Risk Assessment and Risk Management Plan

Application for licence for dealings involving intentional release of a genetically modified organism into the environment

DIR 023/2002

Title: Commercial release of herbicide tolerant (Roundup Ready®) and herbicide tolerant/insect resistant (Roundup Ready®/INGARD®) cotton

Applicant: Monsanto Australia Ltd

June 2003

Office of the
Gene Technology Regulator

Abbreviations

aad	aminoglycoside adenylyltransferase gene
AAD	aminoglycoside adenylyltransferase enzyme
ANZFA	Australia New Zealand Food Authority (now FSANZ)
APVMA	Australian pesticides & Veterinary Medicines Authority (formerly NRA)
AQIS	Australian Quarantine Inspection Service
Bt	*Bacillus thuringiensis*
Btk	*Bacillus thuringiensis* variety *kurstaki*
CaMV	cauliflower mosaic virus
cp4 epsps	5-enolpyruvylshikimate-3-phosphate synthase gene originally isolated from *Agrobacterium* sp. strain CP4, conferring tolerance to glyphosate
CP4 EPSPS	glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase enzyme encoded by *cp4 epsps* gene
cry1Ac	insecticidal *cry1Ac* gene
Cry1Ac	insecticidal Cry1Ac protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DIR	dealing involving intentional release
DNA	deoxyribonucleic acid
epsps	5-enolpyruvylshikimate-3-phosphate synthase gene
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase enzyme
ELISA	enzyme linked immunosorbent assay
EMBL	European Molecular Biology Laboratory
FAO	Food and Agriculture Organisation of the United Nations
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
IgE	immunoglobulin E
IOGTR	Interim Office of the Gene Technology Regulator
IPCS	International Program on Chemical Safety
IRM	Insecticide Resistance Management
MRL	maximum residue limit
mRNA	messenger ribonucleic acid
nos	nopaline synthase gene
nptII	neomycin phosphotransferase II gene
NPTII	neomycin phosphotransferase II enzyme
NRA	National Registration Authority for Agricultural & Veterinary Chemicals (now APVMA)
OGTR	Office of the Gene Technology Regulator
ppm	parts per million
TIMS	Transgenic and Insecticide Resistance Management Strategy
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration

WHO	World Health Organisation
µg	micrograms

TABLE OF CONTENTS

EXECUTIVE SUMMARY

THE REGULATION OF GENETICALLY MODIFIED ORGANISMS

The *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001* (the Regulations) set out requirements which the Gene Technology Regulator (the Regulator) must follow when considering an application for a licence to intentionally release a genetically modified organism (GMO) into the environment.

For a licence to be issued, the Regulator must be satisfied that the release will not pose any risks to human health and safety or the environment that can not be managed. To this end, Section 51 of the Act requires the Regulator to prepare a risk assessment and risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and stakeholders.

THE APPLICATION

Monsanto Australia Ltd (Monsanto) applied for a licence (application number DIR 023/2002) for the continued commercial release of genetically modified (GM) herbicide tolerant Roundup Ready® cotton and herbicide tolerant/insecticidal Roundup Ready®/INGARD® cotton into the environment, for cultivation in the cotton growing regions of New South Wales and Queensland south of latitude 22º South.

Roundup Ready® and Roundup Ready®/INGARD® cotton were approved for general (commercial) release in 2000 (GR-9) and INGARD® cotton in 1996 (GR-3), based on advice from the Genetic Manipulation Advisory Committee (GMAC) which oversaw the previous voluntary system. Section 190 of the Act includes arrangements for such dealings to be 'deemed' as licensed for two years from the commencement of the Act on 21 June 2001, therefore expiring on 21 June 2003. The issuing of a licence in respect of application DIR 023/2002 will allow the continued commercial release of these genetically modified cottons beyond 21 June 2003. Continued commercial release of INGARD® cotton was approved under licence number DIR 022/2002.

Roundup Ready® cotton (GM event 1445) contains a gene that confers tolerance to glyphosate, the active ingredient of the herbicide Roundup®. Conventional cotton is susceptible to glyphosate damage. The use of Roundup Ready® cotton allows the application of glyphosate for the control of weeds that emerge early in the crop (up to the four-leaf growth stage). INGARD® cotton (GM event 531) contains a gene that encodes a highly specific lepidopteran insect toxin. Roundup Ready®/INGARD® cotton was produced by conventional breeding of Roundup Ready® cotton with INGARD® cotton, and expresses both the herbicide tolerance and insecticidal properties. Both Roundup Ready® cotton and Roundup Ready®/INGARD® cotton contain bacterial genes conferring resistance to antibiotics that were used solely as selectable markers in the initial laboratory stages of developing the GM cottons.

