Research Centre 2002b). Cotton is planted when the minimum soil temperature at 10 cm depth is 14°C for at least three successive days. Cotton seedlings may be killed by frost. A minimum of 180–200 frost-free days of uniformly high temperatures (averaging 21–22°C) is required (Duke 1983). Growth and development of cotton plants below 12°C is minimal and a long, hot growing season is crucial for achieving good yields (Constable & Shaw 1988). In the current cotton growing areas of NSW, and southern and central QLD, the growing season for cotton is typically from September/October through to March/April.

55. Crop yields may be lower in southern growing regions as a result of the shorter summer season. The minimum day degrees (heat accumulation, calculated progressively during the season) required from planting of cotton to 60% boll opening is 2050 (information from the Australian Cotton CRC; available at <<http://cotton.pi.csiro.au>>). For example, cotton planted on 1 October near Warren (Macquarie Valley, NSW) could be expected to reach 60% boll opening by 31 March the following year.

56. Cotton can also be grown as dryland crop (Australian Cotton Cooperative Research Centre 2002b). Dryland cotton production strongly depends on rainfall (at the right time during the growing season) and the water holding capacity of the soil. In most areas south of latitude 22° South, the [variability of rainfall during the critical months](#) (January to March) is high and therefore, dryland cotton production may not always be viable.

Table 1.6 Climatic data for some of the current cotton growing regions in Australia.

Average daily temperature and rainfall for summer and winter	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 ^a)	34.2°C/20.3°C	32.3°C/17.3°C	35.6°C/20.3°C	32.4°C/17.6°C
Average daily max/min temperature (winter ^b)	23.3°C/7.8°C	18.9°C/4.5°C	18.9°C/5.5°C	15.8°C/4.6°C
Average monthly rainfall (summer)	84.4 mm	72.5 mm	38.8 mm	28.7 mm
Average monthly rainfall (winter)	27.8 mm	45.7 mm	23.6 mm	32.1 mm

^a December, January, February

^b June, July, August

Source: [Bureau of Meteorology](#)

3.3.2 Possible areas for expansion of the cotton industry

57. Opportunities for further expansion of the cotton industry in southern Australia are limited mainly by the length of growing season in VIC and southern NSW (S. Vaessen, NSW Department of Primary Industries, pers. comm.), or availability of irrigation water in NSW, SA, and WA (S. Vaessen, NSW Department of Primary Industries; N. Wihelm, South Australia Research and Development Institute; R. Wheater, Department of Agriculture, WA; pers. comm., respectively).

58. A study by the Australian Cotton Cooperative Research Centre (Australian Cotton Cooperative Research Centre 2004) indicates considerable potential for expansion of cotton in northern Australia, in WA, NT and QLD. The study suggested at least 200,000 ha of potential irrigation areas that could be developed in the near future (< 10 years). Key factors in determining the suitability of an area for cotton growing include the average temperature

during the growing season, timing of rainfall, and the suitability of the soil. The ACCRC report noted that for many of the potential cotton sites in northern Australia, some of the basic information or long term data on environmental conditions, potential pests of cotton, and the relationship between surface and ground water may limit or delay the introduction of cotton into these areas.

59. The ACCRC study (2004) examined 17 potential sites for cotton growing in northern Australia. Climatic conditions and proposed region specific production systems for five of these sites where the ACCRC is currently involved are given in Table 1.7. These include sites where field trials with GM cottons have been carried out since 2002 and are the most likely sites for the introduction of commercial cotton production. The majority of the arable soils of northern Australia are similar in that they are red and yellow earths and poorly drained cracking clays. The fertility of these soils is moderate to low. Soil type and fertility impact crop nutrition, soil surface management and irrigation systems. There is potential for growing cotton crops in both summer and winter in different locations, particularly in north QLD. However, the wet season (approximately November through to March) in more northern areas of Australia would impact greatly on cotton fibre quality (Eastick 2002; Farrell & Roberts 2002) and the ability to access and operate in the cotton fields. The most suitable growing season for cotton in each of the five regions in northern Australia, as suggested in the ACCRC study, is provided in Table 1.7.

Table 1.7 Climatic data for sites where the Australian Cotton CRC is currently involved in northern Australia.

	Broome Post Office (northern WA)	Kununarra ORIA (northern WA)	Katherine Council (northern NT)	Richmond Post Office (northern QLD)	Lower Burdekin Ayr DPI RS (northern QLD)
Average daily max/min temperature (summer ^a)	33.6°C/26.1°C	36.7°C/25.2°C	35.3°C/24.0°C	36.9°C/22.6°C	31.7°C/22.5°C
Average daily max/min temperature (winter ^b)	28.6°C/14.9°C	31.4°C/16.1°C	30.9°C/14.3°C	26.8°C/9.4°C	25.6°C/12.3°C
Average monthly rainfall (summer)	126.1 mm	171.6 mm	216.4 mm	98.7 mm	182.4 mm
Average monthly rainfall (winter)	10.1 mm	1.8 mm	0.9 mm	9.3 mm	18.2 mm
Growing season	May-November	April-October	March-October	December-July	March-November
Arable soil type	Sandy loam	Cracking clay	Clay loam and sandy clay loam	Cracking clay, some inherent salinity	Cracking clay
Irrigation system	Sub surface drip	Furrow	Sub surface drip/ overhead	Furrow	Furrow
Development status	New area under development or evaluation	Existing (non-cotton) irrigated cropping and/or potential for expansion.	New area under development or evaluation	New area under development or evaluation	Existing (non-cotton) irrigated cropping and/or potential for expansion.

^a December, January, February

^b June, July, August

ORIA: Ord River Irrigation Area

DPI RS: Department of Primary Industries Research Station

Sources: [Bureau of Meteorology](#) and ACCRC (2004)

3.4 Presence of the PAT protein or similar proteins in the receiving environment

60. The PAT protein is widespread in the environment, through the presence of the bacteria from which it is derived. This forms part of the baseline data for assessing any risks from exposure to this protein that may result from the proposed release of the GM cotton.

PAT proteins are produced naturally by the common soil bacteria *Streptomyces viridochromogenes* and *S. hygroscopicus*, encoded by the *pat* and *bar* genes, respectively (Wohlleben et al. 1988; Strauch et al. 1988). The PAT protein expressed in Liberty Link[®] Cotton differs only in one amino acid from the protein naturally produced by *S. hygroscopicus* and the two proteins are functionally equivalent. *Streptomyces* spp. are saprophytic, soil-borne bacteria and are not considered pathogens of plants, humans or other animals (OECD 1999b). A search of the GenBank database reveals that other genes encoding PAT or similar enzymes are present in a wide variety of bacteria including *Mesorhizobium* sp., *Pseudomonas syringae*, *Streptococcus thermophilus*, *Bacillus* sp., *Anaeromyxobacter dehalogenans* and *Vibrio angustum*. Acetyltransferases, the class of enzymes to which PAT belongs, are common enzymes in all microorganisms, plants and animals. Different versions of PAT protein have also been expressed in other GM crop plants trialled in Australia (eg cotton under DIR licences 015/2002, 016/2002, 036/2003, 038/2003, 040/2003 and 044/2003; and canola under DIR licences 010/2001) or commercially approved (canola under DIR licence 021/2003).

3.5 Presence of same or other GM cotton in the receiving environment

61. A number of field trials of GM cotton plants expressing the same or similar PAT protein have previously been approved. These releases are summarised in Section 4. The current DIR 056/2004 licence authorises the field trial of both LLCotton25 (ie Liberty Link[®] Cotton) and LLCotton25/Bollgard II[®] cotton lines in NSW and QLD during the 2005-06 and 2006-07 summer growing seasons. In each season the GM cotton lines may be grown on up to 12 sites covering a maximum area of 500 hectares.

62. The insect resistant Bollgard II[®] GM cotton and the glyphosate tolerant Roundup Ready[®] GM cotton and conventional crosses between them are widely planted in the agricultural environment as a result of their commercial release in southern Australia (authorised under DIR 012/2002 and 023/2002 licences). In the 2005-06 season, 327,000 ha of cotton was planted (38% in QLD and 62% in NSW) and 90% of this was GM cotton (Cotton Australia 2006). The GM cotton was comprised of 81% Bollgard II[®] and 74% Roundup Ready[®] cotton, with stacked traits contributing to some of these percentages (B. Patterson, Monsanto Australia Limited). This forms part of the baseline data for estimating the risks that may result from the proposed release.

63. It should be noted that the commercial release in southern Australia of glyphosate tolerant Roundup Ready Flex[®] MON 88913 (referred to as Roundup Ready Flex[®]) and glyphosate tolerant/ insect resistant (Roundup Ready Flex[®]/ Bollgard II[®]) cotton were recently approved

in February 2006 (under DIR 059/2005 licence) and are expected to replace Roundup Ready and Roundup Ready[®]/ Bollgard II[®] cotton, respectively.

3.6 Agronomic practices for the GM cotton

64. Agronomic management of the GM cotton would differ from the management of non-GM cotton in that glufosinate ammonium herbicide could be applied over the top of the cotton crop to control weeds. The APVMA generally imposes conditions on the use pattern of herbicides to, for example, limit the development of herbicide resistance and to comply with residue limits. All other crop management practices, including application of water, insecticides and fertilizer, are expected to be similar to those for non-GM cotton. As discussed in Section 3.5, 90% of cotton planted in Australia during the 2005/2006 season was GM cotton, either Bollgard II[®], Roundup Ready[®], or Bollgard II[®]/Roundup Ready[®] cotton. Therefore, Liberty Link[®] Cotton would require more insecticide applications compared to Bollgard II[®] or Bollgard II[®]/Roundup Ready[®] cotton and could not be sprayed with Roundup[®] (a herbicide containing glyphosate) as for Roundup Ready[®] and Bollgard II[®]/Roundup Ready[®] cotton lines.

65. High levels of farm hygiene are commonly maintained on cotton farms (eg all equipment is cleaned on entry and exit to a field/farm to prevent the transfer of disease or the spread of weeds). Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in 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. Transport of ginned cotton seed is conducted in covered vehicles to minimise loss of seed.

66. Bayer intends to implement a Reseller and Agronomist Training and Accreditation package and a Technical and Crop Management Plan to optimise and maintain the use of its technology. These will form part of the company's agreement with retailers and growers to purchase and handle Liberty Link[®] Cotton. The risk assessment does not assume compliance with this agreement in order to consider the underlying risks to human health and safety and the environment posed by the proposed commercial release.

Section 4 Previous Australian and international approvals

4.1 Previous Australian approvals of the same or similar GMOs

4.1.1 Previous releases approved by GMAC or the Regulator

67. Liberty Link[®] Cotton (previously known as Liberty[®] or LLCotton25) has been trialled under limited and controlled conditions in Australia under both the current regulatory system and the former voluntary system. Two trials by CSIRO (PR-124X and PR-124X(2)) were authorised by the Genetic Manipulation Advisory Committee (GMAC) and three trials (DIR 015/2002, DIR 038/2003 and DIR 056/2004) have been approved by the Regulator.

68. The limited and controlled releases under licences DIR 015/2002 and DIR 038/2003, held by CSIRO, took place in the current cotton growing regions of NSW and QLD on trial sites ranging from 0.04 to 135 hectares. Licence DIR 056/2004, held by Bayer, authorises the field trial of both GM herbicide tolerant (LLCotton25) and herbicide tolerant/insect resistant (LLCotton25/Bollgard II[®]) cotton lines in NSW and QLD during the 2005-06 and 2006-07 summer growing seasons. In each season the GM cotton lines may be grown on up to 12 sites covering a maximum area of 500 hectares.

69. GM cotton lines very similar to Liberty Link[®] Cotton (ie containing different transformation events with either the same *bar* gene, or related *pat* gene, and different combinations of regulatory sequences) were also field trialled under GMAC (PR-82, PR82-X

and PR-124). Additional GM cotton lines containing either the *bar* herbicide tolerance gene, or the related *pat* gene, as well as introduced insecticidal and/or antibiotic resistance genes, have been (DIR 016/2002 and DIR 040/2003), or are currently being (DIR 036/2003 and DIR 044/2003), trialled in Australia.

4.1.2 Approvals by other Australian government agencies

70. 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 would also have to be met in respect of release of Liberty Link[®] Cotton, including requirements of the Food Standard Australia New Zealand (FSANZ) and Australian Pesticides and Veterinary Medicines Authority (APVMA). This is discussed further in the Technical Summary and in Chapter 4.

71. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved the use of food (containing oil and linters) derived from Liberty Link[®] Cotton (FSANZ report A533).

72. Bayer currently has a research permit for use of glufosinate ammonium in current cotton trials involving this GMO, and the APVMA is currently assessing an application from Bayer to register Liberty[®] 150 Herbicide for the control of various weeds in Liberty Link[®] Cotton.

4.2 International approvals

73. Liberty Link[®] Cotton has been approved for commercial release in other countries:

- The USA - the Animal and Plant Health Inspection Service (US Department of Agriculture) approved the commercial release, and the US Food and Drug Administration approved use in human food and animal feed in 2003
- Japan – the Japanese Ministries of Agriculture, Forestry and Fisheries approved the commercial release in early 2006, and Health, Labour and Welfare approved use in human food in 2004, and in animal feed in early 2006
- Korea – approved the commercial release of Liberty Link[®] Cotton and its use in human food in 2005
- The Canadian Food Inspection Agency gave approval for the use of Liberty Link[®] Cotton in Canada for human food and animal feed use in 2004. Approval for release of Liberty Link[®] Cotton into the environment was not sought by Bayer as cotton is not grown in Canada.

74. Other countries where Liberty Link[®] Cotton has applied for approval include Mexico (for environment, food and feed), the European Union (for environment, food and feed), China (for environment and food) and Brazil (for food and feed).

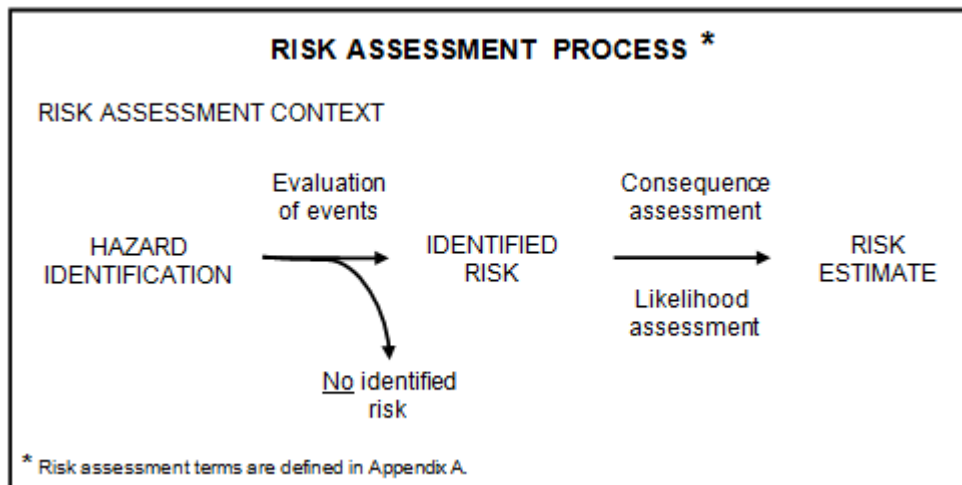
75. Field trials of Liberty Link[®] Cotton are currently in progress in Spain and Brazil.

Chapter 2 Risk assessment

Section 1 Introduction

76. 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 2.1) considers risks from the proposed dealings with the GMO that could result in harm to the health and safety of people or the environment posed by or as a result of gene technology.

Figure 2.1 The risk assessment process.



77. 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 this release of GMOs into the environment.

78. 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 can be an event, a substance or an organism (OGTR 2005).

79. 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 GMO and the receiving environment as a result of the proposed dealings.

80. 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 2005). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same or similar GMOs are also considered.

81. 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

82. The list of events compiled during hazard identification are 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.

83. A risk is identified only when there is some chance that harm will occur. Those events that do not lead to an adverse outcome or could not reasonably occur do not represent an identified risk and will not advance in the risk assessment process. Risks associated with the remaining events are assessed further to determine the seriousness of harm (consequence) and

chance of harm (likelihood). The identified risks must be posed by or result from gene technology.

84. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2005). Harm is assessed in comparison to the parent organism and other GMOs previously approved for commercial release, in the context of the proposed dealing and the receiving environment. The risk assessment process focuses on measurable properties for determining harm.

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

- the proposed dealings, which may include experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMO
- comparisons with the non-GM parent
- routes of exposure to the GMO, the introduced gene and its product
- potential effects of the introduced gene and its product expressed in the GMO
- potential exposure to the introduced gene and its product from other sources in the environment
- the presence of related species in the environment
- properties of the biotic and abiotic environment at the site of release
- agronomic management practices for the GMO
- the size, duration and regions of the release.

86. Limited and controlled releases of the same GMO has occurred in field trials approved under licence DIR 015/2002, DIR 038/2003 and DIR 056/2004. There have been no reports of adverse effects on the health and safety of people or the environment resulting from any of these releases.

87. Twenty eight events that are discussed in detail later in this Section are summarised below in Table 2.1. Events that share a number of common features are grouped together in broader hazard categories as indicated in the table. Three of the events that were characterised are considered to lead to an identified risk that required further assessment.

Table 2.1 Summary of events that may give rise to adverse outcomes

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 to people	1. Ingestion of GM plant materials and food products containing the PAT protein.	Toxicity for people	No	<p>People usually only consume processed products of cotton plants (oil and linters) which do not contain detectable protein or genetic material.</p> <p>Evidence from feeding studies indicates that the PAT protein is of extremely low toxicity for mammals as no acute oral toxicity has been able to be determined.</p> <p>Compositional analysis indicates that cotton seed and raw cotton seed meal from Liberty Link[®] Cotton are compositionally equivalent to that derived from non-GM cotton.</p> <p>FSANZ has approved the use of Liberty Link[®] cotton and other GM crops containing the same or similar PAT protein in food (eg corn, canola and soybean).</p> <p>People are already exposed to PAT protein via bacteria expressing the same or similar protein.</p>
	2. Contact with, or inhalation of, GM plant materials containing the PAT protein via: <ul style="list-style-type: none"> • occupational exposure • general exposure to wider community 	Toxicity for people	No	<p>On the basis that the PAT protein is of extremely low toxicity, it is also expected that it will have very low acute dermal and inhalation toxicity.</p> <p>People are already exposed to bacteria expressing the same or similar protein or via GM canola and other GM cotton lines in field trials without evidence of toxicity.</p>
	3. Consumption of honey produced by bees that pollinated GM plants.	Toxicity for people	No	<p>The level of the PAT protein in pollen is low. The pollen content of honey is less than 0.1%. Therefore, negligible amounts of PAT would be present in honey. The PAT protein exhibits extremely low toxicity.</p>
	4. Consumption of fungi cultivated on GM cotton trash/compost.	Toxicity for people	No	<p>The introduced DNA and expressed protein would be degraded during composting. Mushrooms can only take up break-down products of proteins (eg amino acids), not intact proteins.</p>
SECTION 2.2 Production of a substance allergenic to people	1. Use of GM plant materials in food.	Allergic reactions in people	No	<p>People usually only consume processed products of cotton plants (oil and linters) which do not contain detectable protein or genetic material.</p> <p>Evidence indicates that the PAT protein is not allergenic.</p>
	2. Contact with items containing GM cotton fibre.	Allergic reactions in people	No	<p>Unprocessed lint contains negligible amounts of protein. However, processed lint does not contain detectable amounts of protein.</p>

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	3. Contact with GM plant materials containing the PAT protein via: <ul style="list-style-type: none"> occupational exposure general exposure to wider community 	Allergic reactions in people	No	Evidence indicates that the PAT protein is not allergenic. People have already been exposed to the same GM cotton plant materials containing the same protein as well as similar PAT protein in other GM cotton lines and GM canola during numerous field trials. There have been no reports of allergenicity.
SECTION 2.3 Production of a substance toxic to organisms other than people	1. Ingestion of GM plant materials by vertebrates, including stock.	Toxicity for vertebrates	No	Evidence from feeding studies indicates that the PAT protein has extremely low toxicity for mammals. Compositional analysis indicates that cotton seed and raw cotton seed meal from Liberty Link® Cotton are compositionally equivalent to that derived from non-GM cotton. The same or similar protein is widespread in the environment because of their presence in many microorganisms therefore vertebrates are already exposed to them.
	2. Direct or indirect ingestion of the PAT protein by invertebrates.	Toxicity for invertebrates	No	Evidence suggests that the PAT protein has extremely low toxicity to invertebrates. They are exposed to the same or similar protein through natural sources and through GM cotton lines and GM canola previously and currently being field trialled.
	3. Contact with the PAT protein by microorganisms.	Toxicity for microorganisms	No	Evidence suggests that the PAT protein has extremely low toxicity to microorganisms. They are exposed to the same or similar protein through natural sources and through GM cotton and canola previously and currently being field trialled. The PAT protein is readily degraded by proteases and not expected to accumulate in the soil.
SECTION 2.4 Spread and persistence of the GM cotton in the environment	1. Expression of the <i>bar</i> gene increasing spread and persistence of the GM cotton plants through tolerance to glufosinate ammonium.	Weediness	Yes	See Chapter 3, Event 1.
	2. The presence of the regulatory sequences in the GM cotton plants.	Weediness	No	The presence of regulatory sequences are not expected to have any influence on the spread and persistence of the GM cotton plants.
	3. Exposure of people to the PAT protein as a result of spread and persistence of the GM cotton plants in the environment.	Toxicity/Allergic reactions in people	No	The amount of exposure expected as a result of spread and persistence of the GM cotton would be small in comparison to the exposure from the proposed release of the GM cotton plants. Refer to events 2.1.1 and 2.2.1

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	4. Exposure of organisms other than people to the PAT protein as a result of spread and persistence of the GM cotton plants in the environment.	Toxicity for organisms other than people	No	The amount of exposure expected as a result of spread and persistence of the GM cotton would be small in comparison to the exposure from the proposed release of the GM cotton plants. Refer to event 2.3.
SECTION 2.5 Gene flow by vertical gene transfer	1. Gene transfer to native <i>Gossypium</i> species.	Weediness	No	Well established genetic incompatibility prevents vertical gene transfer to native <i>Gossypium</i> species.
	2. Expression of the <i>bar</i> gene in other <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants (not including commercially released GM cotton lines) providing glufosinate ammonium tolerance.	Weediness	Yes	See Chapter 3, Event 2.
	3. Expression of the <i>bar</i> gene in combination with <i>cp4 epsps</i> gene and/or <i>cryIAc</i> and <i>cry2Ab</i> genes providing dual herbicide tolerance and reducing lepidopteran herbivory.	Weediness	Yes	See Chapter 3, Event 3.
	4. Exposure of organisms (including people) to the PAT protein or in combination with other genes as a result of gene transfer to <i>G. hirsutum</i> or <i>G. barbadense</i> plants.	Toxicity for organisms	No	The amount of exposure to the PAT protein expected as a result of vertical gene transfer would be small in comparison to the exposure from the proposed extensive cultivation of GM cotton plants. None of the individual proteins are toxic/allergenic and stacking is not expected to alter this. Refer to events 2.1.1, 2.2.1 and 2.3
	5. Presence of the introduced regulatory sequences in <i>G. hirsutum</i> or <i>G. barbadense</i> plants as a result of gene transfer.	Unpredictable effects	No	The introduced regulatory sequences do not behave any differently than endogenous regulatory sequences in plants.
	6. Reduced choice of herbicides for the control of cotton volunteers as a result of stacking of herbicide tolerance traits	Weediness	No	Glufosinate ammonium has limited effectiveness in controlling cotton volunteers even at the seedling stage and other herbicides are available. Cultivation/mechanical removal is the best option to remove established cotton plants.
	7. Tolerance to other herbicides as a result of stacking	Weediness	No	Unlikely that resistance to a new herbicide will develop.
SECTION 2.6 Gene flow by horizontal gene transfer	1. Presence of the <i>bar</i> gene, or the introduced regulatory sequences, in other organisms.	Toxicity, weediness or increased pathogenicity	No	Horizontal gene transfer of the introduced sequences to other organisms is not expected to result in any adverse outcomes during this release.
SECTION 2.7 Unintended changes in toxicity	1. Altered levels of innate toxic or anti-nutritional compounds as a result of random insertion of the gene construct into the cotton genome as a result of genetic modification.	Toxicity for people and other organisms	No	Compositional analysis indicates that there are no significant changes in any of the toxic or anti-nutritional compounds in cotton seed or raw cotton seed meal from Liberty Link [®] Cotton compared to non-GM cotton.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	2. Altered metabolism of glufosinate ammonium in the GM cotton plants expressing the PAT protein resulting in the production of toxic compounds.	Toxicity for people and other organisms	No	The toxicity of metabolites from the metabolism of glufosinate ammonium in the GM plants are comparable to or less than that of the parent compound, which is of low acute oral toxicity.
SECTION 2.8 Unintended changes in biochemistry or physiology	1. Altered biochemistry or physiology of the GM cotton plants resulting from insertion or expression of the introduced gene.	Toxicity for people and/or other organisms or weediness	No	Phenotypic and compositional analyses demonstrate that Liberty Link® Cotton is equivalent to non-GM cotton indicating that biochemical pathways and plant physiology are not altered in the GM plants.
SECTION 2.9 Unintended effects on existing pests or weeds	1. Expression of the introduced <i>bar</i> gene resulting in increased disease burden	Increased disease burden	No	Previous releases of the same GM cotton in Australia did not show increased disease burden. No differences were observed in the pest or disease status between Liberty Link® Cotton and non-GM cotton during agronomic performance testing in the USA.
SECTION 2.10 Secondary impacts	1. Consumption of animals that were fed GM plant materials.	Toxicity for people	No	Protein and DNA are rapidly broken down into smaller components in the digestive tract of animals that are fed cotton seed irrespective of whether it is GM or not. As a result, products from these animals would be no different to those from animals that were fed seed from non-GM cotton.
	2. Use of glufosinate ammonium on the GM cotton resulting in changes in the weed spectrum or development of herbicide resistant weeds (in the agricultural environment)	Development of herbicide resistant weeds	No	Development of herbicide resistant weeds is under consideration by the APVMA. The APVMA may impose conditions on the use of the herbicide if it considered this necessary. Bayer has submitted a proposed crop management plan to the APVMA which specifies an integrated weed management strategy. There are integrated weed management guidelines for the Australian cotton industry.

2.1 Production of a substance toxic to people

88. Toxicity is the cascade of reactions resulting from exposure to a dose of a chemical that is sufficient to cause direct cellular or tissue injury, or otherwise inhibit normal physiological processes (Felsot 2000). Toxic proteins are known to act via acute mechanisms rather than through chronic exposure (Sjoblad et al. 1992). Toxicity may occur through ingestion, contact or inhalation. The level of toxicity is often expressed as the LD₅₀. This is the amount of a substance given in a single dose that causes death in 50% of a test population of an organism.

89. Toxicity assays generally use the purified toxin of interest rather than the product that expresses the protein (eg GM plant materials). This is necessary because the aim of the assays is to determine the concentration of toxin at which an adverse effect is seen. PAT is present in cotton tissues at less than 1% of total extractable protein so it would not be possible to feed the test animal large enough quantities of the plant material for a toxicity assay. The level of

expression in the product is used to determine the level of exposure to the toxin and comparison to the results of the toxicity assay indicate whether or not this is a safe level of exposure (OECD 1998; Konig et al. 2004). The use of purified toxin also increases the reproducibility of the assays.

2.1.1 Ingestion of GM plant materials and food products containing the PAT protein

90. Exposure to the PAT protein could occur as a result of ingestion of material from the GM cotton plants. However, people do not normally eat cotton plants or any unprocessed material from cotton plants. The applicant intends to use cotton seed oil and linters from the proposed release in human food (which has received approval by FSANZ). However, protein or genetic material is not detectable in processed cotton seed oil and linters (Sims et al. 1996; USDA 2004). Any ingestion of material from the GM cotton plants containing the PAT protein is expected to be very limited and result in extremely low exposure to the PAT protein.

91. PAT represents less than 1% of the total extractable protein in Liberty Link[®] Cotton roots, leaves, stems and pollen (see Chapter 1, Section 2.4.3 for details). PAT is responsible for detoxifying phosphinothricin (glufosinate ammonium). The potential toxicity of this protein has also been assessed in detail in the RARMP for DIR 021/2002 (available on the [OGTR website](#)).

92. PAT proteins are widespread in the environment, through the presence of naturally occurring bacteria (See Section 3.4, Chapter 1 for details), and therefore people are already widely exposed to PAT proteins.

93. The amino acid sequences of the PAT protein encoded by the *bar* gene was compared for homology with amino acid sequences of known toxic proteins using seven different databases (SwissProt, trEMBL, GeneSeq-Prot, PIR, PDB, DAD and GenPept). No significant homology to any known toxic proteins was detected (Herouet 2002b). This conclusion is supported by others (Van den Bulcke 1997; ANZFA 2001b; Bayer CropScience 2003).

Digestability and degradation studies

94. Wehrmann (Wehrmann et al. 1996) reported that when the PAT protein was subjected to simulated gastric conditions, the protein was degraded within seconds. In more recent *in vitro* digestibility studies conducted by Bayer (Esdaile 2002b; Esdaile 2002c; Herouet et al. 2005), the PAT protein was digested within 30 seconds of incubation in simulated gastric fluid (pH 2), and within seconds of incubation in simulated intestinal fluid (pH 7.5). Another study conducted by Bayer shows that PAT is rapidly degraded both in pig stomach fluids and in bovine abomasum (4th stomach) and rumen (1st stomach) (Bayer CropScience 2003). Other studies have shown that the PAT protein was inactivated within one minute when subjected to typical mammalian stomach conditions (European Commission 1996). The Canadian Food Inspection Agency's report stated that experimental data indicated that the PAT protein is rapidly degraded in the gastric environment and is also readily denatured by heat or low pH (Canadian Food Inspection Agency 1995; Canadian Food Inspection Agency 1998a). Enzyme activity of the PAT protein is destroyed by heating it to 50°C for 10 minutes (Wehrmann et al. 1996), although the band of PAT protein was unchanged on a protein gel after heat treatment up to 90 °C for 60 min indicating that the protein was not degraded, only inactivated (Esdaile 2002a; Herouet et al. 2005).

Toxicity Studies

95. Extensive animal testing has shown that the PAT protein is not likely to be toxic to humans.

96. In a 14-day acute toxicity study, mice fed with high levels of the recombinant PAT protein (2500 mg/kg bodyweight) showed no significant, treatment-related toxic effects (Merriman 1996). In this study, ten mice (five male and five female) were administered a single dose of His-tag PAT/kg body weight. A His-tag is a stretch of amino acid (Histidine) residues attached to the protein molecule that aid in its purification by columns that bind Histidine. Body weights of the test animals were determined prior to dosing (day 0), and on days 7 and 14 after dosing and the animals were observed daily for any clinical abnormalities or mortality. No mortality occurred during the study. Following scheduled euthanasia of test animals on day 14, no gross internal abnormalities were observed. Based on this test, the acute oral LD50 was estimated to be greater than 2500 mg of His-tag PAT/kg body weight.

97. In addition, a study by Pfister *et al.* (1996, cited in Bremmer & Leist 1996) also investigated the toxicity of purified PAT protein in a repeated dose oral toxicity study in rats. Groups of 5 male and 5 female rats were fed PAT protein for 14 days at levels of 0, 0.5 or 5% of their diet (equivalent to 0, 707 and 7792 mg/kg body weight/day). No adverse effects or mortality were observed during the study, even at the highest dose of the PAT protein. At day 14 the rats were euthanised and the following parameters were investigated at necropsy:

- total and differential white blood count
- spleen and thymus weight
- histological examination of spleen, thymus, mesenteric lymph node, Peyer's patches
- bone marrow.

98. No significant differences were observed for any of these parameters upon necropsy, even at the highest dose of PAT protein. Based on this study, the LD50 of PAT was estimated to be greater than 7792 mg/kg body weight.

99. To ensure maximum systemic exposure to the PAT protein, a study was conducted to assess the intravenous toxicity of the PAT protein, encoded by the *bar* gene. This route of exposure excludes the confounding effects of an unknown amount of proteolytic degradation, digestion and absorption. It also enables a much higher dose to be administered compared to the maximum potential dose that could be absorbed after oral exposure. No toxic effects were observed in mice after acute intravenous administration of 10 mg/kg body weight (Kennel 2002; Herouet *et al.* 2005).

Compositional analyses

100. In Australia, oil and linters derived from cotton are used in food. Compositional analyses of refined deodorised cotton seed oil and linters derived from Liberty Link[®] Cotton grown in the USA are presented (Table 2.2 and 2.4). The antinutrients present in refined deodorised cotton seed oil were also analysed (Tables 2.4). Refined deodorised cotton seed oil was analysed for fatty acids, tocopherols, gossypols, malvalic acid, sterculic acid and dihydrosterculic acids (Table 2.2 and 2.3). Linters were analysed for proximates and crude fibre (Table 2.4).

Table 2.2: Compositional analysis of refined deodorised cotton seed oil from non-GM and Liberty Link[®] Cotton plants

	Non-GM (Coker 312)	Liberty Link[®] unsprayed	Liberty Link[®] sprayed
Fatty acids (% relative)			
Total saturated	24.02	23.55	23.58
Total monounsaturated	14.52	14.72	14.88
Total polyunsaturated	59.88	60.05	59.77
Tocopherols (ppm)			
Alpha	528 ± 100	521± 108	512 ± 87
Gamma	427 ± 63	425 ± 15	410± 10
Delta	<1	<1	<1
Total tocopherols	955 ± 163	944 ± 122	922 ± 97

Table 2.3: Anti-nutrients in refined deodorised cotton seed oil from non-GM and Liberty Link[®] Cotton plants

Anti-nutrient	Non-GM (Coker 312)	Liberty Link[®] unsprayed	Liberty Link[®] sprayed
Total gossypol (%dm)	<0.002	<0.002	<0.002
Malvalic acid (% relative)	0.4 ± 0.06	0.41±0.02	0.4±0
Sterculic acid (% relative)	0.24±0.03	0.23±0.02	0.23±0.02
Dihydrosterculic acid (% relative)	0.21±0.12	0.17±0.05	0.17±0.07

101. No statistically significant differences are present between the refined deodorised oil derived from the Liberty Link[®] and control cotton plants for all the constituents or anti-nutritionals measured (Tables 2.2 and 2.3). Cotton seed oil derived from Liberty Link[®] Cotton was therefore considered to be compositionally equivalent to that derived from non-GM cotton.

102. The only noticeable difference observed between linters derived from non-GM cotton and Liberty Link[®] Cotton is the mean protein value of the Liberty Link[®] Cotton plants sprayed with Liberty[®], which is higher than the non-GM cotton plants (Table 2.4). However, the value is still within the standard deviations and within the reported literature range for current commercial cotton varieties. Linters derived from Liberty Link[®] Cotton were therefore considered to be compositionally equivalent to those derived from non-GM cotton

Table 2.4: Compositional analysis of cotton linters from non-GM and Liberty Link[®] Cotton plants

	Non-GM (Coker 312)	Liberty Link[®] unsprayed	Liberty Link[®] sprayed
Moisture (%dm)	7.98±1.47	8.42±0.76	7.90±0.59
Fat (%dm)	1.41±0.68	1.53±0.37	1.53±0.55
Protein (%dm)	3.66±1.23	4.75±1.34	3.27±0.68
Ash (%dm)	2.49±0.92	2.55±0.61	2.68±0.54
Total carbohydrates (%dm)	92.44±2.83	91.17±2.32	92.51±1.77
Crude fibre (%dm)	82.82±5.82	79.59±3.35	82.79±0.16

103. In some countries, including the USA (but not Australia), cotton seed meal is approved for use in human diets (OGTR 2002). The composition and antinutrients present in cotton seed meal are discussed in Section 2.3.1 of this Chapter. Levels of PAT protein in seed from Liberty Link[®] Cotton that had been sprayed with Liberty Herbicide have been measured at 127 µg/g dry weight. A 70 kg person would need to consume approximately 4291 kg of dried cotton seed at one sitting in order to be acutely exposed to 7792 mg/kg body weight. As indicated above, no toxic effects were observed in mice at this level.

Assessments by other agencies

104. The toxicity of the PAT protein expressed in genetically modified plants has been assessed by a number of regulatory bodies in Australia, USA, Canada, and Europe (FDA 1995; FDA 1997; European Scientific Committee on Plants 1998a; European Scientific Committee on Plants 1998b; ANZFA 2001b).

105. FSANZ has recently approved the use of oil and linters derived from Liberty Link[®] Cotton in human food. They concluded in the report A533 (FSANZ 2005a) that food derived from the cotton line LL25 (ie Liberty Link[®] Cotton) is as safe and wholesome as food derived from other cotton varieties. FSANZ has approved the use of food derived from other GM plants containing either the *bar* or *pat* gene, including GM corn (applications A375, A380 and A446), canola (A372) and soybean (A481) containing PAT, concluding that the PAT protein is not toxic (ANZFA 2001a; ANZFA 2001b; ANZFA 2001c; FSANZ 2003; FSANZ 2004a). The studies submitted in support of the food uses for this protein indicate that it has none of the properties associated with protein toxins.

106. The United States Environmental Protection Agency has determined that PAT, and the genetic material necessary for its production is exempt from the requirement to establish a maximum permissible level for residues in plants due to its very low toxicity and allergenicity (EPA 1997). The OECD states that there is no evidence available indicating that the PAT protein is toxic to either humans or animals (OECD 1999b). As detailed in Chapter 1, Section 4.2, Liberty Link[®] Cotton was approved for human food and animal feed in the US, Japan and Canada, and human food in Korea. In total, 22 food and feed approvals have been obtained for the *bar/pat* genes in 8 different crop species including sugar beet, *Brassica napus*, *Brassica rapa*, chicory, cotton, maize, rice and soybean (AGBIOS database <http://www.agbios.com/dbase.php>)

Conclusion

107. In conclusion, any ingestion of material from the GM cotton plants containing the PAT protein is expected to be very limited and evidence indicates that the PAT protein has extremely low toxicity for mammals. Therefore, **no risk is identified** and the potential for toxicity for people as a result of ingestion of the PAT protein will not be assessed further.

2.1.2 Contact with, or inhalation of, GM plant materials containing the PAT protein

108. Exposure to the PAT protein could occur as a result of contact with, or inhalation of, material from the GM cotton plants. This may occur via occupational exposure or general exposure to the wider community from living near the proposed release.

109. Processed cotton lint and linters contain no detectable DNA or protein (Leffler & Tubertini 1976; Sims & Martin 1996). In US field trials carried out over two seasons and using different herbicide regimes and site locations, most measurements of fibre characteristics (length, strength, fineness) of Liberty Link[®] Cotton showed no significant differences to the non-GM parent variety, and all characteristics measured for the GM cotton

were within the expected range of existing commercial cotton cultivars (Freyssinet 2002a; Freyssinet 2002b). In Australia, research carried out under DIRs 015/2002 and 038/2003 showed that the presence of the *bar* gene in Liberty Link[®] Cotton had no deleterious effects on fibre quality or yield (CSIRO annual reports for 2002/2003 and 2003/2004 seasons submitted to the OGTR). Therefore, the safety of wearing cotton clothing or using other products made from Liberty Link[®] Cotton is not likely to be different from that of current commercially approved GM cotton lines or non-GM cotton.

110. Humans working with cotton plants will be exposed primarily to the outer waxy cuticle layer at the plant surface, to the seed coat or to the cotton fibres, all of which are essentially free of protein. Exposure to proteins (including the PAT protein expressed in the GM cotton) or to other cellular components of the cotton plants will only occur if plant cells are ruptured. If the plant cells did rupture, exposure to the PAT protein expressed in Liberty Link[®] Cotton will be very low, as the protein is only present at low levels in the GM cotton tissues (see Chapter 1 Section 2.4.3 for details). For example, the amount of PAT protein as percent of the total crude protein in Liberty Link[®] Cotton roots, stems, and leaves is 0.08, 0.23 and 0.19%, respectively. However, the PAT protein is not toxic to humans (See Section 2.1.1 of this Chapter).

111. The primary processing of cotton at cotton gins, and the bulk handling of cotton seed and cotton fibre, can create and stir up fine dust and lint particles. Inhalation of this dust by millworkers can cause byssinosis, an asthma-like condition, in sensitive individuals. Preventative measures such as the use of facemasks have been successful in lowering the incidence of this condition. Since cotton lint contains negligible amounts of protein and the fibre characteristics of Liberty Link[®] Cotton are equivalent to non-GM cotton varieties, the cotton lint derived from Liberty Link[®] Cotton is no more likely to induce adverse responses in workers than is conventional cotton or other commercially released GM cotton lines.

112. Dermal and inhalation toxicity studies have not been conducted with the PAT protein. However, on the basis that it has extremely low acute oral toxicity, it is also expected to be of extremely low acute dermal and inhalation toxicity. In Australia, during field trials with GM cotton and canola containing the same or similar PAT proteins, there have been no reports of any adverse impacts on people working with them. Liberty Link[®] Cotton has been approved for commercial release in the US, Japan and Korea with no reports of adverse effects.

113. Furthermore, PAT proteins are widespread in the environment, through the presence of naturally occurring bacteria (See Section 3.4, Chapter 1 for details), and therefore people are already widely exposed to PAT proteins. Therefore, **no risk is identified** and the potential for toxicity for people as a result of contact with, or inhalation of, GM plant materials containing the PAT protein will not be further assessed.

2.1.3 Consumption of honey produced by bees that pollinated GM plants

114. Honey usually contains some pollen grains. The average pollen content of sieved honey is normally less than 0.1% (Agrifood Awareness Australia 2001). Pollen grains contain protein and, therefore, may also contain the PAT protein. However, the level of the PAT protein in pollen from the GM cotton is expected to be very low. Levels of PAT protein in pollen from Liberty Link[®] Cotton have been measured at 19.3 µg/g fresh weight. So the average PAT protein content of honey will be less than 0.019µg/g or 0.00000019%.

115. Furthermore, the PAT protein has extremely low toxicity and GM crops (including Liberty Link[®] Cotton) containing PAT proteins are approved for use in food (see Section 2.1.1 of this Chapter). Therefore, **no risk is identified** and the potential for toxicity for people

as a result of consumption of honey produced by bees that pollinated GM plants will not be assessed further.

2.1.4 Consumption of fungi cultivated on cotton trash/compost

116. Cotton trash can be used as a bulking agent to improve the efficacy of animal manure composting. The compost may be used for cultivation of fungi. However, the introduced DNA and expressed protein would be degraded during composting. DNA degradation has also been shown to occur during silaging (Phipps et al. 2005).

117. Fungi are heterotrophic (ie require carbon in organic form) and release enzymes into their environment that help degrade organic matter. Only break-down products of proteins (eg amino acids), not intact protein, can be taken up by the growing fungi.

118. Furthermore, the PAT protein has extremely low toxicity and GM crops (including Liberty Link[®] Cotton) containing PAT proteins are approved for use in food (see Section 2.1.1 of this Chapter).

119. Therefore, **no risk is identified** and the potential for toxicity for people as a result of consumption of fungi cultivated on cotton trash/compost will not be assessed further.

2.2 Production of a substance allergenic to people

120. The possibility that exposure of people to the PAT protein encoded by the introduced *bar* gene in the GM cotton plants may result in an allergic reaction is considered. Routes of exposure to the PAT protein could include consumption of food containing cotton products, accidental ingestion of material from the cotton plants, contact with clothing or household items containing cotton, or contact with material from cotton plants, either as a result of occupational exposure or exposure to the wider community from living near the proposed release.

2.2.1 Use of GM plant materials in food

121. The applicant intends to use cotton seed oil and linters from the proposed release in human food and has received approval by FSANZ for this use. Protein or genetic material is not detectable in processed cotton seed oil and linters (Sims et al. 1996; USDA 2004). GM canola, corn and soybean containing a similar PAT protein have previously been approved by FSANZ for use in human food.

122. The molecular weight of the PAT protein is 22-23 kD which is in the typical range for allergenic proteins. However, it does not possess potential glycosylation sites, nor is it stable in the mammalian digestive system, decreasing the probability that it is allergenic (Bayer CropScience 2003; Herouet et al. 2005). Furthermore, it is only present as a minor component of the GM cotton (see Chapter 1, Section 2.4.3).

123. The amino acid sequences of the PAT protein encoded by the *bar* gene was compared for homology with amino acid sequences of known allergens (aeroallergens and food allergens) of both plant and animal origin using seven large separate databases (SwissProt, trEMBL, GeneSeq-Prot, PIR, PDB, DAD and GenPept). No significant homology to any known allergen was detected (Herouet 2002a; Herouet 2002b; Herouet et al. 2005).

124. Identified epitopes of allergenic proteins tend to have an optimal length of between 8 and 12 amino acids for binding to T-cells and it has been proposed that an immunological significant sequence identity requires a match of at least eight contiguous amino acids (Metcalf et al. 1996). A search for homology with known allergens of the PAT protein was

conducted based on detecting identities of eight contiguous amino acids and no sequence homologies were detected (Van den Bulcke 1997; Herouet et al. 2005).

125. A more refined method for detecting possible allergenic epitopes has recently been published (Kleter & Peijnenburg 2002; McCoy & Bannon 2003; Kleter & Peijnenburg 2003). The method is based on detecting identities of six amino acids with known IgE epitopes. The method was applied to the amino acid sequences of the PAT protein introduced into GM canola plants. No identities with known IgE epitopes were found confirming the previous results.

126. Many allergens are glycosylated, with the most common type being N-glycosylation. *In silico* and *in vitro* analysis for N-glycosylation revealed no potential or actual glycosylation (Herouet et al. 2005).

127. As detailed in Section 2.1.1, PAT proteins encoded by both the *bar* and *pat* genes are rapidly digested in simulated gastric and intestinal fluid (Wehrmann et al. 1996; Bayer CropScience 2003; Herouet et al. 2005).

128. The PAT protein expressed in Liberty Link Cotton is not derived from a known allergenic source. A number of GM plants containing the *pat* or *bar* gene have been field trialled in Australia (See Chapter 1 for details) as well as overseas and no adverse effects on humans, animals or the environment have been reported. The UK Royal Society have concluded that there is, at present, no evidence that available GM foods cause allergic reactions, and that the risks posed by GM plants are in principle no greater than those posed by conventional breeding or by plants introduced from other areas of the world (The Royal Society 2002).

129. Therefore, **no risk is identified** and the potential for allergic reactions in people resulting from exposure through food will not be assessed further.

2.2.2 Contact with items containing GM cotton fibre

130. Fibres are removed from the surface of cotton seed in two separate processes (Gregory et al. 1999). During ginning, the long fibres are removed from the seed. These fibres are called cotton lint. Cotton fabrics, used in clothing, upholstery, towels and other household products, are made from cotton lint. Following the ginning process, the seeds can be delinted, which involves the removal of the shorter fibres (linters) still attached to the seed. These cotton linters are used in a variety of products including medical dressings, felt, fine quality paper (including banknotes in many countries), twine and mops. Cellulose derivatives produced from the linters may be used in pharmaceuticals, cosmetics, toothpaste, lacquers, paints, ice cream, salad dressing and a variety of plastics (Gregory et al. 1999; FSANZ 2004b). Cotton fibre is widely used in pharmaceutical and medical applications because of its very low allergenicity.

131. A general study of the accumulation of mineral nutrients in cotton fruit found no detectable protein in fibre fractions (Leffler & Tubertini 1976). However, using more sensitive methods, specific proteins were detected at very low levels in raw, but not processed, linters and lint (Sims et al. 1996). Therefore, the safety of wearing cotton clothing or using other products made from cotton is not expected to be affected by the genetic make-up of the cotton plants from which these components have been derived, that is, whether or not it is derived from GM or non-GM cotton plants. Therefore, **no risk is identified** and the potential for allergic reactions in people resulting from exposure through cotton fibre will not be assessed further.

2.2.3 Contact with GM plant materials containing the PAT protein

132. People working with cotton plants would be exposed primarily to the outer waxy cuticle layer at the plant surface, to the seed coat or to the cotton fibres, all of which are essentially free of protein. However, dermal exposure to proteins (including the PAT protein) or to other cellular components of the cotton plants may occur if damage to the plants during handling results in rupture of plant cells.

133. Inhalation of pollen by workers or people living near cotton crops could potentially result in allergic reactions if the PAT protein was allergenic and was expressed in the pollen. The PAT protein has been shown to be present in most parts of the GM cotton plants including pollen, leaves and seed (See Table 2.1, Chapter 1). However, evidence indicates that the PAT protein is not allergenic (see Section 2.2.1 of this Chapter for details).

134. The primary processing of cotton at cotton gins, and the bulk handling of cotton seed and cotton fibre, can create and stir up fine dust and lint particles. Use of personal protective equipment by exposed workers is commonplace in such facilities to prevent respiratory irritations such as byssinosis.

135. Furthermore, PAT proteins are widespread in the environment, through the presence of naturally occurring bacteria (See Section 3.4, Chapter 1 for details), and therefore people are already widely exposed to PAT proteins.

136. Therefore, **no risk is identified** and the potential for allergic reactions in people resulting from contact with GM plant materials containing the PAT protein will not be assessed further.

2.3 Production of a substance toxic to organisms other than people

137. A range of organisms may be exposed directly, through feeding on the GM cotton plants, or indirectly through eating organisms that feed on GM cotton plants. These organisms include vertebrates, invertebrates and microorganisms.

138. Cotton tissue (from either GM or non-GM plants), particularly the seeds, can be toxic to mammals if ingested in large quantities because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropanoid fatty acids (eg dihydrosterculic, sterculic and malvalic acids).

139. Mammals generally avoid feeding on cotton plants. The presence of gossypol and cyclopropanoid 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. Inactivation or removal of these components during processing enables the use of some cotton seed meal for farmed fish, poultry and swine. The meal and hulls of cotton seed can also be used for cattle feed. Its use as stockfeed is limited, nonetheless, to a relatively small proportion of the diet and it must be introduced gradually, to avoid potential toxic effects.

140. Neither cotton trash nor stubble are used as animal feed, due to the possible presence of pesticide residues.

2.3.1 Ingestion of GM plant materials by vertebrates, including stock

141. Mammals generally avoid feeding on cotton plants due to both the presence of toxic, anti-nutritional substances and the morphology of the plant (OGTR 2002). In the field, seed cotton is present as large lint-covered bolls that are unattractive to avian species (OGTR 2002), so birds are not likely to be exposed to the PAT protein in the seeds of the GM cotton.

142. Cotton seed and pollen from the release are not expected to enter aquatic habitats in any significant quantities (OGTR 2002); therefore the level of exposure of aquatic vertebrates to the GM cotton will be low. Irrigation practices (Good Management Practice of cotton

industry) used by cotton growers in 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.

143. As discussed in detail in Section 2.1.1 of this Chapter as well as in the RARMP for DIR 021/2002, available on the OGTR website, studies aimed at determining the level of acute oral toxicity of the PAT protein for mammals and other vertebrates, have been performed and did not find any evidence of acute toxicity. Therefore, it is concluded that the PAT protein has extremely low toxicity to vertebrates.

144. PAT enzymes are produced naturally by the common soil bacteria *Streptomyces viridochromogenes* and *S. hygroscopicus*, encoded by the *pat* and *bar* genes, respectively (Wohlleben et al. 1988; Strauch et al. 1988). The PAT protein expressed in Liberty Link[®] Cotton differs only in one amino acid from the protein naturally produced by *S. hygroscopicus*. Since the same or similar proteins are widespread in the environment, the introduced PAT protein is not expected to be a novel source of harm for vertebrates.

145. Vertebrates will also have been exposed to the same or similar PAT proteins through expression in other GM cotton lines and GM canola currently and previously being field trialled (see Chapter 1, Section 4.1.1). No adverse effects have been reported from these releases.

USDA-APHIS has approved LLCotton25 (ie Liberty Link[®] Cotton) for commercial release in the USA and reported that no toxicity or altered population levels have been observed for birds or other species that frequent cotton fields (USDA-APHIS 2003a).

146. Extensive compositional analyses performed on whole and linted cotton seeds, lint, as well as different cotton seed products (e.g. linters, hulls, delinted seeds, meal, toasted meal, crude oil and refined deodorised oil), have demonstrated that the levels of important nutritional and anti-nutritional components in Liberty Link[®] Cotton are comparable to the parental non-GM variety (Coker312) and are within established ranges for current commercial cotton varieties (information supplied by the applicant, refer also to Section 2.1.1).

147. The most likely component to be fed to stock is cotton seed meal. Some of the compositional and antinutrients analyses of raw cotton seed meal derived from Liberty Link[®] Cotton grown in the USA are presented in Table 2.5 and 2.6.

Table 2.5: Compositional analysis of cotton seed meal from non-GM and Liberty Link[®] Cotton plants

	Non-GM (Coker 312)	Liberty Link[®] unsprayed	Liberty Link[®] sprayed
Moisture (%dm)	2.91±1.05	3.83±1.87	8.30±8.38
Fat (%dm)	4.11±0.66	4.15±0.68	3.25±1.50
Protein (%dm)	45.47±1.80	49.70±1.31	49.72±0.06
Ash (%dm)	6.86±0.52	7.05±0.19	7.15±0.24
Total carbohydrates (%dm)	43.56±0.62	39.11±0.83	39.87±1.82
Crude fibre (%dm)	13.04±1.23	10.69±1.56	11.45±0.03

Table 2.6: Anti-nutrients in cotton seed meal from non-GM and Liberty Link[®] Cotton plants

Anti-nutrient	Non GM (Coker 312)	Liberty Link [®] unsprayed	Liberty Link [®] sprayed
Free gossypol (%dm)	0.08±0.02	0.10±0.01	0.08±0.05
Total gossypol (%dm)	1.38±0.14	1.34±0.06	1.43±0.42
Phytic acid (% relative)	2.86±0.87	3.01±1.40	3.16±1.96
Malvalic acid (% relative)	0.38±0.02	0.34±0.03	0.32±0.04
Sterculic acid (% relative)	0.22±0.03	0.20±0.01	0.20±0.03
Dihydrosterculic acid (% relative)	0.16±0.01	0.14±0	0.14±0.01

148. Only minor differences were observed between Liberty Link[®] Cotton, either sprayed or unsprayed, and the non-GM cotton for cotton seed meal (Table 2.5 and 2.6). The high moisture content in the cotton seed meal from sprayed Liberty Link[®] Cotton was attributed to an artefact of processing of one particular sample, with all other samples assessed not significantly different to non-GM cotton. Free gossypol was also higher in unsprayed Liberty Link[®] Cotton but the value is still within the reported literature range for current commercial cotton varieties. Therefore, cotton seed meal from Liberty Link[®] Cotton was therefore considered to be compositionally equivalent to that derived from non-GM cotton.

149. No animal feeding studies have been reported with Liberty Link[®] Cotton. However, there have been a number of studies using other crops containing the PAT protein.

150. A recent study investigated the effects of feeding glufosinate ammonium tolerant corn silage to dairy cows (Phipps et al. 2005). Measurements were made of dry matter intake, milk yield, milk composition and body weight for each cow during the 12 week experiment. No significant differences were observed between the treatments.

151. A study on pigs compared glufosinate ammonium tolerant rice with a near isogenic conventional rice (Cromwell et al. 2005). Pigs were fed >72% of rice in their diet supplemented with soybean meal. The trial compared the glufosinate ammonium tolerant variety rice grown with or without glufosinate herbicide sprays, the near isogenic cultivar and a commercially milled variety. The compositions of the grain from the four types of rice were similar. Weight gain, feed intake and carcass weight did not differ significantly between treatments. The results therefore indicated that the glufosinate ammonium herbicide tolerant rice was similar in composition and nutritional value to conventional rice used to feed pigs.

152. The Cromwell study also cites a study by Schat et al 1999 in which broiler chickens were fed 30% rice diets containing glufosinate ammonium tolerant rice or non-GM rice for 42 days (Cromwell et al. 2005). These chickens had essentially equivalent growth rates, feed intake, weight gain efficiency and % fat, independent of which diet they received.

153. Experiments at the German Institute of Animal Nutrition were carried out in pigs with glufosinate ammonium tolerant sugar beet and maize and in sheep with glufosinate ammonium tolerant sugar beet and sugar beet leaf silage (Bohme et al. 2001; Aulrich et al. 2006). No significant differences in digestibilities of organic matter, crude nutrients as well as energy content of isogenic and transgenic fodder.

154. A review of 66 papers on feeding studies using GM crops, including glufosinate ammonium tolerant corn and beets (Aumaitre 2004) concluded that the health of animals, their physiological characteristics and their survival rate was not affected.

155. Therefore, **no risk is identified** and the potential for toxicity for vertebrates resulting from the ingestion of GM plant materials will not be assessed further.

2.3.2 Direct or indirect ingestion of the PAT protein by invertebrates

156. Invertebrates may be directly exposed to the GM cotton and the PAT protein through feeding on the Liberty Link[®] Cotton plants. Exposure may also occur indirectly through eating other organisms which have previously fed on the GM cotton plants. Exposure in the soil may occur either when cotton tissues decompose or as a result of root exudation. Relative exposure will be greatest for herbivorous species feeding on the cotton plants. Pollinator species and various adult insects that feed on pollen will also be exposed to the protein. Sap feeders, such as aphids, will have minimal exposure, as the sap is composed primarily of sugars and mineral salts dissolved in water.

157. Pollinator species and various insects that feed on pollen will have lower exposure to the PAT protein, because of the much lower expression levels in pollen, relative to that in other plant tissues (see Chapter 1, Section 2.4.3).

158. Major pests of cotton, which include a subset of lepidopteran insects, moths and butterflies such as *Helicoverpa armigera* and *H. punctigera*, and spidermites such *Tetranychus uticae* (OGTR 2002), as well as minor pests of cotton will be exposed to the GM cotton expressing the PAT protein. For many other species of insects, cotton is not the preferred food source, and their populations would be maintained on other types of plants found around cotton fields.

159. In USA field trials conducted between 1999 and 2001, no differences in either beneficial insect diversity, which included lady beetles (*Hippodamia convergens*), lacewings (*Chrysopa spp.*) and honey bees (*Apis mellifera*), or pest species diversity, which included cotton boll worms (*Helicoverpa zea*), whiteflies (*Aleyrodidea* family), cotton aphids (*Aphis spp.*) and cutworms (*Agrotis ipsilon*), were observed between Liberty Link[®] Cotton sprayed with Liberty[®] herbicide and non-GM cotton plots (information supplied by the applicant). Although no detailed studies have been conducted in Australia, similar observations were made during routine inspections of field trials, with insect species appearing to have little preference for either Liberty Link[®] Cotton plants or commercial non-GM cotton cultivars (information supplied by the applicant).

160. More information on the PAT protein in relation to toxic effects to invertebrates including insects can be found in the risk assessment and risk management plan for glufosinate ammonium tolerant canola expressing the PAT protein (DIR 010/2001 and DIR 021/2002, available at www.ogtr.gov.au). In summary, no significant differences were found in the species composition, population density and activity behaviour of epigeal fauna (invertebrates living or occurring on or near the surface of the ground) in fields containing conventional canola and GM glufosinate ammonium tolerant canola. Furthermore, the USDA-APHIS (USDA-APHIS 1999) concluded that there was no potential for deleterious effects on beneficial organisms such as earthworms, based on the knowledge of the mode of action and the lack of known toxicity of the PAT protein.

161. USDA-APHIS has approved Liberty Link[®] Cotton for commercial release in the USA and stated in its environmental assessment report that APHIS has never encountered impacts on organisms associated with the expression of PAT from any glufosinate ammonium tolerant crops that have been assessed (USDA-APHIS 2003a).

162. PAT proteins are produced naturally by the common soil bacteria *Streptomyces viridochromogenes* and *S. hygroscopicus*, encoded by the *pat* and *bar* genes, respectively

(Wohlleben et al. 1988; Strauch et al. 1988). The PAT protein expressed in Liberty Link[®] Cotton differs only in one amino acid from the protein naturally produced by *S. hygroscopicus*. Since the same or similar proteins are widespread in the environment, the introduced PAT protein is not expected to be a novel source of harm for invertebrates.

163. In conclusion, invertebrates are already widely exposed to the same or similar PAT protein present in soil bacteria and there is no evidence suggesting that PAT proteins are toxic to invertebrates. Therefore, **no risk is identified** and the potential for toxicity for invertebrates as a result of the expression of the introduced gene will not be assessed further.

2.3.3 Contact with the PAT protein by microorganisms

164. Microorganisms, particularly soil microorganisms, will be exposed to the GM cotton plants and the expressed PAT protein during the growth and decomposition of plant material. After harvest of lint and seed, the remaining cotton plant residues are typically tilled into the soil, so that soil microorganisms are likely to be exposed to the PAT protein as the residues are broken down.

165. Although no formal evaluation of the biodegradability of Liberty Link[®] Cotton plant materials has been carried out in Australian soil, the PAT protein is easily degraded by proteases (Wehrmann et al. 1996) and is not expected to accumulate in soil.

166. More information on the PAT protein in relation to toxic effects to microorganisms including microbial communities associated with rhizospheres can be found in the risk assessment and risk management plan for glufosinate ammonium tolerant canola expressing the *bar* gene (DIR 010/2002 and DIR 021/2002, available at www.ogtr.gov.au). In summary, the several studies using GM glufosinate ammonium tolerant canola with the introduced *pat* gene showed very few significant effects on microbial communities compared to non GM canola. Greater impacts on microbial communities tended to be related to soil type, stage of plant development, season and site heterogeneity (Sessitsch et al. 2005; Dunfield & Germida 2001; Becker et al. 2001; Gyamfi et al. 2002; Aumaitre 2004)

167. The effect of the presence of the *pat* gene in GM glufosinate ammonium tolerant maize and sugar beet on rhizosphere microflora by using a genetic profiling technique based on PCR amplification of 16S ribosomal RNA genes and single-strand conformation polymorphism (PCR-SSCP) has been investigated (Schmalenberger & Tebbe 2002). No significant differences were observed in microbial communities between GM and non-GM plants of maize or sugar beet, nor was there any significant effect from the application of glufosinate ammonium herbicide (Schmalenberger & Tebbe 2002; Schmalenberger & Tebbe 2003a; Schmalenberger & Tebbe 2003b). Differences in the microflora were observed for maize plants (both GM and non-GM) at different growth stages (Schmalenberger & Tebbe 2002) and for sugar beets between seasons and also between site heterogeneity (Schmalenberger & Tebbe 2003b).

168. PAT proteins are present in common soil bacteria and therefore are widespread in the environment. For example, in a study undertaken in Germany using soil samples taken from a barley field, 6 bacterial isolates from a total of 300 (2%) contained the PAT protein enzymatic activity (Bartsch & Tebbe 1989). Microorganisms are also exposed to the PAT protein through trials with GM cottons and canola in Australia (See Chapter 1 for details).

169. In conclusion, microorganisms are already widely exposed to the same or similar PAT protein and there is no evidence suggesting that PAT proteins are toxic to microorganisms. Therefore, **no risk is identified** and the potential for toxicity for microorganisms as a result of the expression of the introduced gene will not be assessed further.

2.4 Spread and persistence of the GM cotton in the environment

2.4.1 Expression of the bar gene increasing spread and persistence of the GM cotton plants through tolerance to glufosinate ammonium

170. The GM cotton plants produce sufficient PAT protein to provide tolerance to glufosinate ammonium throughout the growing season. In environments where glufosinate ammonium is used to control weeds, the GM cotton plants would have some selective advantage. This could lead to spread and persistence of the GM cotton. Therefore, **a risk is identified** for weediness of the GM cotton as a result of the expression of the *bar* gene. The level of risk of weediness from this event is estimated in **Chapter 3** as **Event 1**.

2.4.2 The presence of the regulatory sequences in the GM cotton plants

171. The introduced regulatory sequences in the GM cotton are not expected to have any impact on the spread or persistence of the GM cotton plants. The introduced regulatory sequences behave no differently to endogenous regulatory sequences in cotton. Therefore, **no risk is identified** and the potential for weediness of the GM cotton as a result of the presence of the introduced regulatory sequences will not be assessed further.

2.4.3 Exposure of people to the PAT protein as a result of spread and persistence of the GM cotton plants in the environment

172. Spread and persistence of the GM cotton plants in the environment could lead to increased exposure of people to the expressed protein. This could result in toxicity for people or allergic reactions.

173. However, the introduced PAT protein has extremely low toxicity and other GM crops containing the same or similar PAT protein are approved for use in food (see Section 2.1.1 of this Chapter for details). Evidence also indicates that the PAT protein is not allergenic (See Section 2.2.1 of this Chapter) and people are already widely exposed to the protein via bacteria expressing the protein naturally.

174. The amount of exposure expected as a result of spread and persistence of the GM cotton would be small in comparison to the exposure from the proposed commercial release of the GM cotton plants.

175. Therefore, **no risk is identified** and the potential for toxicity or allergic reactions in people as a result of spread and persistence of the GM cotton plants in the environment will not be assessed further.

2.4.4 Exposure of organisms other than people to the PAT protein as a result of spread and persistence of the GM cotton plants in the environment

176. Spread and persistence of the GM cotton plants in the environment could lead to increased exposure of organisms other than people to the expressed PAT protein. This could result in toxicity to these organisms.

177. Organisms are already widely exposed to PAT proteins in nature through their presence in bacteria. The level of exposure expected as a result of spread and persistence of the GM cotton plants would be minimal, since the numbers of GM cotton plants resulting from spread and persistence will be small in comparison to the proposed commercial release of Liberty Link[®] Cotton.

178. Furthermore, the PAT protein has extremely low toxicity to organisms (See Section 2.3 of this Chapter for details).

179. Therefore, **no risk is identified** and the potential for toxicity for organisms other than people as a result of spread and persistence of the GM cotton plants in the environment will not be assessed further.

2.5 Gene flow by vertical gene transfer

180. Transfer of genetic material to offspring by reproduction (vertical gene transfer) could result in the transfer of the introduced gene or the associated regulatory elements to other plants. The only sexually compatible species present in Australia that could receive genes from the GM cotton are *G. hirsutum* (including both cultivated (GM and non-GM) and naturalised cotton populations) and *G. barbadense*.

181. There are a number of commercially approved GM cotton lines grown in southern Australia as of August 2006. Glyphosate tolerant Roundup Ready[®] GM cotton contains the *cp4 epsps* gene which encodes the 5-enolpyruvylshikimate-3-phosphate synthase protein and the *nptII* gene which encodes the neomycin phosphotransferase II protein. Insect resistant Bollgard II[®] GM cotton contains the *cryIAc* and *cry2Ab* genes which encode crystal insecticidal proteins, the *nptII* gene, and *uidA* gene which encodes the β -glucuronidase protein. Roundup Ready[®]/ Bollgard II[®] GM cotton, the conventional crossing (stacking) of the above two GM cotton lines, contains all of the genes present in the parental GM cotton lines. These three GM cotton lines are widely planted in the agricultural environment south of latitude 22° South (authorised under DIR 012/2002, 022/2002, and 023/2002 licences). In the 2005-06 season, it has been estimated that 90% of all cotton planted in Australia were these three GM cotton lines (Cotton Australia 2006).

182. Insect resistant INGARD GM cotton was previously grown but has now been replaced by Bollgard II[®] cotton. Glyphosate tolerant Roundup Ready Flex[®], containing two copies of the *cp4 epsps* gene, and glyphosate tolerant/ insect resistant (Roundup Ready Flex[®]/ Bollgard II[®]) cotton were recently approved for commercial release in southern Australia in February 2006 (authorised under DIR 059/2005 licence).

183. The GM cotton proposed for release is not insect resistant and will therefore be sprayed with insecticides in the same way as non-GM cotton. The insecticide sprays would also kill pollinator species and this is expected to reduce pollination rates (as compared to those for the now widely grown insect resistant Bollgard II[®] GM cotton).

2.5.1 Gene transfer to native *Gossypium* species

184. As discussed in the *Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002), Australian flora contains 17 native *Gossypium* species. The centre of native *Gossypium* diversity in Australia is in northern Western Australia and the Northern Territory.

185. Most of the Australian *Gossypium* species have limited distributions and occur at considerable geographic distance from agriculturally used areas. However, some native *Gossypium* populations occur near roads where GM cotton seed may be transported and GM cotton volunteers may establish.

186. There is well established genetic incompatibility between native *Gossypium* species and the cultivated cotton (OGTR 2002). All native *Gossypium* species are diploid (C, G or K genomes), while the cultivated cotton species are tetraploid (AD genomes). The GM cotton proposed for release does not have increased ability to cross with native cotton species (compared to non-GM cotton).

187. The native cotton species with highest potential for hybridising with *G. hirsutum* is *G. sturtianum*. Hybrids between these two species have been produced under field conditions, without application of plant hormones, when plants were grown in close proximity to each

other. However, these hybrids were sterile, effectively eliminating any potential for introgression of *G. hirsutum* genes into *G. sturtianum* populations under natural conditions. There are no reports of hybrids between *G. hirsutum* and any other native *Gossypium* species occurring under natural conditions.

188. Hybrids between *G. hirsutum* and native *Gossypium* species have been produced under artificial conditions in the glasshouse (ie emasculation, hand-pollination and application of plant hormones) but the resulting hybrids were sterile, with the exception of six K-genome species which had some level of female fertility (Brubaker et al. 1999; Brubaker & Brown 2001). Backcrosses between the *G. hirsutum* x K-genome species (ADK) hybrids and *G. hirsutum* (AD) resulted in the production of pentaploid progeny (AADDK). These successful backcrosses were possible due to the production of unreduced gametes in the hybrid (Brubaker & Brown 2001). The pollen from these pentaploid plants was functionally sterile which would limit the possibility of introgression of genes into the native K-genome species.

189. The introgression of the introduced genes is further limited because the pentaploid hybrids would contain a single set of K-genome chromosomes, which can not pair up during meiosis. Thus, in subsequent backcrosses to either cultivated GM cotton or the native *Gossypium* K-genome species, the K-genome chromosomes would be lost and this process would continue until all the K-genome chromosomes are lost. In addition, the introduced genes are carried on the A and/or D genomes of the GM cotton (*G. hirsutum* (AD)) and could only be maintained in the K-genome cotton if they are transferred to a balanced set of K-chromosomes. Transfer of introduced genes by recombination between chromosomes of different genomic origin is thought to be extremely rare, as demonstrated by studies in hexaploid wheat (Hedge & Waines 2004). This is likely due to the spatial separation of chromosomes from different genomes during the cell cycle as observed in hexaploid wheat which contains 3 genomes (Avivi et al. 1982) and the F1 hybrid generated by crossing barley and wild rye (Leitch et al. 1991). Thus, the potential for introgression of introduced genes into any of the K-genome *Gossypium* species is negligible.

190. Therefore, **no risk is identified** and the potential for weediness in these sexually incompatible species as a result of gene transfer will not be assessed further.

2.5.2 Expression of the bar gene in other *G. hirsutum* or *G. barbadense* cotton plants (not including commercially released GM cotton lines) providing glufosinate ammonium tolerance

191. Sexually compatible plants (ie naturalised, volunteer or commercially grown non-GM *G. hirsutum* or *G. barbadense* cotton plants) expressing the *bar* gene as a result of vertical gene transfer could become tolerant to glufosinate ammonium. This could confer a fitness advantage on the plants in environments where glufosinate ammonium is used to control weeds. Therefore, **a risk is identified** for weediness as a result of vertical gene transfer of the *bar* gene construct into non-GM *G. hirsutum* or *G. barbadense* plants. The level of risk of weediness from this event is estimated in **Chapter 3 as Event 2**.

2.5.3 Expression of the bar gene in combination with cp4 epsps gene and/or cry1Ac and cry2Ab genes providing dual herbicide tolerance and reducing lepidopteran herbivory

192. GM cotton plants expressing the *bar* gene in combination with the *cp4 epsps* and/or the *cry1Ac* and *cry2Ab* genes present in commercially approved GM cotton lines as a result of vertical gene transfer could become tolerant to glufosinate ammonium as well as tolerant to glyphosate and/or resistant to lepidopteran insects. In environments where either glufosinate

ammonium or glyphosate is used to control weeds or where cotton plants are controlled by lepidopteran insects, these plants could survive and may become persistent in the environment. Therefore, **a risk is identified** for weediness as a result of vertical gene transfer of the *bar* gene into commercially approved GM cotton varieties. The level of risk of weediness from this event is estimated in **Chapter 3** as **Event 3**.

2.5.4 Exposure of organisms (including people) to the PAT protein or in combination with other genes as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants

193. Expression of the introduced gene in sexually compatible plants (ie naturalised, volunteer or commercially grown *G. hirsutum* or *G. barbadense* cotton plants, including other GM cotton lines) could lead to increased exposure of organisms, including people, to the expressed PAT protein. Additionally, exposure to the introduced *bar* gene or its product in combination with introduced genes or their products present in commercially approved GM cotton lines could also occur from gene flow between Liberty Link[®] Cotton and other commercially approved GM cotton lines. These events could result in toxicity for organisms, including people or allergic reactions in people.

194. However, the PAT protein has extremely low toxicity (see Sections 2.1 and 2.3 of this Chapter) and the GM cotton products (oil and linters) have been approved for use in food (see Section 2.1.1 of this Chapter for details). Evidence also indicates that the PAT protein is not allergenic to people (see Section 2.2). Furthermore, organisms including people are already widely exposed to the protein via bacteria naturally expressing it (see Section 2.1).

195. As discussed in the DIR 012/2002, 022/2002, 023/2002 and 059/2005 RARMPs, all of the expressed proteins in GM cotton lines currently approved for commercial scale cultivation have low acute oral toxicity. If conventional crossing between Liberty Link[®] cotton and other commercially approved cotton lines occurred, no interactions between the proteins expressed as a result of the introduced genes are expected as all of the proteins are produced via independent biochemical pathways. Therefore, it is not expected that there will be any unintended biochemical changes that may give rise to toxicity or allergenicity for people or other organisms.

196. Vertical gene transfer to sexually compatible cotton plants would be minimal, since outcrossing of cotton is localised around the pollen source and decreases significantly with distance (OGTR 2002 and references therein). The amount of insecticide sprays used on Liberty Link[®] Cotton may also reduce the number of pollinators of cotton and thus further reduce the amount of gene transfer. Furthermore, the level of exposure expected as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants would be minimal, since the numbers of GM cotton plants resulting from spread and persistence will be small in comparison to the proposed commercial release of Liberty Link[®] Cotton.

197. Therefore, **no risk is identified** and the potential for toxicity or allergic reactions in organisms, including people, as a result of vertical gene transfer of the introduced gene into other *G. hirsutum* or *G. barbadense* plants will not be assessed further.

2.5.5 Presence of the introduced regulatory sequences in *G. hirsutum* or *G. barbadense* plants as a result of gene transfer

198. All of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to cotton plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. The impacts from the introduced regulatory elements are equivalent to and no greater than the endogenous regulatory elements.

Therefore, **no risk is identified** and the potential for an adverse outcome as a result of vertical gene transfer of introduced regulatory sequences will not be assessed further.

2.5.6 Reduced choice of herbicides for the control of cotton volunteers as a result of stacking of herbicide tolerance traits

299. GM cotton plants expressing the *bar* gene in combination with *cp4 epsps* gene present in commercially approved cotton lines as a result of vertical gene transfer could become tolerant to both glufosinate ammonium and glyphosate and therefore these herbicides could not be used to control the cotton volunteers.

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

201. Herbicides can be used to control seedling cotton volunteers. Glyphosate has been the most common herbicide to control these volunteers but, with the uptake of Roundup Ready[®] GM cotton since 2000, alternative herbicides are being used. Glufosinate ammonium is one of the options. However, its use is limited on cotton volunteers as its effectiveness on cotton seedlings at the 4 and 8 leaf stage offers 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 2002a).

202. Established or ratoon cotton plants, whether GM or non-GM, are difficult to control by herbicides alone. For example, glyphosate is not generally used to control established cotton plants because it does not kill the plants and they can recover. Instead, established or ratoon cotton plants are most effectively controlled by mechanical methods involving mulching, root cutting and cultivation (Roberts et al. 2002).

203. Therefore, **no risk is identified** and the potential for reduced choice of herbicides to control cotton volunteers as a result of vertical gene transfer of the *bar* gene to other commercially approved GM cotton lines containing the *cp4 epsps* gene will not be assessed further.

2.5.7 Tolerance to other herbicides as a result of stacking

204. GM cotton plants expressing the *bar* gene in combination with *cp4 epsps* gene present in commercially approved GM cotton lines as a result of vertical gene transfer could become tolerant to both glufosinate ammonium and glyphosate as well as other herbicides.

205. Herbicides containing glufosinate ammonium are classified into Group N and herbicides containing glyphosate are in Group M and therefore these herbicides affect different biochemical pathways in plants. Therefore, it is unlikely that the presence of both the *bar* and *cp4 epsps* gene in cotton plants will result in unintended biochemical interactions and that the plants will develop resistance to a different type of herbicide.

206. A study on stacking of glyphosate and glufosinate ammonium herbicide tolerance traits in GM canola showed no susceptibility to other, unrelated herbicides and no gene silencing (Senior et al. 2002).

207. Therefore, **no risk is identified** and the potential for tolerance to other herbicides as a result of vertical gene transfer of the *bar* gene to other commercially approved GM cotton lines containing the *cp4 epsps* gene will not be assessed further.

2.6 Gene flow by horizontal gene transfer

2.6.1 Presence of the *bar* gene, or the introduced regulatory sequences, in other organisms

208. Transfer of the *bar* gene, or the introduced regulatory sequences, from the GM plants to sexually incompatible plants, animals or microorganisms (horizontal gene transfer) could occur only rarely without human intervention.

209. Transfer of the *bar* gene to other organisms could cause the spread of this gene in the environment, leading to tolerance to glufosinate ammonium in other organisms. There are a number of soil bacteria that have been identified as naturally tolerant to glufosinate ammonium (Bartsch & Tebbe 1989) and there are likely to be many more. These include *S. hygrosopicus* from which the *bar* gene was isolated from (Murakami et al. 1986), and *S. viridochromogenes* from which the *pat* gene was isolated (Wehrmann et al. 1996).

210. Acetyltransferases with the capacity to acetylate phosphinothricin, although with weak affinity, have also been identified from *S. griseus*, *S. coelicolor*, *S. lividans* and *Aliccaligenes faecalis* (Bedford et al. 1991; Wehrmann et al. 1996). Thus, insensitivity to glufosinate ammonium is already widespread in microbial populations.

211. Since the introduced gene is already widely present in bacterial species, any transfer of this gene is much more likely to occur between bacteria, or bacteria and other organisms, than between the GM plants and other organisms (De Vries & Wackernagel 2004).

212. Transfer of the regulatory sequences to other organisms could alter the expression of endogenous genes in unpredictable ways. However, all of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to cotton plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. As there is no difference between the two events, this does not represent a novel adverse outcome as a result of the genetic modification.

213. Horizontal gene transfer has been examined in detail in a number of other RARMPs (most recently DIR 059/2005 and DIR 061/2005), which are available from the [OGTR website](#) or by contacting the Office. These assessments have concluded that horizontal gene transfer from plants to other sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. Reports of horizontal gene transfer from plants to bacteria occurring during laboratory experiments have relied on the use of highly similar sequences to allow homologous recombination to occur, and conditions designed to enhance the selective advantage of gene transfer events (Nielsen et al. 1998; Mercer et al. 1999; Nielsen et al. 2000; Gebhard & Smalla 1998; De Vries et al. 2001). Horizontal gene transfer is not expected to produce any adverse outcomes during this proposed release. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of horizontal gene transfer will not be assessed further.

2.7 Unintended changes in toxicity

2.7.1 Altered levels of innate toxic or anti-nutritional compounds as a result of random insertion of the gene construct into the cotton genome as a result of genetic modification

214. Cotton tissue (from either GM or non-GM plants), particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional compounds including gossypol and cyclopropanoid fatty acids (eg dihydrosterculic, sterculic and malvalic

acids). There is potential for the GM cotton plants proposed for release to have increased levels of toxic or allergenic compounds as a result of the genetic manipulation.

215. Compositional and antinutrient analyses of Liberty Link[®] Cotton has been conducted (see Section 2.1.1 and 2.3.1 of this Chapter) and no significant differences were detected compared to other commercially grown cotton varieties.

216. A detailed compositional analysis of Liberty Link[®] Cotton in comparison to the parental line was assessed by FSANZ in deciding to approve Bayer's application to use materials containing oil and linters derived from this GM cotton in human food. FSANZ concluded in its assessment report that food derived from Liberty Link[®] Cotton is as safe as food derived from other cotton varieties (FSANZ 2005b).

217. Therefore, **no risk is identified** and the potential for toxicity as a result of unintended changes in toxicity will not be assessed further.

2.7.2 Altered metabolism of glufosinate ammonium in the GM cotton plants expressing the PAT protein resulting in the production of toxic compounds

218. 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).

219. Phosphinothricin acetyl transferase (PAT), encoded by either the *bar* or *pat* gene, 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).

220. The metabolism of glufosinate ammonium in tolerant GM plants and in non-GM (non-tolerant) plants has recently 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).

221. 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 either metabolized to:

- 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-methylphosphonico-2-hydroxy-butanoic acid (MHB), which may be further converted to 4-methylphosphonico-butanoic acid (MPB), a final and stable product (Droge-Laser et al. 1994; Ruhland et al. 2002; Ruhland et al. 2004).

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

223. The metabolism of glufosinate ammonium has been investigated in herbicide-tolerant, genetically modified canola, maize, tomato, soybean and sugar beet by Thalacker (1994, cited in Food and Agriculture Organization 1998), and the OECD (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.

224. Studies using cell cultures of tolerant (GM) and sensitive canola gave similar results, with N-acetyl-L-glufosinate ammonium being the major metabolite in the glufosinate ammonium tolerant cells (Ruhland et al. 2002). D-glufosinate ammonium and N-acetyl-L-glufosinate ammonium are readily transported in the phloem of glufosinate ammonium tolerant canola (Beriault et al. 1999).

225. N-acetyl-L-glufosinate and MPP are non-toxic to both plants and mammals, including humans (OECD 1999a; 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.

226. Therefore, **no risk is identified** and the potential for toxicity as a result of altered metabolism of glufosinate ammonium in the GM plants will not be assessed further.

2.8 Unintended changes in biochemistry or physiology

2.8.1 Altered biochemistry or physiology of the GM cotton plants resulting from insertion or expression of the introduced gene

227. 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 changes in chromatin structure, methylation patterns or transcriptional read-through
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions of an introduced gene product with endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in the biochemical pathway of the introduced gene product.

228. Such unintended effects might result in adverse outcomes such as toxicity or allergenicity; weediness, pest or disease burden; or reduced nutritional or agronomic value as compared to the parent organism. However, accumulated experience with genetic modification of plants indicates that 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).

229. Unintended changes in gene expression could alter either the biochemistry or the physiology of the GM cotton plants. Biochemical or physiological changes to the GM cotton proposed for release could occur either as a result of the expression of the introduced gene or of the transformation process itself. However, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

230. The site where the introduced DNA is inserted in Liberty Link[®] Cotton has been fully sequenced (Aerts & De Beuckeleer 2003) and does not have homology to any known gene in current databases. Therefore, it is unlikely that the expression or function of an important endogenous gene has been disrupted as a result of the transformation event.

231. Agronomic performance trials were conducted on a total of 9 sites at 6 different locations in the USA during 2000 and 2001 and monitored closely for differences between the GM Liberty Link[®] Cotton (in the Coker 312 background or in 6 different genetic backgrounds) and parental non-GM cotton varieties. Overall the trials demonstrated the agronomic equivalence of Liberty Link[®] Cotton and non-GM cotton in terms of germination, growth habit, plant morphology, fibre characteristics (length, strength, elongation, and fineness) and

disease susceptibility (Freyssinet 2002a; Freyssinet 2002b). All characteristics measured were in the range expected for existing commercial cotton cultivars.

232. As expected, some differences were observed between the different genetic backgrounds of Liberty Link[®] Cotton for some yield components (lint yield, seed per boll, seed index and sympodia⁵ length), some maturity components (node of first fruiting branch and percent open bolls) and some aspects of fibre quality. However, these were all in the range expected for existing commercial cotton cultivars.

233. Liberty Link[®] Cotton was also assessed for tolerance to glufosinate ammonium herbicide application. When Liberty Link[®] Cotton was treated with glufosinate ammonium at 1x and 4x the recommended rate, agronomic performance was equal to or better than cotton treated under a conventional herbicide program.

234. Four years of small and medium scale field trials of Liberty Link[®] Cotton in Australia (conducted under PR124X(2), DIR 015/2002 and DIR 038/2003) have not indicated any secondary, pleiotropic effects.

235. Extensive compositional analyses performed on whole and linted cotton seeds, lint, as well as different cotton seed products (e.g. linters, hulls, delinted seeds, meal, toasted meal, crude oil and refined deodorised oil), have demonstrated that the levels of important nutritional and anti-nutritional components in Liberty Link[®] Cotton are comparable to the parental non-GM variety (Coker312) and are within established ranges for current commercial cotton varieties (See Sections 2.1.1 and 2.3.1 for details).

236. The PAT protein expressed in the GM plants functions by acetylating the L-glufosinate ammonium which does not inhibit glutamine synthase. 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). The metabolites produced by the GM cotton are non-toxic to both plants and mammals, including humans (OECD 1999a; OECD 2002). See Section 2.8.2 of this Chapter for further detail.

237. The demonstration of agronomic and compositional similarity of Liberty Link[®] Cotton and non-GM cotton indicates that no significant pleiotropic or epistatic effects (that is, unintended effects of a genetic change on other, apparently unrelated, plant genes or plant characteristics) have occurred. Therefore, **no risk is identified** and the potential for toxicity or weediness as a result of unintended changes in biochemistry or physiology will not be assessed further.

2.9 Unintended effects on existing pests or weeds

2.9.1 Expression of the introduced bar gene resulting in increased disease burden

238. Weed management practices in Liberty Link[®] Cotton would differ compared to non-GM cotton, herbicide tolerant (Roundup Ready[®]) GM cotton lines and insecticidal (Bollgard[®] II) GM cotton lines, such as the types of herbicides used and the amount of mechanical cultivation required. Management of insect pests in Liberty Link[®] Cotton would also differ to the most commonly grown cotton, Bollgard[®] II, by the number of insecticide sprays required. These different management practices may have an impact on the disease and pest status of the Liberty Link[®] Cotton.

239. Previous releases of the same GM cotton in Australia (conducted under DIR 015/2002 and DIR 038/2003) did not show increased occurrence of disease or pest status compared to

⁵ segmented fruiting branches where flowers and resulting bolls grow

non-GM cotton. Similarly, no increase in disease pressure or pest status was detected during agronomic performance testing conducted in the USA over a 3 year period from 1999 to 2001 (information supplied by the applicant).

240. Expression of the *bar* gene in a variety of crop plants (for example canola and corn), over several years of agronomic performance testing and commercial cultivation, has not been linked to increased susceptibility to disease, as assessed by regulatory authorities of other countries, including the Animal and Plant Health Inspection Agency (APHIS) of the US Department of Agriculture (e.g. USDA-APHIS 2003b) and the Canadian Food Inspection Agency (e.g. Canadian Food Inspection Agency 1998b); also see Gene Files (Gene Files 2002) for links to further safety assessments.

241. Therefore, **no risk is identified** and the potential for increased disease burden as a result of the expression of the PAT protein will not be assessed further.

2.10 Secondary impacts

2.10.1 Consumption of animals that were fed GM plant materials

242. Mammals generally avoid feeding on cotton plants. 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. Inactivation or removal of these components during processing enables the use of some cotton seed meal for farmed fish, poultry and swine. The meal and hulls of cotton seed can also be used for cattle feed. Its use as stockfeed is limited, nonetheless, to a relatively small proportion of the diet and it must be introduced gradually, to avoid potential toxic effects.

243. Protein and DNA of plant material are rapidly broken down into smaller components (eg protein and DNA fragments, amino acids, sugars etc.) in the digestive tract of animals. The PAT protein is rapidly degraded in mammalian digestive systems (see Section 2.1.1 of this Chapter for details). As a result, meat produced from animals fed Liberty Link[®] Cotton seed would be no different to meat from animals that were fed seed from non-GM cotton.

244. In a study where cows were fed silage made from glufosinate ammonium tolerant corn, no PAT protein or the gene was detected in milk by ELISA and PCR, respectively (Phipps et al. 2005).

245. Meat from cattle that were fed seed from commercially released Roundup Ready[®] and Bollgard II[®] cotton lines has been consumed for several years (since 2000 and 2002, respectively) with no adverse effects reported.

246. As discussed in Section 2.1.1, the PAT protein has extremely low toxicity.

247. Therefore, **no risk is identified** and the potential for toxicity for people as a result of consumption of animals that were fed GM plant materials will not be assessed further.

2.10.2 Use of glufosinate ammonium on the GM cotton resulting in changes in the weed spectrum or development of herbicide resistant weeds

248. The proposed release of Liberty Link[®] Cotton will enable the spraying of the Liberty[®] 150 Herbicide (containing glufosinate-ammonium as the active ingredient) for in-crop weed control. Glufosinate ammonium is classified as a Group N Herbicide for weed resistance management in Australia and is also a member of the glycine group of herbicides (label information). Its mode of action is as an inhibitor of glutamine synthetase.

249. The spraying of the Liberty[®] 150 Herbicide may impact on agricultural management practices. Changes in agricultural practices (eg adoption of minimal tillage) may cause weed

population shifts. Any change in weed management practices (eg changes in herbicide use) will also cause a shift in the weed spectrum. For example, weed species that are inherently more resistant to the herbicide used than other weed species may become more problematic (Owen & Zelaya 2005; Nandula et al. 2005). A change in the weed spectrum may occur where Liberty[®] 150 Herbicide is used to replace other weed management practices. This could result in the emergence of weeds that are more difficult to control.

250. Bayer has prepared a draft Liberty Link[®] Cotton and Liberty[®] 150 Herbicide crop management plan, which has been endorsed by the Transgenic and Insect Management Strategy (TIMS) committee of the Australian Cotton Growers Research Association. This crop management plan specifies an integrated weed management strategy and would be used in conjunction with the agreement between the grower and Bayer. There are also integrated weed management guidelines for the Australian cotton industry (Roberts and Charles, 2002).

251. Herbicide use on weed communities can exert selective pressure that leads to the development of herbicide resistant weeds. The repetitive use of a single herbicide, or herbicide group, increases the chance that development of herbicide resistant weeds will occur. Integrated weed management practices help to avoid selection of resistant weed biotypes (Avcare 2003). Integrated weed management has prevented the development of herbicide resistant weeds in Australian cotton fields up to this point (Roberts & Charles 2002; Charles et al. 2004).

252. Studies have shown a number of plant species with varying degrees of susceptibility to glufosinate ammonium application (Ridley & McNally 1985; Steckel et al. 1997). There was no discussion as to whether resistance in these plants was innate or had developed due to the use of glufosinate ammonium containing herbicides. Tolerance to the herbicide can be affected by a number of factors including the rate of the herbicide that is applied, the timing of application, the stage of plant development and climatic conditions.

253. In Australia, there are no reports that the use of herbicides containing glufosinate ammonium have resulted in the evolution of glufosinate ammonium resistant weeds.

254. The Herbicide Resistance Action Committee (HRAC) is an international body whose aim is a cooperative approach to the management of herbicide resistance and they support a worldwide survey of resistant weeds initiated by the Weed Science Society of America. The HRAC classified glufosinate-ammonium in group H for weed resistance management and places it in the phosphinic acid chemical family rather than the glycine group as it is designated in Australia. [Information prepared by HRAC](#) does not list any weeds with resistance to glufosinate-ammonium (Group H).

255. In a study on herbicide application, no weeds resistant to glufosinate ammonium were detected in an orchard sprayed with the herbicide 4 times per year for 9 years (Bulcke & Callens 1998).

256. The APVMA operates the national system that evaluates, registers and regulates agricultural and veterinary chemical products. Any changes to the use of a product that is already on the market must also be referred to the APVMA. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits for experimental work that allow restricted use of an agricultural chemical, for example for a limited period of time or for a limited area.

257. In considering applications for registration or permits, as well as considering potential health and environmental impacts, the APVMA also considers a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as efficacy and the trade implications of residues. The hazard of development of resistance to agricultural

chemicals is also part of the APVMA's assessment of agricultural chemical use. The APVMA may impose conditions on the use of chemical products in both registrations and permits. These conditions may include restrictions on use, implementation of a resistance management plan, and ongoing reporting on compliance.

258. Bayer has a research permit for use of glufosinate ammonium in current cotton trials involving this GMO, and the APVMA is currently assessing an application from Bayer to register Liberty[®] 150 Herbicide for the control of various weeds in Liberty Link[®] Cotton. Bayer also proposes a crop management plan, which specifies an integrated weed management strategy to prevent the evolution of glufosinate ammonium resistant weeds. The APVMA may also impose conditions on the use of the herbicide (eg restrictions on the number of applications that can be made and at what stage of crop growth these can be made) if it considers this necessary to manage any identified risks.

259. Therefore, **no risk is identified** as the potential for the use of glufosinate ammonium on the GM cotton resulting in development of herbicide resistant weeds will be considered by the APVMA.

Section 3 Risk estimate process for identified risks

260. Three events from the hazard identification process (Events 1–3 in Table 2.1) are considered to lead to identified risks for the same adverse outcome of weediness.

261. Chapter 3 provides detailed assessment of the consequences and likelihood of these three events in order to obtain estimates of the level of risk. The risks are assessed against the baselines established by reference to characteristics of the parent organism and aspects of the receiving environment (including agronomic management practices and other GM cotton lines previously approved for commercial release).

262. Information contained in the application (including information required by the Act and the Regulations on the GMO, 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 with experts, agencies and authorities and the public (Appendix B to E) were also considered.

263. The consequence assessment considers the seriousness of the harm that could potentially result from each 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 2.2). During the consequence and likelihood assessments, consideration is also given to areas of uncertainty, if any, that arise from a lack of data.

Figure 2.2 The OGTR Risk Estimate Matrix (OGTR 2005)

		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			

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.

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

265. After an estimate is obtained for each identified risk, risks higher than negligible are evaluated to determine if risk treatment measures are required to mitigate potential harm (see Chapter 4—Risk Management).

Chapter 3 Risk estimates for weediness

266. This Chapter estimates the risks associated with three events that could lead to the adverse outcome of weediness arising from this proposed release. The risk estimates are based on the consequence and likelihood assessments of each event.

Section 1 Background

267. Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens. In addition, plants may also be considered weeds if they are growing where they are not wanted.

268. Weediness in Australia is often correlated with weediness of the species, or a close relative, elsewhere in the world (Panetta 1993; Pheloung et al. 1999; Pheloung 2001). The chance of weediness is increased by repeated intentional introductions of plants outside their natural geographic range that increase the opportunity for the plants to establish and spread into new environments (eg escapes of commonly used garden plants) (Mulvaney 2001; Groves et al. 2005).

269. Negative characteristics of weeds may include spread and persistence, competitiveness, rambling or climbing growth, toxicity, production of spines, thorns or burrs, or parasitism. In addition, the spread and persistence of weeds is a measure of their potential invasiveness, which may give rise to negative environmental impacts such as:

- reduced biodiversity (including genetic, species and ecosystem diversity) that results from lower abundance of desirable species, reduced species richness, or undesirable changes in species composition
- interference with the intended use of the land they occupy
- degradation of landscape/ecosystems, such as altered water or nutrient availability.

270. Complex interactions between a plant and its environment (including availability of water, nutrients and light) determine the degree to which that plant can spread and persist in the environment. A number of measurable properties of plants that may influence spread and persistence or competitiveness are listed below:

- germination, survival and reproduction under a wider range of environmental conditions
- rates of seedling growth
- rates of growth to reproductive stage
- degree of self-pollination
- use of non-specialist pollinators or wind when out-crossing
- period of seed production
- seed output
- degree of seed dispersal
- longevity of seed and degree of dormancy
- allelopathy (effect on the germination and/or growth of neighbouring plants through chemical exudates)
- resistance to pests or pathogens.

271. 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 GMO, or of sexually compatible relatives that may receive the introduced gene. Alterations in these characteristics may indicate potential for weediness.

272. The GM cotton proposed for release expresses one protein as a result of the genetic modification. Events that may give rise to weediness were considered in Chapter 2.

Section 2 Consequence and likelihood assessments

273. Consideration is given to the three events identified in Chapter 2 (Hazard identification) that may give rise to weediness (Event numbers 1-3). For each event 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).

274. The Regulator can only consider risks 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 weediness of the non-GM parent and the environment in which the GM cotton plants are proposed for release. Therefore, widespread planting in both the current cotton growing regions in NSW and QLD as well as in potential future cotton growing regions is considered, along with the distribution of other commercially approved GM cotton lines, when assessing the risks posed by the proposed commercial release of Liberty Link[®] Cotton.

2.1 Weediness of non-GM cotton

275. 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 and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002). This document concludes that non-GM cotton is not a serious weed in Australia, because abiotic and biotic factors including temperature, soil moisture, nutrient levels and roadside management practices limit the establishment and/or persistence of cotton outside of agricultural and other disturbed environments. Additional limiting abiotic and biotic factors that determine whether cotton will persist in the environment include frost, short summer seasons, soil type, fire, competition from other plants, herbivory (insects and other animals), and physical destruction such as trampling (Eastick 2002, Farrell and Roberts 2002). The relative impact of each of these factors is dependent on whether the cotton plants are in coastal or inland 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.

276. 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. When grown in a glasshouse, they tend to have poor architecture and produce small bolls and seed with sparse, grey lint. They also produce mainly tufted rather than fuzzy seeds, which is a strong indication that they are not derived from modern cultivars which are all fuzzy seeded cotton plants (Curt Brubaker and Lyn Craven, CSIRO, pers. comm.).

277. Tufted seeded cotton plants were originally used when hand delinting was required, before the advent of mechanical saw gins in the late 18th century. Tufted seeded cotton plants were subsequently replaced by fuzzy seeded varieties with better lint characteristics and disease resistance. It seems likely, therefore, that many naturalised cotton populations result from attempts in the early 19th century to establish cotton industries in northern Queensland and the Northern Territory (Curt Brubaker and Lyn Craven, CSIRO, pers. comm.).

278. A small number of 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 in northern Australia.

279. An important indicator of potential weediness of a particular plant is its history of weediness in any part of the world and its taxonomic relationship to declared weeds (Bergelson et al. 1998; Panetta 1993; Pheloung 1995). Cotton has been grown for centuries throughout the world without any reports that it is a serious weed. Likewise, cotton is not considered to be a serious weed in Australia (Groves et al. 2000; Groves et al. 2002; Groves et al. 2003). 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).

280. The weed status of cotton has also been considered previously in many of the RARMPs produced during the assessment of a variety of GM cotton lines (eg DIRs 012/2002, 022/2002, 023/2002, 055/2004, 056/2006 and 059/2005). In addition to the information in the Biology and Ecology document (OGTR 2002), these RARMPs have considered new data that has been collected during previous releases of GM cotton lines in Australia.

281. 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, mainly in Queensland (OGTR 2002). The presence of remnants of some of these populations has not been confirmed.

2.2 Event 1: Expression of the introduced bar gene increasing spread and persistence of the GM cotton plants through tolerance to glufosinate ammonium.

282. The applicant is seeking approval for the unrestricted commercial scale planting of the GM cotton in all current cotton growing areas and potential future areas with environmental conditions suitable for cultivation in Australia. This would include conventional breeding, sale of seed for commercial planting, use in human food and stockfeed, sale of lint, export of seed and unrestricted transport. Therefore, GM cotton plants could potentially persist in the agricultural environment where originally grown, or GM cotton plants may establish and persist in the wider environment as a result of GM cotton seed dispersal via transport, stockfeeding, animals or flooding.

283. The risk of weediness of the GM cotton plants as a result of the expression of the *bar* gene construct would depend on the weediness of non-GM cotton plants, the importance of the use of glufosinate ammonium 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 level of risk is assessed against the baseline of the low weediness potential in the non-GM parent organism, in the context of the large scale of the proposed release, and the receiving environment for the proposed release, which includes the commercial release of other GM cotton lines.

284. Discussions of the risk from expression of *bar* gene increasing the weediness of cotton plants in Australia is provided in the risk assessment documents for DIRs 015/2002, 038/2003 and 056/2004, available at <<http://www.ogtr.gov.au>>. These risk assessments concluded that expression of the *bar* gene, which provides tolerance to glufosinate ammonium herbicide does not enhance the weediness potential of these GM cotton plants (in comparison to non-GM cotton plants) in the cotton growing regions of Australia.

285. A number of studies have investigated whether the introduction of glufosinate ammonium tolerance results in increased weediness. Four different glufosinate ammonium tolerant crops, oilseed rape, potato, maize and sugar beet were grown in 12 different habitats

and monitored over a period of 10 years (Crawley et al. 2001). The genetically modified crops were not found to be more invasive or more persistent than their conventional counterparts in any of the 12 habitats. Oilseed-rape expressing tolerance to the herbicide glufosinate showed significantly lower seedling establishment when compared with conventional canola lines in six out of twelve cases and significantly higher in two cases. Another study found no differences in competitive ability of glufosinate ammonium tolerant canola lines and non-GM cultivars when grown either in monoculture or in mixture with barley (Poulsen et al. 1999). The GM canola lines only behaved differently from non-GM cultivars when glufosinate ammonium herbicide was applied.

286. During previous field trials of Liberty Link[®] Cotton under DIR's 015/2002, 038/2003 and 056/2004, there have been no difficulties in controlling volunteers as reported in annual reports and as observed during monitoring of field trial sites by staff from the OGTR.

2.2.1 Consequence assessment

287. The *bar* gene construct could confer a selective advantage in areas where glufosinate ammonium is used to control weeds. This could result in spread and persistence of the GM cotton in the environment.

288. Glufosinate ammonium is not totally effective in the control of seedlings of non-GM and GM cotton lines currently approved for commercial release, and is not effective on established cotton plants, irrespective of whether they are GM or non-GM (Roberts et al. 2002).

289. Glufosinate ammonium herbicide has limited use in Australia, one of the reasons being because of its higher cost than some other commonly used herbicides. Although, it is listed in the 100 most commonly used herbicides in Australia (Radcliffe 2002), the only registered broad acre crop use is for weed control in crops of GM glufosinate ammonium tolerant InVigor[®] hybrid canola (which is currently not grown commercially in Australia). It is not currently registered for use on cotton. Products containing glufosinate ammonium are also registered for use around various fruit trees and vines, in home gardens and in some non-agricultural settings such as roadsides.

290. In the presence of glufosinate ammonium, the small competitive advantage of the GM cotton is offset by abiotic and biotic factors (such as water availability, temperature, soil type and nutrients) that limit the spread and persistence of all cotton in Australia (see Section 2.1 of this Chapter).

291. Therefore, the consequences of the expression of the *bar* gene increasing the spread and persistence of the GM cotton plants proposed for release through tolerance to glufosinate ammonium are assessed as **marginal**.

2.2.2 Likelihood assessment

292. As discussed above, the applicant is seeking approval for the unrestricted commercial scale planting of the GM cotton in all current cotton growing areas and potential future areas with environmental conditions suitable for cultivation in Australia. Therefore, GM cotton plants could potentially persist in the agricultural environment where originally grown, or GM cotton plants may establish and persist in the wider environment as a result of GM cotton seed dispersal via transport, stockfeeding, animals or flooding.

Agricultural environment

293. For the proposed release, Bayer anticipates a phased introduction of the GM cotton over three years in the current cotton growing areas of NSW, and southern and central QLD. The

area is expected to increase in subsequent years and may include plantings in other areas that are suitable for growing cotton. Wide spread commercial planting in northern areas of Australia may be limited because the GM cotton proposed for release is not insect resistant and the major insect pests are highly likely to impact on cotton productivity if insecticides are not constantly applied throughout the growing season. Therefore, spread and persistence of the GM cotton in the north due to cultivation may be limited.

294. Some dispersal of GM cotton seed may also occur in areas where cotton seed is stored. Seed is stored on farms in various ways (eg in sheds) that maintain its quality and protect it from animals and weathering. Dispersal of GM cotton seed during storage is expected to be restricted to areas immediately surrounding these storage areas.

295. Cotton volunteers are actively managed on-farm by mechanical methods involving mulching, root cutting and cultivation (using cultivators, graders, excavators or chippers), application of herbicides (if in the seedling stage) or burning (Australian Cotton Cooperative Research Centre 2002a; Roberts et al. 2002; Charles et al. 2002). Volunteer Liberty Link[®] Cotton plants could not be controlled by the application of glufosinate ammonium but could easily be controlled by alternative herbicides or these other methods.

296. In the on-farm environment, a range of herbicides may be used to control cotton volunteers (at the seedling stage) that emerge after harvest. Herbicides containing glyphosate, carfentrazone-ethyl, or paraquat and diquat as active constituents are currently registered by the APVMA for control of volunteer cotton ([APVMA Pubcris database](#)).

297. Integrated weed management strategies stress the need to avoid relying on one control method (Roberts & Charles 2002). To avoid the development of glufosinate ammonium resistant weeds for example, application of glufosinate ammonium herbicide alone should not be used as the sole management strategy. Alteration of various strategies would result in the destruction of glufosinate ammonium tolerant GM cotton volunteers. Consistent with this, the applicant has developed an integrated weed management strategy in the proposed Liberty Link[®] Cotton and Liberty[®] 150 Herbicide crop management plan that will be considered by the APVMA in assessing Bayer's application to register Liberty[®] 150 Herbicide for use on the GM cotton.

298. As discussed in Section 2.2.1, a small selective advantage of the GM cotton would only occur in the presence of glufosinate ammonium. However, glufosinate ammonium is a non-persistent herbicide and therefore after application, the duration of any selective action due to the glufosinate ammonium would be limited.

299. Therefore, the likelihood of Liberty Link[®] Cotton plants persisting in the agricultural environment is not expected to be greater than other commercially grown cotton plants.

Dispersal during transportation

300. Some GM cotton seed may be dispersed during transport of GM cotton seed for storage, planting, ginning, processing and stockfeed. The amount of cotton seed being transported and the distances transported would depend on the amount of the GM cotton grown each year and its end use. This can be highly variable. For example, the use of cotton seed as stockfeed increases significantly during drought.

301. As cotton does not compete well with other plants and has high water and nutrient requirements (see Section 2.1 of this Chapter), volunteer establishment is mainly expected in disturbed, favourable habitats such as ditches and roadside drains.

302. The type of seed dispersed has a large impact on the likelihood of germination (Eastick 2002). Black seed, which has been ginned and acid delinted and is used for planting, has the

highest germination rate at >80%. This seed is unlikely to be accidentally dispersed as it is transported in smaller quantities and is of higher value. Fuzzy seed, which has been ginned, is often transported and used for cattle feed. This germinates much less readily than the black seed. The seed cotton, directly harvested from the plant, has the highest potential for unintentional dispersal during transport but germinates relatively poorly.

303. A survey of the transport routes between Emerald (in the cotton growing region in central Queensland) and the Atherton Tablelands (north of latitude 22° South in Queensland), 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). Only four plants were observed in 1200 km of road surveyed north of latitude 22° South. Details of the study can be found in the risk assessment prepared for [DIR 059/2005](#). The study concluded that cotton volunteers 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 volunteers in the roadside environment were identified: competition from already established vegetation, low quantity of seed escapes, high disturbance in areas requiring frequent maintenance and high rate of seed desiccation.

304. These results are supported by Eastwick 2002 (Eastick 2002). Cotton seed germination was highest in disturbed habitats especially when the seed was buried rather than remaining exposed on the soil surface. Persistence of cotton plants for more than 1-2 years was only seen in habitats with increased water availability or nutrition such as cattle yards.

305. Slashing appeared to be the common method of roadside weed control, and herbicide use tended to be limited to around fixtures (eg signs and guide posts) and drainage points where slashing is difficult. This suggests that glufosinate ammonium tolerance is not likely to provide a significant selective advantage. Studies on the presence of GM Roundup Ready[®] volunteers did not indicate a selective advantage of Roundup Ready[®] cotton compared to other cotton varieties (Details available in the risk assessment prepared for [DIR 059/2005](#). Again, this suggests that herbicide tolerance is not likely to provide a significant selective advantage.

306. Although some GM cotton plants may establish along transport routes, the expression of the *bar* gene construct is not expected to alter susceptibility to the abiotic and biotic factors that limit the establishment and persistence of cotton (eg plant competition, soil type, fire, herbivory, frost and variable availability of water and nutrients).

Dispersal via use as stockfeed

307. 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). As a result, cotton volunteers could establish in areas where livestock is fed cotton seed (eg feedlots, cattle yards or dairy farms) or grazes after being fed. Areas where stock is fed are nutrient rich, disturbed habitats and cotton volunteers are expected to establish.

308. The amount of cotton seed being used in stockfeed each year can be highly variable. For example, its use increases significantly during drought. However, the quantity of cotton seed used is generally limited to a relatively small proportion of the diet, and must be introduced gradually, to avoid potential toxic effects due to the presence of anti-nutrients (ie gossypol and cyclopropenoid fatty acids) that are normally present in cotton.

309. Other GM cotton lines are currently in commercial cultivation, Roundup Ready[®] and Roundup Ready[®]/INGARD[®] cotton since 2000 (DIR 023/2002), and Bollgard II[®] and Bollgard II[®]/Roundup Ready[®] cotton since 2002 (DIR 012/2002). Since their commercial release, cotton seed from these GM cotton lines has been used as stockfeed in northern Australia. Over this period there has been no evidence that these GM cotton lines have become problematic weeds.

310. For example, Farrell and Roberts (Farrell & Roberts 2002) found cotton volunteers at seven of nine dairy farms surveyed (Atherton Tablelands, March 2002), with GM cotton (Roundup Ready[®], Roundup Ready[®]/INGARD[®] or INGARD[®] cotton) identified on four of these. Volunteers were all close to dairy infrastructure, suggesting that their ability to invade is negligible. Such volunteers generally do not complete an entire reproductive cycle to produce new seedlings, being limited by physical damage (eg trampling and grazing), disease and competition, and do not spread into other areas of the farms or natural environment or lead to the development of self-sustaining populations. On farms where both GM and non-GM volunteers were found, there was no indication that the GM plants had enhanced survivorship or reproductive potential in any situation. The Liberty Link[®] cotton is not expected to be different to non-GM and other commercially approved GM cotton lines.

311. Eastick (2002) found that although cotton growing in cattle yards may reach reproductive maturity, persistence and seed dispersal from these areas is limited by trampling and grazing, as well as plant competition, and no cotton volunteers were found in undisturbed bush habitats.

312. Results from a survey conducted over the 2002–03 cotton growing season (as part of research required under licences for DIR 023/2002 and DIR 022/2002) on the incidence of cotton volunteers in areas in northern Queensland where stock are fed cotton seed, or graze after being fed cotton seed, indicate that very little cotton seed is used as stockfeed. Where it has been used, the incidence of cotton volunteers was never observed to be problematic, and volunteer plants never reached flowering or maturity. Cotton seed had not been used for stockfeed in Northern Territory and northern Western Australia and these areas were therefore excluded from this survey.

313. Although some GM cotton plants may establish where stock is fed cotton seed or where stock grazes after being fed cotton seed, the expression of the *bar* gene construct is not expected to alter susceptibility to the abiotic and biotic factors that limit the establishment and persistence of cotton in these situations (eg plant competition, fire, herbivory and variable availability of water and nutrients). The chance of volunteer GM plants establishing as weeds by finding suitable ecological niches would be no greater than for the non-GM parent organism.

Dispersal via animals

314. In the field, seed cotton is present as large lint-covered bolls. Mammals, including rodents, generally avoid feeding on cotton plants and therefore are unlikely to carry bolls any greater distance from the cotton fields. The cotton bolls are also unattractive to avian species, so birds are unlikely to transport seeds of the GM cotton (OGTR 2002).

Dispersal via flooding or other extreme environmental conditions

315. Some seed from the GM cotton plants may be dispersed from areas where the cotton is grown or harvested or from areas used for stockfeed and storage of GM cotton seed during flooding or other extreme environmental conditions such as cyclones. Seed may also be

washed into drains, creeks, rivers and sinkholes close by. As a result, cotton volunteers may establish near areas used for stockfeed and storage, or along waterways close by.

316. Flooding does occasionally occur, especially in northern parts of Australia and GM cotton seed may be dispersed by flooding. Much of this dispersed seed is not expected to survive as the viability of cotton seed is affected by moisture (Hallowin 1975). Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in 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. This practice would also reduce the dispersal of seed.

317. Although habitats close to waterways may be favourable for cotton establishment, tolerance to glufosinate ammonium is not expected to provide a significant selective advantage, compared to non-GM cotton, in these environments. Other environmental factors such as plant competition and herbivory by insects and other animals are expected to limit the establishment and persistence of cotton plants in these areas.

Conclusions

318. Some GM cotton seeds may spread from the release sites, germinate and survive if conditions are suitable following the release. However, glufosinate ammonium is not used to control established cotton volunteers and other methods are available. The expression of the *bar* gene construct is not expected to alter susceptibility to the abiotic and biotic factors that limit the spread and persistence of cotton. The chance of volunteer GM plants establishing as weeds by finding suitable ecological niches would be no greater than for the non-GM parent organism or GM cotton lines currently approved for commercial release. Therefore, the likelihood of weediness as a result of event 1 is assessed as **highly unlikely**.

2.3 Event 2: Expression of the *bar* gene construct in non-GM *G. hirsutum* or *G. barbadense* cotton plants providing glufosinate ammonium tolerance

319. The risk of weediness as a result of transfer of the *bar* gene construct to non-GM *G. hirsutum* or *G. barbadense* plants would depend on the importance of the use of glufosinate ammonium in limiting the spread and persistence of cotton (consequence assessment), the chance of gene transfer occurring and the chance of progeny establishing as weeds following gene transfer (likelihood assessment). The level of risk is assessed in the context of the large scale of the proposed release, the distribution of non-GM cultivated or naturalised cotton plants growing in the vicinity of the crops and the conditions necessary for cross-pollination.

320. Transfer of the PAT to commercially approved GM cotton plants is considered separately in Event 3, Section 2.4 of this Chapter.

2.3.1 Consequence assessment

321. Transfer of the introduced *bar* gene construct to other sexually compatible plants such as non-GM *G. hirsutum* and *G. barbadense* could result in the expression of the PAT herbicide tolerance protein in these plants. The *bar* gene construct could confer some selective advantage as discussed in Section 2.2 of this Chapter. This could result in spread and persistence of these cotton plants in an environment where the use of glufosinate ammonium is the major constraint on cotton survival.

322. As discussed in Section 2.2.1 of this Chapter, glufosinate ammonium is not used to control established cotton volunteers.

323. There are no commercially cultivated non-GM *G. hirsutum* or *G. barbadense* in northern areas of Australia, only small populations of naturalised cottons. As mentioned in Chapter 1, only 10% of the 2005/2006 cotton crop was non GM cotton, all of which was grown in NSW, and southern and central QLD. Transfer of the *bar* gene construct to other non-GM cotton plants is not expected to alter the fact that cotton is not a serious weed in Australia due to a number of abiotic and biotic factors that limit the spread and persistence of cotton in Australia (as discussed in Section 2.1 of this Chapter). The expression of the *bar* gene construct is not expected to alter susceptibility to these factors.

324. In the presence of glufosinate ammonium, the small competitive advantage of the GM cotton is offset by abiotic and biotic factors (such as water availability, temperature, soil type and nutrients) that limit the spread and persistence of all cotton in Australia (see Section 2.1 of this Chapter).

325. Therefore, the consequences of expression of the *bar* gene construct in non-GM *G. hirsutum* or *G. barbadense* cotton plants providing glufosinate ammonium tolerance are assessed as **marginal**.

2.3.2 Likelihood assessment

326. Weediness resulting from an increase in the spread and persistence of other cotton plants is contingent on both of the following steps:

- transfer of the introduced *bar* gene construct to other cotton plants
- weediness of the recipient plants as a result of expression of the introduced gene.

327. Cotton is primarily self-pollinating with pollen that is large, sticky and heavy and not easily dispersed by wind (Jenkins 1992; OGTR 2002). 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 2002 and references therein). In Australia, honeybees and native bees are the most likely insects responsible for any cross-pollination in cotton (OGTR 2002). A study on the fate of pollen on *Helicoverpa armigera* as a possible vector for long distance pollen transport showed the quality and quantity of cotton pollen decreased rapidly in contact with *H. armigera* proboscis and therefore this is unlikely to promote wide pollen dispersal (Richards et al. 2005). For vertical gene transfer to occur, the GM cotton would need to be growing within pollination distance of other *G. hirsutum* or *G. barbadense* plants (including naturalised cotton populations).

328. The requirement for multiple applications of insecticides would further limit the amount of insect pollination in Liberty Link[®] Cotton plants compared to insecticidal GM cotton lines.

329. *G. barbadense* is the closest relative of *G. hirsutum* occurring in Australia (OGTR 2002). It is commercially grown on a small scale in Australia. 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.).

330. Transfer of the *bar* gene construct to naturalised cotton populations could also occur. The herbarium records for *G. hirsutum* and *G. barbadense* may not indicate current naturalised populations of these plants. A comparison of shires where cotton is cultivated and shires where feral cotton populations occur (in Queensland) indicates that feral populations occur in only three cotton production shires, and one shire adjacent to a cotton production

shire. Where significant geographic distances between naturalised populations and the cotton growing regions of NSW and Queensland exist, this will decrease the frequency of gene transfers.

331. The proposed release could result in the extensive cultivation of GM cotton plants in current and potential areas that are suitable for growing cotton, which would increase the occurrence of gene transfer events. However, cotton is primarily in-breeding and gene transfer to other cotton plants is expected to occur in close proximity and at low frequencies. Following transfer of the *bar* gene construct to any of these cotton plants, the likelihood of it causing weediness in these plants is expected to be the same as for the GM cotton plants (see event 1). Glufosinate ammonium is not widely used and GM cotton volunteers can be controlled by mechanical means or, if still at the seedling stage, alternative herbicides. Therefore, the likelihood of weediness as a result of event 2 is assessed as **highly unlikely**.

2.4 Event 3: Expression of the bar gene in combination with cp4 epsps gene and/or cry1Ac and cry2Ab genes providing dual herbicide tolerance and reducing lepidopteran herbivory.

332. The risk of weediness as a result of transfer of the *bar* gene construct to other commercially approved herbicide tolerant and/or insect resistant GM cotton plants would depend on the importance of the use of the relevant herbicides and lepidopteran herbivory in limiting the spread and persistence of cotton (consequence assessment), the chance of gene transfer occurring and the chance of progeny establishing as weeds following gene transfer (likelihood assessment). The level of risk is assessed against the baseline of the low weediness potential of the non-GM parent organism and in the context of the large scale of the proposed release, the distribution of other commercially approved GM cotton plants growing in the vicinity of the crops and the conditions necessary for cross-pollination.

333. The following GM cotton lines are currently approved for commercial release in Australia south of latitude 22° South:

- insect resistant INGARD[®] cotton (DIR 022/2002)
- glyphosate tolerant Roundup Ready[®] cotton (DIR 023/2002)
- glyphosate tolerant/insect resistant (Roundup Ready[®]/INGARD[®]) cotton (DIR 023/2002)
- insect resistant Bollgard II[®] cotton (DIR 012/2002)
- insect resistant/glyphosate tolerant (Bollgard II[®]/Roundup Ready[®]) cotton (DIR 012/2002).
- glyphosate tolerant Roundup Ready Flex[®] cotton (DIR 059/2005)
- glyphosate tolerant/insect resistant (Roundup Ready Flex[®]/Bollgard II[®]) cotton (DIR 059/2005)

334. In 2004, INGARD[®] cotton was withdrawn from the market in favour of Bollgard II[®] cotton.

335. Roundup Ready Flex[®] and Roundup Ready Flex[®]/ Bollgard II[®] cotton were only recently approved in February 2006 (under DIR 059/2005 licence) and are expected to replace Roundup Ready[®] and Bollgard II[®]/Roundup Ready[®], respectively in future seasons.

336. In the 2005-06 season, 90% of cotton grown in Australia was GM (Cotton Australia 2006) consisting of Bollgard II[®], Roundup Ready[®] and Bollgard II[®]/Roundup Ready[®] cotton lines.

337. There is currently no commercial cultivation of cotton (GM or non-GM) in northern Australia. However, field trials of insect resistant and insect resistant/herbicide tolerant cotton lines have been conducted under limited and controlled conditions since 1998.

338. The main introduced genes in the commercially approved 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/or the *cry1Ac* and *cry2Ab* genes in Bollgard II[®] which confers resistance to lepidopteran insect herbivory. Detailed consideration of adverse outcomes from these various introduced genes alone or in combination increasing the weediness of GM cotton plants in Australia is provided in the risk assessment document for DIR 059/2005, available at <<http://www.ogtr.gov.au>>. This risk assessment concluded that the risk estimates for the adverse outcome of weediness of the GM cotton lines as a result of the expression of the proteins in various combinations was negligible in the cotton growing regions of Australia south of latitude 22° South.

2.4.1 Consequence assessment

339. The limited effectiveness of glufosinate ammonium in controlling cotton is discussed in event 1. The limited effectiveness of glyphosate in controlling cotton beyond the seedling stage is discussed in the risk assessment prepared for DIR 059/2005. The risk assessment prepared for DIR 012/2002 concluded that lepidopteran herbivory is not an important limiting factor in determining cotton distribution in southern Australia compared to other environmental factors. Therefore, in the presence of glufosinate ammonium, and glyphosate and/or lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by abiotic and biotic factors (such as water availability, temperature, soil type and nutrients) that limit the spread and persistence of all cotton in Australia (see Section 2.1 of this Chapter).

340. The herbicide tolerance and insecticidal genes operate through independent, unrelated biochemical mechanisms. There is no evidence to suggest that the *bar* gene will interact with the *cry* genes or the *cp4 epsps* gene, their proteins or their metabolic pathways, resulting in unintended effects and no reason to expect that this is likely to occur. Therefore, cotton volunteers containing the *bar* gene with any or all of the introduced genes present in commercially approved GM cotton lines are expected to be able to be controlled by other herbicides or by cultivation, similar to the parental GM cotton lines.

341. Therefore, the consequences of the expression of the *bar*, *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in various combinations increasing the spread and persistence of the GM cotton plants proposed for release through tolerance to glufosinate ammonium as well as glyphosate and/or reduced lepidopteran herbivory are assessed as **minor**.

2.4.2 Likelihood assessment

342. The proposed release would result in the extensive cultivation of GM cotton plants in current and potential areas of Australia that are suitable for growing cotton. Bayer anticipates a phased introduction of the GM cotton over three years in the current cotton growing areas of NSW, and southern and central QLD. The area is expected to increase in subsequent years and may include plantings in other areas that are suitable for growing cotton.

343. Currently, stacked GM cottons are not likely to occur in northern areas of Australia as cotton (both GM and non-GM) is not commercially cultivated in these areas. However, it should be noted that the OGTR is currently assessing an application for the commercial release of five types of GM cotton lines in northern Australia: Bollgard II[®], Roundup Ready[®], Bollgard II[®]/Roundup Ready[®], Roundup Ready Flex[®] and Roundup Ready Flex[®]/Bollgard II[®]. These assessments will consider the implication of stacking with Liberty Link[®] Cotton.

344. Extensive cultivation of Liberty Link[®] Cotton would increase the occurrence of gene transfer events. Some GM cotton seeds may spread from the release sites, germinate and persist in the environment following the release. However, cotton is primarily in-breeding and the main mechanism for gene transfer, via insect mediated pollen flow, would only be expected to occur in close proximity and at low frequencies. Such transfer would be further reduced by the requirement to apply insecticides to the GM cotton.

345. As mentioned earlier, cotton is not a serious weed in Australia because of a number of abiotic and biotic factors. In southern Australia, where GM cotton lines are currently grown on a commercial scale and where stacking with Liberty Link[®] Cotton may occur, frost and soil moisture are particularly significant in relation to limiting seedling germination and plant growth. The expression of the *bar*, *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination is not expected to alter susceptibility to the environmental conditions that limit the spread and persistence of cotton in Australia.

346. Glufosinate ammonium and glyphosate are not used to control established cotton volunteers as other methods are more commonly used, such as mechanical means or, if still at the seedling stage, alternative herbicides.

347. GM cotton plants with either the glyphosate tolerance trait, insect resistance trait, or a combination of the two traits have been grown in NSW, and southern and central QLD since 2002 and have not become problematic weeds. These commercially approved GM cotton lines are subject to transport conditions in northern Australia and have only been used as stockfeed in northern QLD and planted as field trials under limited and controlled conditions in northern areas of Australia.

348. The chance of volunteer GM plants establishing as weeds by finding suitable ecological niches would be no greater than for Liberty Link[®] Cotton, other commercial GM cotton or non-GM cotton. Therefore, the likelihood of weediness as a result of event 3 is assessed as **highly unlikely**.

Section 3 Risk estimates

349. 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).

350. The risk estimates for the adverse outcome of weediness of the GM cotton as a result of the expression of the *bar* gene construct have been made relative to the baselines of the weediness of non-GM cotton growing in Australia, the current widespread cultivation and use of other GM cotton lines in southern Australia (Roundup Ready[®], Bollgard II[®], and Bollgard II[®]/Roundup Ready[®], and their expected replacement by Roundup Ready Flex[®] and Roundup Ready Flex[®]/Bollgard II[®]) and the absence of commercial cotton cultivation (both GM and non-GM) in northern Australia.

351. The consequences of increased spread and persistence of cotton resulting from the presence of the *bar* gene in the GM cotton (event 1) have been assessed as **marginal**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore the risk estimate is **negligible**.

352. The consequences of increased spread and persistence resulting from the presence of the *bar* gene construct in other *G. hirsutum* and *G. barbadense* cotton plants, as a result of gene transfer (event 2), have been assessed as **marginal**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore the risk estimate is **negligible**.

353. The consequences of increased spread and persistence of cotton resulting from the presence of the *bar* gene in combination with the *cp4 epsps* gene and/or *cry1Ac* and *cry2Ab*

genes (event 3), as a result of gene transfer, have been assessed as **minor**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore the risk estimate is **negligible**.

354. The risks of three events that may lead to weediness are estimated to be negligible and therefore, no risk treatment measures for weediness are proposed.

Table 5.1 Summary of risk assessment

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p>Event 1 Expression of the <i>bar</i> gene increasing spread and persistence of the GM cotton plants through tolerance to glufosinate ammonium</p>	<p>Marginal</p> <ul style="list-style-type: none"> • Glufosinate ammonium is not effective for the control of established cotton volunteers. • In the presence of glufosinate ammonium, the small competitive advantage of the GM cotton is offset by abiotic and biotic factors (such as water availability, temperature, soil type and nutrients) that limit the spread and persistence of all cotton in Australia. 	<p>Highly unlikely</p> <ul style="list-style-type: none"> • Glufosinate ammonium is not a widely used herbicide for the control of cotton volunteers as other methods are more commonly used, such as 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 (eg transportation, use as stockfeed, via animals or flooding) finding suitable ecological niches and establishing as weeds would be no greater than for non-GM and commercially approved GM cotton lines. 	<p>Negligible</p>	<p>No</p>
<p>Event 2 Expression of the <i>bar</i> gene in other <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants (not including commercially released GM cotton lines) providing glufosinate ammonium tolerance</p>	<p>Marginal</p> <ul style="list-style-type: none"> • Glufosinate ammonium is not effective for the control of established cotton volunteers. • In the presence of glufosinate ammonium, the small competitive advantage of the GM cotton is offset by abiotic and biotic factors (such as water availability, temperature, soil type and nutrients) that limit the spread and persistence of all cotton in Australia. 	<p>Highly unlikely</p> <ul style="list-style-type: none"> • Cotton is primarily self-pollinating and gene transfer to other cotton plants is only expected to occur in close proximity and at low frequencies. • The requirement to apply insecticides to herbicide tolerant GM cotton will further reduce the chance of gene transfer via insects. • Glufosinate ammonium is not a widely used herbicide for the control of cotton volunteers as other methods are more commonly used, such as mechanical means or, if still at the seedling stage, alternative herbicides. 	<p>Negligible</p>	<p>No</p>

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p>Event 3 Expression of the <i>bar</i> gene in combination with <i>cp4 epsps gene</i> and/or <i>cry1Ac</i> and <i>cry2Ab</i> genes providing dual herbicide tolerance and reducing lepidopteran herbivory</p>	<p>Minor</p> <ul style="list-style-type: none"> • Glufosinate ammonium and glyphosate is not effective for the control of established cotton volunteers. • In the presence of glufosinate ammonium, and glyphosate and/or lepidopteran herbivory, the small competitive advantage of any stacked GM cotton is offset by abiotic and biotic factors (such as water availability, temperature, soil type and nutrients) that limit the spread and persistence of all cotton in Australia. • The <i>bar</i> gene operates independently of the herbicide tolerant and insecticidal genes present in other GM cotton lines and there is no evidence of any interactions. 	<p>Highly unlikely</p> <ul style="list-style-type: none"> • The current commercially approved GM cotton lines are only authorised for unrestricted release in southern areas of Australia. Stacking is not expected to occur in northern areas of Australia as field trials with GM cotton in northern Australia are required to be conducted under limited and controlled conditions. • Cotton is primarily self-pollinating and gene transfer to other cotton plants is expected to occur in close proximity and at low frequencies. • The requirement to apply insecticides to herbicide tolerant GM cotton will further reduce the chance of gene transfer via insects. • Glufosinate ammonium and glyphosate are not used to control established cotton volunteers as other methods are more commonly used, such as mechanical means or, if still at the seedling stage, alternative herbicides. 	<p>Negligible</p>	<p>No</p>

Chapter 4 Risk management

355. This Chapter evaluates the risks assessed in Chapter 3 to determine whether or not specific treatments are required to mitigate harm that may arise during the proposed release. Other risk management considerations required under the Act are also addressed in this Chapter.

Section 1 Background

356. 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.

357. All licences are required to be subject to three conditions prescribed in the Act. In summary, section 63 requires that each licence holder inform relevant people of their obligations under the licence, section 64 requires that licence holders provide access to premises by authorised persons, and section 65 requires that in certain circumstances the licence holder is to provide information to the Regulator. These provisions are reproduced in full in each licence.

358. It is a further requirement that the licence be subject to any conditions imposed by the Regulator. Examples of the matters to which conditions 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 Other Australian regulators

359. Australia's gene technology regulatory system operates as part of an integrated legislative framework (OGTR 2005). Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, TGA, NICNAS, NHMRC and AQIS. Dealings conducted under any licence issued by the Regulator may also be subject to regulation by one or more of these agencies.

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

361. FSANZ is responsible for human food safety assessment and has recently approved food (containing oil and linters) derived from Liberty Link[®] Cotton (FSANZ 2005a).

362. The use of Liberty[®] 150 Herbicide on the GM cotton proposed for release is subject to regulation by the APVMA. Bayer has a research permit for use of glufosinate ammonium in current cotton trial involving this GMO, and the APVMA is currently assessing an application from Bayer to register Liberty[®] 150 Herbicide for the control of various weeds in Liberty Link[®] Cotton. The APVMA generally imposes conditions on the use pattern of herbicides to, for example, limit herbicide resistance development and comply with residue limits (refer to 2.10.2 in Chapter 2 for detailed discussion).

363. The Regulator has liaised closely with FSANZ and the APVMA during the assessment of applications pertaining to this commercial release of GM cotton.

Section 3 Risk treatment measures for identified risks

364. The detailed risk assessment of Events 1–3 contained in Chapter 3 concluded that the risk estimates are **negligible** for all three events. These events were considered in the context of the large scale of the proposed release and the receiving environment for this proposed release, including other commercially approved GM cotton lines.

365. The *Risk Analysis Framework* (OGTR 2005), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

Section 4 General risk management

4.1 Other risk management considerations

366. All DIR licences issued by the Regulator contain a number of general conditions. These include, for example:

- applicant suitability
- contingency and compliance plans
- auditing and monitoring
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment.

4.1.1 Applicant suitability

367. 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.

368. Before making the decision to issue a licence for this application (DIR 062/2005), the Regulator determined that Bayer CropScience Pty Ltd is suitable to hold a licence.

369. Conditions in the licence 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.

370. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.1.2 Compliance plan

371. The licence requires Bayer to submit a plan detailing how it intended to ensure compliance with the licence conditions and document that compliance. This plan is required before the planting of the GM cotton occurs.

372. Bayer is also required to provide a method to the Regulator for the reliable detection of the presence of the GMO and the introduced genetic material in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

4.1.3 Auditing and Monitoring

373. 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 observe a condition of the licence, 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. This requirement applies whether or not the condition is written into a licence, but as a matter of practice, and for the removal of doubt, the Regulator inserts the condition into all licences.

4.1.4 Reporting structures

374. The licence obliges the licence holder 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 release
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the release.

375. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence.

Section 5 Conclusions of the RARMP

376. The risk assessment concludes that this commercial release of herbicide tolerant Liberty Link[®] GM cotton poses negligible risks to the health and safety of people and the environment as a result of gene technology.

377. The risk management plan concludes that the negligible risks do not require specific risk treatment measures. Licence conditions that have been imposed relate to ongoing licence holder suitability; auditing and monitoring provisions; reporting requirements, including a compliance plan, annual report and other relevant information; and a suitable detection methodology.

Chapter 5 Licence conditions

Section 1 Interpretations and Definitions

Dealings permitted by this licence may be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

In this licence:

- (a) unless defined otherwise in this licence, words and phrases used in this licence have the same meanings as they do in the Act and the *Gene Technology Regulations 2001*;
- (b) words importing a gender include any other gender;
- (c) words in the singular include the plural and words in the plural include the singular;
- (d) words importing persons include a partnership and a body whether corporate or otherwise;
- (e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;
- (f) where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word or phrase has a corresponding meaning.

In this licence:

‘Act’ means the *Gene Technology Act 2000* (Cth) and equivalent provisions in corresponding State law.

‘Annual Report’ means a written report provided to the Regulator within 90 days of each anniversary of the date of issue of this licence containing all the information required by this licence to be provided in the Annual Report.

‘Cotton’ means plants of the species *Gossypium hirsutum* L.

‘Deal with’ in relation to a GMO means one or more of the following:

- (a) conduct experiments with the GMOs;
- (b) make, develop, produce or manufacture the GMOs;
- (c) breed the GMOs;
- (d) propagate the GMOs;
- (e) use the GMOs in the course of manufacture of a thing that is not the GMOs;
- (f) grow, raise or culture the GMOs;
- (g) import the GMOs;

and includes the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing mentioned in any of paragraphs (a) to (g).

‘GM’ means genetically modified.

‘GMOs’ means the genetically modified organisms listed in Attachment B and authorised for release by this licence.

‘OGTR’ means the Office of the Gene Technology Regulator.

‘Personal information’ means information or an opinion (including forming part of a database), whether true or not, and whether recorded in a material form or not, about an individual whose identity is apparent, or can reasonably be ascertained, from the information or opinion.

‘Location’ means an area of land where the GMOs are planted and grown for the purposes of a licence.

‘Regulator’ means the Gene Technology Regulator.

Section 2 Licence Conditions

Duration of licence

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

Holder of licence

2. The holder of this licence (‘the licence holder’) is Bayer CropScience Pty Ltd.

Project Supervisor

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

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

Persons covered by this GMO licence

5. Subject to condition 6, any person, including the licence holder, may conduct any Dealing with the GMOs.

6. Where the GMOs authorised by this licence are planted or in any other way Dealt with as part of a subsequent licence, or subsequent licence variation, authorising a dealing under the Act, then, for purposes of the subsequent licence, or subsequent licence variation,

- (a) only the persons covered by the subsequent licence or licence variation are permitted to grow or otherwise deal with the GMOs, and,
- (b) the conditions of the subsequent licence or the licence containing the licence variation, and not the conditions of this licence, will apply to the dealing with the GMOs.

Example: If a subsequent licence contemplates the planting of these GMOs in a Location containing another GMO authorised by that later licence, the conditions of the subsequent licence and not the conditions of this licence will apply to the GMOs for purposes of the dealings conducted under that licence.

Informing people of their obligations

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

- (a) the particular condition (including any variations of it);
- (b) the cancellation or suspension of the licence;
- (c) the surrender of the licence.

8. The licence holder must notify the project supervisor and all persons covered by a licence to whom a condition of this licence applies that Personal Information collected by the licence holder which is relevant to the administration and/or enforcement of the licence may be released to the Regulator.

Licence holder to notify of circumstances that might affect suitability

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

- (a) any relevant conviction of the licence holder occurring after the commencement of this licence;
- (b) any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment;
- (c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of this licence to meet the conditions in it.

Licence holder must provide information on matters related to suitability

10. The licence holder must provide information related to the licence holder's ongoing suitability to hold a licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

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

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

Remaining an accredited organisation

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

Section 3 Growing the GMOs

GMOs covered by this licence

13. The GMOs covered by this licence ('the GMOs') are identified and described at **Attachment B**.

Section 4 Reporting and Documentation Requirements

Additional information to be given to the Regulator

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

- (a) becomes aware of additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or
- (b) becomes aware of any contraventions of the licence by a person covered by the licence; or
- (c) becomes aware of any unintended effects of the dealings authorised by the licence.

Note: The Act requires, for the purposes of the above condition that:

- (d) *the licence holder will be taken to have become aware of additional information of a kind mentioned in subsection (1) if he or she was reckless as to whether such information existed; and*
- (e) *the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in subsection (1) if he or she was*

reckless as to whether such contraventions had occurred, or such unintended effects existed.

15. The licence holder must provide the information required by paragraphs (a) (b) and (c) of condition 14 to the Regulator as soon as practically and reasonably possible and must also include the information in the Annual Report.

Compliance management plan

16. Prior to growing the GMOs, a written Compliance Management Plan must be provided to the Regulator. The Compliance Management Plan must describe in detail how the licence holder intends to ensure compliance with each of these conditions and document that compliance.

Annual Report

17. The licence holder must provide an Annual Report to the Regulator.

Testing Methodology

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

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Appendix A Definitions of risk analysis terms

(* terms defined as in Australia New Zealand Risk Management Standard AS/NZS 4360:2004)

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

Uncertainty

imperfect ability to assign a character state to a thing or process; a form or source of doubt

Appendix B Summary of submissions received from prescribed experts, agencies and authorities⁶ on the application

All issues raised relating to risks to human health and safety and the environment were considered in the context of the currently available scientific evidence that was used in the preparation of the consultation RARMP.

Issues relating to the Risk Assessment and where they have been considered:

- Compositional characterisation requirements (see Chapter 2)
- Human health effects (see Chapter 2)
- Development of herbicide resistant weeds (see Chapter 2)
- Risks from expansion into new areas (see Chapters 1 and 2)
- Toxicity (see Chapters 2)
- Risk of weediness (see Chapters 2 and 3)
- Risks arising from gene flow to other GM or non GM cotton plants including related species (see Chapters 2 and 3)
- Potential for gene stacking with other GM cotton crops (see Chapters 2 and 3)
- Environmental effects (see Chapters 2 and 3)
- Potential for unintended genetic effects (see Chapter 2)

Issues relating to the Risk Management Plan:

- Changed herbicide use patterns as a result of the release (the Australian Pesticides and Veterinary Medicines Authority considers this issue—refer Chapter 2 and 4)

Issues that were outside the scope of assessments conducted under the *Gene Technology Act 2000*:

- Market viability, segregation and unintended presence concerns
- Labelling concerns
- General social, economic, ethical and political concerns.

⁶ Gene Technology Technical Advisory Committee, State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and local councils where the release may occur. For this application all councils in Australia were consulted.

Appendix C Summary of public submissions received on the application

All issues raised relating to risks to human health and safety and the environment were considered in the context of currently available scientific evidence that was used in the preparation of the consultation RARMP.

One submission was received that raised the following issues:

- Risks to human health and safety and the environment (Chapters 2 and 3)
- Use of GM products in human food and animal feed (see Chapter 2)
- Gene transfer to microorganisms (Chapter 2)
- Stability of the genetic modification (Chapters 1 and 2).

Appendix D Summary of submissions received from local councils on the consultation RARMP

None of the experts, agencies and authorities prescribed for consultation under the *Gene Technology Act 2000*, other than local councils, raised any issues on the RARMP relating to human health and safety and the environment that required further consideration.

Thirteen of the 675 local councils consulted provided submissions that raised a number of concerns, as well as some matters that are outside the scope of assessments required by the Act. A summary of the submissions and how they were considered is provided below.

All issues relating to risks to human health and safety and the environment were 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.

ISSUE RAISED	Consideration in RARMP	SUMMARY OF CONSIDERATION
Risks from expansion into new areas	Chapter 2	The GM cotton has the same water, soil type and climatic requirements as non-GM and other commercially approved GM cotton. Therefore, if expansion occurred it would only be into regions suitable for growing cotton. The impact of expansion would be comparable to the introduction of any crop species into a new area because changes to agricultural systems from the introduction of new crops are not unique to GMOs.
Human health effects	Chapter 2	The potential toxicity and allergenicity of the introduced protein has been assessed and no risks were identified. Oil and linters derived from the GM cotton line have been approved by FSANZ for use in human food.
Toxicity and allergenicity to other organisms	Chapters 2	The toxicity and allergenicity of the PAT protein, encoded by the introduced gene, for other organisms is discussed in Chapter 2. No risks were identified.
Risk of increased spread and persistence (weediness)	Chapters 2 & 3	Three events were identified in Chapter 2 that might result in increased weediness. The three events were assessed in detail in Chapter 3. The risks were estimated as negligible for all three events. Herbicides are not effective for the control of established cotton volunteers and mechanical options are most successful. The GM cotton is susceptible to the same biotic and abiotic factors that limit the persistence of other GM and non-GM cottons.
Risks arising from gene flow to other GM and non-GM cotton plants and related species	Chapters 2 & 3	The consequences and likelihood assessments of the transfer of the <i>bar</i> gene to conventional cotton, other GM cottons and related cotton species resulting in weediness were detailed in Chapter 2 & 3 (Events 2 & 3). The risks were estimated as negligible.
Unintended genetic effects	Chapter 2	No unintended effects have been observed in field trials in Australia or when grown overseas (Liberty Link [®] Cotton is approved for commercial release and food use in the USA, Japan and Korea).
Market viability, segregation and unintended presence concerns	—	This issue is outside the scope of assessments conducted under the <i>Gene Technology Act 2000</i> . State and Territory governments have legislation relating to marketing issues.
Benefits of the GM cotton	—	Benefits of GM technology are outside the scope of the assessment.

ISSUE RAISED	Consideration in RAMP	SUMMARY OF CONSIDERATION
General social, economic, ethical and political concerns.	—	These issues are outside the scope of assessments conducted under the <i>Gene Technology Act 2000</i> . The Gene Technology Ethics Committee is in place to advise the Regulator and to identify and explore ethical issues relating to gene technology. However, no specific ethical concerns were identified.

Appendix E Summary of public submissions received on the consultation RARMP

The Regulator received 11 submissions from the public on the consultation RARMP. All were analysed in detail. Five were from organisations with direct experience of cotton growing that supported the application. Six were from interest groups and individuals that raised a range of concerns about the use of the GM cotton. These included issues regarding the use of agricultural chemicals and the development of resistance that fall within the regulatory responsibilities of the APVMA.

All issues raised relating to risks to human health and safety and the environment were 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. These are summarized in order of receipt of submission, in the table below.

Abbreviations used:

APVMA: Australian Pesticides and Veterinary Medicines Authority; **Ch:** Chapter; **FSANZ:** Food Standards Australia New Zealand; **LC:** licence; **OSA:** Outside the scope of the assessment

Issues raised: **AG:** agricultural practices; **E:** ethical concerns; **EN:** environmental risks; **G:** gene transfer; **GS:** genetic stability; **H:** Health concerns; **HR:** herbicide resistance; **HU:** herbicide use; **IR:** insecticide resistance; **M:** market and trade concerns; **RARMP:** risk assessment and risk management plan; **Res:** further research; **S:** gene stacking; **T:** toxicity; **W:** weediness.

^a **Submission from:** **A:** agricultural/industry organisation; **IG:** interest group; **I:** Individual

Sub. No.	Type ^a	Summary of issues raised	Issue	Consideration of issue
1	IG	Protests against GM cotton and GMO crops in general and questions their ethics and safety.	E, EN, H	The <i>Gene Technology Act 2000</i> requires the Regulator to identify and manage risks to human health and safety and the environment posed by the release of GMOs. For this application these were determined to be negligible. The Gene Technology Ethics Committee is in place to advise the Regulator and to identify and explore any ethical issues relating to gene technology. However, no specific ethical concerns were identified.
		During the period of chemical farming, resistance to herbicides and pesticides have developed and more spraying is required. The introduction of GM resistant crops may further create a more serious problem.	HR, IR	The Liberty Link cotton proposed for commercial release is only tolerant to herbicides containing glufosinate ammonium as the active ingredient. The GM cotton does not contain genes that confer insect resistance. The management of herbicide resistance development comes under the scope of assessments conducted by the APVMA.
		Monoculture farming is not sustainable and the use of pesticides is seen as a quick fix	AG	Agricultural practices are outside the scope of assessment under the <i>Gene Technology Act 2000</i> .

	<p>Genes from GMOs may transfer to related plants and give an evolutionary advantage over other species, creating superweeds. For example, a superweed was created in France when sugar-beets crossed with a wild relative.</p>	G	<p>Glufosinate ammonium tolerant plants remain susceptible to other herbicides and other forms of control. Other cottons are the only related species to which gene flow can occur in Australia and this would only occur at a very low frequency. The transfer of the herbicide resistance gene from Liberty Link Cotton to other cotton plants will only confer an advantage where glufosinate ammonium limits the persistence of the plants. The transfer of this gene is not expected to alter susceptibility to the abiotic and biotic factors that limit the spread and persistence of all cottons (eg water, availability, temperature, soil type and nutrients).</p>
	<p>As GM crops are generally only tolerant to a single herbicide, dependence on a single herbicide will make it easier for the transfer of a herbicide-resistant gene from GM plants to weeds. The question is not so much about if resistance will occur, as when it will occur. Resistance has already happened in the US, Malaysia and Australia and cases will increase.</p>	HR	<p>OSA. The development of herbicide resistance is considered by the APVMA as it comes under their scope of assessment according to the Ag. Vet. Chem. Code. Integrated weed management practices adopted by the cotton industry have prevented the development of herbicide resistance in cotton crops in Australia. Note: there are no weed species that are sexually compatible with cotton.</p>
	<p>Organic farmers are particularly worried about GM crops affecting insect resistance to some of their best pesticides. For example Bt sprays are biodegradable and non-toxic to humans and their potency will diminish faster because of GM crops with Bt genes. Farmers do not wish to change Bt sprays to keep up with ever increasing speed of insect resistance.</p>	IR	<p>The Liberty Link cotton proposed for commercial release is only tolerant to herbicides containing glufosinate ammonium as the active ingredient. The GM cotton does not contain genes that confer insect resistance.</p>
	<p>More herbicide can be sprayed on the herbicide resistance GM crops. Although the biotech companies say less will be used, there is nothing to stop farmers using more as insurance, resulting in greater residues in food and more runoff into the environment.</p>	HU	<p>OSA. APVMA is responsible for the regulation of agricultural and chemical products. FSANZ is responsible for regulating residues in food.</p>
	<p>Farmers will be forced to use the manufacturers herbicide on GM herbicide tolerant crops instead of cheaper or safer herbicides produced by competitors.</p>	HU, EC	<p>OSA. Herbicide use and economic issues are outside the scope of assessment conducted under the Act.</p>
	<p>GM crops can survive up to 20 times the level of herbicide application used in normal crops. Overuse of herbicide may mean herbicide resistant weeds will cross into weeds.</p>	G	<p>Use of a herbicide does not impact on the likelihood of gene transfer.</p>

		Gene technology is unpredictable and cannot be sufficiently controlled to guarantee safety. Commercial development of GMOs should be delayed for ten years to allow for debate and for better testing. More small scale scientific studies should be done on their effects on humans and the environment.	Res	Introduced genes have been shown to be stably integrated over several generations and in a variety of genetic backgrounds when conventionally crossed with various cotton cultivars. Risks to human health and safety and the environment were determined to be negligible. There have been a number of field trials in Australia as well as commercial releases internationally with GM plants containing the <i>bar</i> gene and there have been no reports of adverse outcomes of a result of these plants.
2	I	Opposes the introduction of GM cotton into Australia		Noted.
		Australia is in the unique position to avoid cross pollination because it is an island continent.	G	Cotton is primarily self-pollinated and cross-pollination occurs at low frequencies within a few metres of the cotton plant.
		Australia is losing its advantage over other countries by allowing GM crops and GM free crops will be much sought after.	M	OSA. Marketing issues relating to GM crops are the responsibility of the States and Territories.
3	I	Objects to the release because:		
		➤ Lack of published evidence that soil ecology is not disrupted	Res, T	The introduced gene is derived from a common soil bacterium. The same PAT protein or similar proteins are naturally present in soil and all published data shows that the PAT protein has extremely low toxicity for all organisms tested. Therefore the GM cotton is not expected to disrupt soil ecology (Refer to Ch 2 of RARMP).
		➤ Lack of published evidence that human or animal health is unaffected by consumption of GE cotton by-products	Res, T	The PAT protein is of extremely low toxicity to humans and animals. Oil and linters do not contain detectable protein or genetic material.
		➤ Probability of genetic instability when released into the environment.	GS	The genetic modification has been shown to be stable over several generations and in a variety of genetic backgrounds when conventionally crossed with various cotton cultivars.
		➤ Overproduction of cotton is delirious to environment		OSA. (Agricultural practices)
		➤ Monoculture of genetically identical life forms is unnatural (the closer to nature our techniques are, the more benign and sustainable they are)		OSA. (Agricultural practices)
		➤ The release is a major alteration to the environment and has not been democratically decided.		OSA. (Agricultural practices). Assessment of this application has been done as required under the <i>Gene Technology Act 2000</i> .
4	A	The company relies on an intact nature for their bees to give the best honey available without spoilage from GMOs.	T	The PAT protein encoded by the introduced gene is of extremely low toxicity to humans and animals. Insect populations (including bees) are not adversely affected by crops expressing the PAT protein (discussed in Ch 2 of RARMP and in the RARMPS for DIRs 010/2001 and 021/2002). The pollen content of honey is generally around 0.1%. Therefore, the average PAT protein content of honey will be less than 0.0000019%.

		The company markets the produce as 'Free from GMO' and any permission to grow GM crops would jeopardise the product on the world market.	M	OSA (marketing issues). 90% of cotton grown in Australia is currently GM.
5	A	Supports the application by Bayer for the commercial release of Liberty Link [®] Cotton.		Noted.
		The basis of this support is on the understanding that the draft RARMP has determined that the technology is safe to humans and the environment.	H, EN	Noted.
		In addition, the GM cotton offers options in cost effective and sustainable cotton production.		OSA (Benefits of GMO).
		In terms of agronomics, Liberty Link [®] Cotton promotes sustainable cotton production as the new mode of action, assists weed resistance management and provides better performance on hard-to kill weeds like peach vine, which are less susceptible to current in-crop herbicide regimes.		OSA (Benefits of GMO, herbicide use).
6	A	Supports the application		Noted.
		The adoption of the GM cotton with insect resistance and herbicide tolerance traits has resulted in a substantial reduction in use of traditional chemistry. The industry has been proven to be capable of managing GM crops according to the requirements determined by all regulators (eg Resistance Management Audits have been conducted effectively).	HU	Noted.
		Liberty Link [®] Cotton offers growers the opportunity to a herbicide with an alternative mode of action to traditional product, assisting with the management and prevention of weed resistance.	HR	Noted.
		Herbicides (including glyphosate) and other management practices are available to the cotton grower to control cotton plants tolerant to glufosinate ammonium.	W	Noted. Considered in Ch 3, event 1.
		The current management practices, in particular cultivation, minimize the likelihood of the spread or development of weed resistance.	HR	Noted.
		Glufosinate ammonium is not widely used to control rogue cotton plants and therefore no selective advantage of the GM cotton.	HU,W	Noted. Considered in Ch 3, event 1.
		Liberty Link [®] Cotton allows rotation with existing herbicide tolerant cotton allowing better management of re-growth of cotton plants	HU, W	Noted.
		There is no evidence available to support any claim that this technology or the herbicide partner product will represent any significant risk to the health and safety of people or the environment.	H, EN	Noted. Considered in Ch 2 and 3.

7	IG	<p>Fundamentally opposed to the establishment or expansion of broad scale irrigated agriculture in northern Australia as it is unsustainable and causes significant and unacceptable impacts. Opposed to the introduction of genetically manipulated cotton and other crops through trials or commercial release in northern Australia.</p>	AG	Noted. (OSA, Agricultural practices)
		<p>We request that all raw data from these trials be publicly available and accessible to people commenting on the application.</p>	Res	<p>The OGTR has invited people widely to comment on the RARMP for DIR062 via newspaper advertisements and on our website which indicate how copies of the RARMP and other documents including the application can be obtained. Unless a declaration of commercial confidential information (CCI) is made, all information submitted by an applicant is available to the public.</p>
		<p>There has been only one trial of GM cotton with the PAT gene in northern Australia (DIR044) which was limited in size and for only one season, which would limit the reliability of the information obtained.</p>	Res	<p>The limited and controlled release under licence DIR 044/2003 was one of several DIRs referred to in the RARMP to indicate previous trials of the same or similar GM cotton in Australia and/or northern Australia have occurred, and to point out that no adverse effects resulting from these trials were reported to the OGTR. The results from DIR 044 were not used to substantiate any other claims in the RARMP for DIR062. The only difference between Liberty Link[®] Cotton and non-GM cotton is herbicide tolerance and therefore data from southern Australia is applicable to the north.</p>
		<p>DIR056/2004 is the only application by Bayer with all other trials in relation to PAT gene LLcotton25 (Liberty Link[®] Cotton) undertaken by CSIRO. It is difficult to see how any data resulting from DIR056/2004 grown during the 2005/06 season could be incorporated into this application which was signed and dated by the applicant on 25 November 2005. The trial would have only been in the first month of the growing season, having been planted in Sept/Oct.</p>	Res	<p>Both CSIRO and Bayer have undertaken field trials involving the controlled and limited release of Liberty Link[®] Cotton (containing the <i>bar</i> gene). References to DIR056 in the DIR062 RARMP were included to indicate previous releases of the same GM cotton, and that at the time of finalising the RARMP, one season for DIR 056/2004 had been completed to inform the Regulator of any adverse or unintended outcomes resulting from this release. The results of this trial were not used to substantiate any other claims in the RARMP for DIR062.</p>

	<p>The expression of the PAT gene could be affected by higher temperatures and humidity in northern Australia. There could be other interactions with metabolic processes affecting toxicity, efficacy of the gene and/or other agronomic aspects. As there has been only one small trial of Liberty Link[®] Cotton in northern Australia there are unknown risks and a range of critical knowledge gaps relating to the risk of growing Liberty Link[®] Cotton in northern Australia.</p>	<p>HU, T, UE</p>	<p>The PAT protein encoded by the introduced <i>bar</i> gene has extremely low toxicity and therefore even if expression levels were higher, this would not alter the toxicity of the PAT protein. The current cotton regions in southern Australia all have high temperatures during the growing season and previous trials of Liberty Link[®] Cotton and other similar GM cottons expressing the PAT protein have shown no unintended effects. Thus the potential for adverse outcomes due to higher temperatures and humidity in northern Australia appears to be unlikely.</p> <p>The introduced <i>bar</i> gene has been reported as stable through several generations. Expression levels of the PAT protein are discussed in Ch 1 of the RARMP.</p>
	<p>There have been links made between phosphinothricin and neurological respiratory, gastrointestinal and haematological toxicities and birth defects in mammals, including humans which were not mentioned in the RARMP (Hooper, 2002).</p>	<p>HU, T, UE</p>	<p>OSA. Herbicide use is regulated by the APVMA.</p>
	<p>Research is needed to determine the toxicity and other impacts on ectothermic and poikilothermic insectivorous species such as goannas, frogs and other reptiles and amphibians. There have been no dermal and inhalation toxicity studies on the PAT protein on the basis of its low acute oral toxicity, however research should be undertaken to demonstrate the safety of Liberty Link[®] Cotton</p>	<p>Res, T, UE</p>	<p>The same PAT protein or similar proteins are widespread in the environment and all published data shows that it has extremely low toxicity for all organisms tested. The PAT protein is unlikely to have adverse effect on reptiles and amphibians because they are not known to consume cotton. There have been no reports of adverse impacts due to unexpected interactions from the release of GM cotton or other GM crops expressing the PAT protein from trials conducted around the world.</p>
	<p>It is entirely likely that many sites would be cleared to establish cultivation thus increasing opportunities for interaction with native species, increased chances of seed dispersal through irrigation and/or high rainfall events. The Cotton CRC acknowledges that long term data on environmental conditions, potential pests, hydrological relationships between surface and ground water is critical to informing the roll out of cotton in new areas, ie northern Australia. Therefore, the Regulator needs to consider the key characteristics of potential release sites in northern Australia.</p>	<p>AG, G, W</p>	<p>Issues relating to agricultural expansion are outside the scope of assessment conducted under the <i>Gene Technology Act 2000</i>. Consideration of potential sites would be the same for Liberty Link[®] Cotton and non-GM cotton.</p> <p>There may be increased dispersal of seed due to irrigation and/or high rainfall events, but dispersal of the GM cotton seed would be no greater than for non-GM cotton and the likelihood of spread and persistence of the GM cotton due to expression of the <i>bar</i> gene was assessed as highly unlikely (see event 1, Ch 3 of RARMP).</p>
	<p>There is no data or analysis ascertaining the nature of the risk associated with the release of Liberty Link[®] Cotton on soil structure, microorganisms and function in potential northern Australian commercial cotton sites. The broad assumption that there is universal exposure to the PAT protein in all soil types at the same levels and therefore that there are no risks to soil microorganisms is unsubstantiated.</p>	<p>Res, T</p>	<p>The PAT protein is derived from soil borne bacterium and the same or similar proteins are present in a wide range of soil bacteria. Thus, microorganisms are exposed to the PAT protein through the environment. All published data shows that the PAT protein has extremely low toxicity for all organisms tested. The PAT protein is readily degraded by proteases and not expected to accumulate in the soil.</p>

	<p>Nitrogen fixing soil bacteria and other beneficial soil bacteria and fungi are also inhibited by glufosinate ammonium (Ho and Ching, 2003, p.vii) Further research is required to ascertain the interaction between glufosinate ammonium and soil pathogens. Other research has indicated that glufosinate ammonium is toxic to beneficial microorganisms and to some aquatic organisms (Jewell and Buffin, 2001).</p>	<p>HU, T</p>	<p>OSA. The use of herbicides containing glufosinate ammonium on the GM cotton proposed for release is subject to regulation by the APVMA.</p>
	<p>There is research that has highlighted the possibility of Bt toxins enhancing the persistence of glufosinate in soil (Accinelli et al 2004) and this research would be relevant if Liberty Link[®] Cotton was crossed with Bollgard II[®] cotton or some other Bt construct in the future.</p>	<p>S</p>	<p>DIR 062/2005 proposes the release of GM cotton containing the PAT protein only. Gene stacking with other GM cotton such as Bollgard II[®]/Roundup Ready[®] cotton as a result of this release was considered in the RARMP (Ch 3, Event 3) and no risks to people of the environment were identified. Any future applications for the commercial release of stacked GM cottons containing the PAT and Cry proteins would be assessed at that time. Use of herbicides and their residues are subject to regulation by the APVMA.</p>
	<p>There have been no specific weediness studies on Liberty Link[®] cotton. Reliance is made on a limited number of studies including Rowena Eastick's 2002 report. This report is yet to be published in a peer reviewed journal and should not be treated as scientifically robust and acceptable until it has been. The Eastick research does not put beyond reasonable doubt that an advantage is conferred by the Bt genetic manipulation. At best it provides an ambiguous result. There are significant shortcomings to the Eastick report.</p>	<p>Res, W</p>	<p>Cotton is not considered a problematic weed in Australia. Liberty Link[®] Cotton is susceptible to the same biotic and abiotic factors that limit the persistence of other GM and non-GM cottons and would only have a selective advantage in areas where glufosinate ammonium is used (Refer to Ch 3, Event 1 of RARMP). The GM cotton proposed for release does not have an insect resistance trait.</p>
	<p>Reference to a study by Farrell and Roberts (2002) into cotton volunteers from cotton seed transported from Emerald to Atherton in the RARMP fails to quantify the level of risk based on: (1) the overall quantity of seeds transported and provided to the dairies, (2) the proportion of GM cotton seeds of the overall quantity provided to the dairies, and (3) the number of trips to transport the seed. Quantifying the risk of cotton volunteers establishing in these circumstances would facilitate accurate extrapolation for increases in volunteers establishing as a result of increased transport of cotton seed resulting from an approval for commercial release of Liberty Link[®] Cotton. The Farrell and Roberts (2002) report does not provide this level of information and as it is not in a peer reviewed journal should not be treated as scientifically robust and acceptable until it is so published.</p>	<p>W</p>	<p>As discussed in the RARMP, Event 1, Ch 3, the likelihood of spread and persistence of the GM cotton due to expression of the <i>bar</i> gene was assessed as highly unlikely and would not be greater than the spread and persistence of non-GM cotton. Expression of the PAT protein would only offer a selective advantage in areas where glufosinate ammonium is used to control cotton plants. Mechanical means are commonly used to control cotton volunteers as herbicides are not effective on established cotton plants. The presence of volunteer plants resulting from the cultivation of a commercial crop does not necessarily indicate the establishment of self-sustaining weedy populations.</p>

	<p>The risk of increase in the number of volunteers due to transport needs to be properly ascertained and assessed by the Regulator, applying the Precautionary Principle (s.4(aa), <i>Gene Technology Act 2000</i>).</p> <p>Cotton seed can pass through the gut of cattle and remain viable, and may spread the cotton seed in bush land areas.</p> <p>In northern Australia greater consideration needs to be given to the risk of seed dispersal through water, particularly flooding events.</p>	W	<p>The risk of weediness of the GM cotton as a result of seed dispersal via various mechanisms is considered in Event 1, Ch 3 of RARMP. The risk was considered as negligible.</p>
	<p>The use of glufosinate ammonium will change the weed spectrum, therefore new integrated weed management strategies will be required. How will the risks from changes to weed species be ascertained and assessed? How will effective, adaptive herbicide resistance management systems be developed?</p>	HU, HR	<p>OSA. The APVMA consider issues relating to herbicide use and managing the development of herbicide resistant weeds. Changes to the weed spectrum and the development of herbicide tolerant weeds are briefly discussed in Ch 2, Section 2.10.2 of the RARMP. The Australian cotton industry applies integrated weed management practices.</p>
	<p>Diseases such as Fusarium wilt, Alternaria leaf spot, and/or bacterial blight may have an unknown interaction with the PAT protein.</p>	UE	<p>Unintended effects, such as an increased disease burden in the GM cotton were considered in Chapter 2, Section 2.9.1 of the RARMP. The diseases mentioned exist in the current cotton growing areas of Australia and susceptibility to these diseases is very much cultivar dependent. Previous releases of the same GM cotton in these areas did not show increased disease burden. No differences were observed in the pest or disease status between Liberty Link[®] Cotton and non-GM cotton during agronomic performance testing in the USA (see Ch 2, Section 2.3 of RARMP).</p>
	<p>In the absence of comprehensive scientific information it is impossible to ascertain the potential level of pesticide use that might be required to manage pests in the north due to cultivation of Liberty Link[®] Cotton. Ascertaining sustainable pest management approaches for cultivating Liberty Link[®] Cotton in northern Australia is vital to determining the level of risk the release poses to insect populations, other species and the broader northern Australian environment.</p>	HU	<p>Liberty Link[®] Cotton has been modified for increased tolerance to the herbicide glufosinate ammonium not insect resistance. It has shown no enhanced resistance or susceptibility to pests. The APVMA consider issues relating to herbicide use (OSA).</p>

	<p>Out crossing rates for cotton may vary seasonally or regionally. Work on pollinators and pollination rates on GM cotton in Australia have provided a recommendation for buffer zones of 20m for field trials. It is well acknowledged that small scale trials cannot provide sufficiently accurate information on potential gene flow rates of larger releases, therefore recommended buffers for small scale trials are unlikely to be effective in containing biological flows from large scale commercial releases.</p>	G, LC	<p>The proposal in DIR062 is for an unrestricted commercial release of Liberty Link[®] Cotton and therefore there is no buffer zone. Cotton is primarily self-pollinated and cross-pollination occurs at low frequencies within a few metres of the cotton plant. The risk of an adverse impact on human health and safety or the environment arising from gene flow to other cottons is estimated as negligible. (see Ch 3, Events 2 and 3).</p>
	<p>The only research in northern Australia on pollination and pollinators was undertaken in the Ord, Kununurra in the 1960s (Thompson 1966). This information has limited or no application to the current potential pollination of GM cotton crops, or native or weedy relative of cotton in the area There will be regional differences in pollinator insect populations and different pesticide applications affecting pollinator populations and rates of pollination. As this information is not available for the potential area in northern Australia, it is impossible for the Regulator to make an accurate assessment of the risk posed to the environment.</p>	G	<p>Cotton is primarily self-pollinated and cross-pollination occurs at low frequencies within a few metres of the cotton plant. The application of insecticides will further reduce the number of pollinators. The risk of an adverse impact on human health and safety or the environment arising from gene flow to other cottons is estimated as negligible (See Ch 2, Section 2.5).</p>
	<p>We disagree with the Regulator's opinion in the RARMP that there was no risk identified with respect to the reduced choice of herbicides to control volunteers which may be resistant to glyphosate or glufosinate ammonium and suggest there should be licence conditions imposed regarding management of volunteers.</p>	HU, LC	<p>Herbicides are not used to control established cotton plants (both GM and non-GM) and cultivation is the most effective option (See Ch 2, Section 2.5.6).</p>
	<p>Stacking of genes is more likely in areas where the Bollgard II[®]/Roundup Ready[®] cotton is grown adjacent to Liberty Link[®] cotton, as would clearly be the case in southern cotton growing areas, and this needs to be considered as a potential future risk in the north where both cottons may eventually be grown.</p>	S	<p>Gene stacking with commercially released GM cottons such as Bollgard II[®]/Roundup Ready[®] cotton was considered for southern areas in the RARMP (Ch 3, Event 3). Stacking in northern Australia would be considered in the context of any future applications to grow GM cotton on a commercial scale.</p>
	<p>The RARMP also states there are no reports within Australia of glufosinate ammonium herbicide resistant weeds. Due to the limited use of glufosinate ammonium as a herbicide it is too early to determine whether resistant weeds could develop due to its use and the absence of scientifically published data about weed resistance in Australia/northern Australia represents a further critical knowledge gap.</p>	HR	<p>OSA. Herbicide use and the development of herbicide resistant weeds are subject to regulation by the APVMA.</p>

	<p>The behaviour of Liberty 150 Herbicide with the higher temperatures and humidity in northern Australia is unknown and may result in changed efficacy. There could also be interaction between metabolites of glufosinate ammonium and the higher temperature and humidity which would have unknown effects. There is no information to assess the potential impact on this herbicide on native species, microorganisms, soil biochemistry and so on in the north or whether the herbicide could be used appropriately and effectively.</p>	UE, Res	<p>OSA. The use of herbicides containing glufosinate ammonium on the GM cotton proposed for release is subject to regulation by the APVMA.</p>
	<p>The RARMP notes that the trash or stubble Liberty Link[®] cotton is not suitable for animal feed due to possible pesticide residues, however there is no consideration of the potential risks associated with native species from feeding on this material.</p>	HU	<p>Animals are generally deterred from feeding on cotton plants or seeds because of its plant morphology and innate anti-nutritional or toxic compounds, so it is unlikely that native species will feed on cotton. The use of pesticides on GM and non-GM cotton, including residue levels, is subject to regulation by the APVMA.</p>
	<p>We have concerns that the OGTR may not set sufficiently stringent conditions on the commercial use of this cotton to ensure protection to human health and safety and the environment. We are further concerned about the capacity of the OGTR to ensure compliance with licence conditions including appropriate levels of monitoring to encourage compliance and pursue enforcement of breaches. There are no reported monitoring activities by the OGTR in the relevant Quarterly Reports since the commercial release of Bollgard II[®]/Roundup Ready[®] cotton in southern Australia.</p>	RMP, LC	<p>Noted. The OGTR can audit the holder of commercial licence. The RARMP concludes that risks to human health and safety or the environment from this release are negligible, hence no specific management conditions are imposed. To date, there has been no information in Annual Reports or other advice provided to the Regulator to indicate that monitoring of the commercial release of Bollgard II[®]/Roundup Ready[®] cotton in southern Australia is required.</p>
	<p>Could the Regulator please provide us with details as to how the OGTR is going to effectively manage commercial release activities to ensure compliance with licence conditions and the ongoing protection of the environment and human health from any adverse impacts?</p>	RMP, LC	<p>The company must submit a compliance plan which details how licence conditions relating to suitability, auditing and reporting requirements will be met. There is a statutory requirement under the <i>Gene Technology Act 2000</i> to report any unintended effects and the ability of the OGTR to audit licence holders.</p>

		<p>Recommends an integrated assessment of environmental risks and impacts of GM crops as well as intergovernmental action between the OGTR and the APVMA, Department of Environment and Heritage, and the States and Territories.</p> <p>Recommends that the Gene Technology Ministerial Committee takes a more proactive role in the release and management of GMOs.</p>		<p>Australia's gene technology regulatory system operates as part of an integrated legislative framework. The Act establishes the Regulator as an independent decision maker, but requires two rounds of consultation with a range of prescribed experts, agencies and authorities which includes the APVMA, FSANZ, the Minister for Environment and Heritage and the States and Territories on all DIR applications as part of the assessment process. The <i>Gene Technology (Consequential Amendments) Act 2000</i> requires other regulatory agencies to consult the Regulator for the purposes of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO. FSANZ has previously approved the use of oil and linters from Liberty Link[®] Cotton in food. The use of herbicide on the herbicide tolerant GM cotton is subject to regulation by the APVMA. The APVMA is currently assessing an application from Bayer to register Liberty[®] 150 Herbicide for the control of various weeds in Liberty Link[®] Cotton. There has been close liaison between the organisations during the assessment of these applications.</p>
8	A	<p>The cotton industry has been proven to be capable of managing GM technology responsibly in accordance to the requirements set down by regulatory authorities and accordingly has received major benefits including reduced use and dependence of traditional pesticides.</p>	HU	Noted.
		<p>Supports the introduction of Liberty Link[®] Cotton as it provides an alternative herbicide tolerant technology which will assist in the management and prevention of weed resistance.</p>	HR	Noted.
		<p>Herbicide resistance gene technology has contributed significantly to on-farm management of occupational health and safety issues (eg reduced need for cotton chippers and reduced pesticide exposure)</p>	HU	Noted. OSA (Benefits of the technology and reduced pesticide use).
		<p>A range of management practices are available to control cotton plants tolerant to glufosinate ammonium.</p>	W	Noted.
		<p>Confident that the cotton industry has the technology, management practices and industry monitoring in place to manage potential weed resistance.</p>	HR	Noted.
		<p>Assumes that the APVMA will conduct assessments of widespread use of glufosinate ammonium with respect of human safety, environmental risk and residue risks.</p>	HU	Noted. (APVMA considers herbicide use).

9	A	Strongly supports the commercial release of herbicide tolerant Liberty Link Cotton and are satisfied that the RARMP sufficiently covers the minimal risks attached to this technology.		Noted.
		Lists the benefits of GM cotton to public and environment including decreased pesticide use, insect resistant cottons are sustainable, quality product, use of safer chemicals, and less restriction on rotational crops.		Noted. OSA (benefits of GM technology and herbicide use).
		Combining GM technology with the Cotton Industry Best Management Practise Program continues to have a significant impact of the environment performance and sustainability of the Australian Cotton Industry.		Noted.
		Specific benefits of herbicide tolerance technology include reduction in application of pre-emergent products, mechanical cultivation, leeching of chemicals, erosion, topically applied herbicides, exposure to chemicals		Noted
		Liberty Link Cotton and the use of glufosinate ammonium will assist with resistance management.	HR	Noted
		Agrees with the RARMP that the risk of weediness of the GM cotton is negligible. 40 years of cotton growing (including GM cotton in the more recent years) with spills of seed on farm and on the road network has not resulted in cotton establishing itself as a weed.	W	Noted. Considered in Ch 2 and 3, event 1.
10	IG	Same issues raised as in submission number 7		See comments under submission 7
11	IG	Same issues raised as in submission number 7		See comments under submission 7