It is intended that GM cotton plants and their by-products, including cottonseed, be used in the same manner as non-GM cotton, including for human food and stockfeed. Cottonseed is processed for oil that is used in a variety of foods, and for cotton linters (short fibres that do not contain genetic material or protein) that are used as a cellulose base in some foods. Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority, ANZFA) has already approved the use of oil and linters from Roundup Ready® cotton and INGARD® cotton in human

food. Cottonseed from Roundup Ready® and Roundup Ready®/INGARD® cotton has also been used as stockfeed, including in northern Australia, since their commercial release in 2000.

The Australian Pesticides and Veterinary Medicines Authority (APVMA, formerly National Registration Authority for Agricultural and Veterinary Chemicals, NRA) is responsible for approving use patterns of GM cotton carrying insecticidal genes (including INGARD® cotton, Roundup Ready®/INGARD® cotton, Bollgard II® cotton and Roundup Ready®/Bollgard II® cotton), due to these plants' ability to produce an insecticidal substance. The APVMA currently limits planting of these GM cottons to 30% of the cotton crop in any region, to guard against the emergence of resistant insects. The APVMA is also responsible for setting conditions on the use of Roundup Ready® herbicide on Roundup Ready® cotton crops, including conditions relating to herbicide resistance management.

Monsanto proposes to phase-out Roundup Ready/INGARD® cotton over the next 2 cotton growing seasons while the GM herbicide tolerant/insecticidal Roundup Ready/Bollgard II® cotton (approved for commercial release under licence number DIR 012/2002) is phased-in over the same period.

Prior to commercial release of Roundup Ready® cotton, 23 limited and controlled releases involving Roundup Ready® cotton and 12 involving Roundup Ready®/INGARD® cotton were conducted under the former voluntary system overseen by GMAC. There have been no reports of adverse effects on human health or the environment resulting from any of these releases, nor have there been any such reports since INGARD® and Roundup Ready® cotton were released commercially in 1996 and 2000 respectively.

THE EVALUATION PROCESS

Licence application DIR 023/2002 from Monsanto has been evaluated, and a risk assessment and risk management plan (RARMP) prepared, in accordance with the Act and the Regulations, using a Risk Analysis Framework. This framework was developed by the Regulator in consultation with the public and key State, Territory and Commonwealth government stakeholders and the Gene Technology Technical Advisory Committee, and is available at www.ogtr.gov.au/pdf/public/raffinal.pdf.

Details of the process that the Regulator must follow, including the prescribed consultation process on the application, and the matters that must be considered in preparing a RARMP, are set out in Appendix 8 of the RARMP. The complete RARMP can be obtained from the OGTR or from the OGTR's web site at www.ogtr.gov.au.

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

As mentioned above, the use of Roundup Ready® herbicide (a formulation of glyphosate) on Roundup Ready® and Roundup Ready®/INGARD® cotton crops in Australia is registered by the APVMA. As part of their assessment of this use, the APVMA consider potential human health and environmental effects, for example arising through occupational exposure or residues. Thus risks

associated with the use of glyphosate are not generally considered in the risk assessment of these GM cottons.

Through this process, potential hazards to human health and safety or the environment that may be posed by release of Roundup Ready® cotton and Roundup Ready®/INGARD® cotton were identified. These have been evaluated on the basis of the likelihood of each hazard occurring and the likely impact of the hazard, were it to be realised. The identified potential hazards relate to:

- **toxicity and allergenicity for humans:** could Roundup Ready® cotton or Roundup Ready®/INGARD® cotton be more toxic or allergenic than non-GM cotton, as a result of the novel gene products or because of unforseen or unintended effects?
- **toxicity for non-target organisms:** could Roundup Ready® cotton or Roundup Ready®/INGARD® cotton be harmful to non-target organisms as a result of the novel gene products or because of unforseen or unintended effects?
- **weediness:** could Roundup Ready® cotton or Roundup Ready®/INGARD® cotton be harmful to the environment because of inherent weediness or increased potential for weediness?
- **transfer of introduced genes to other organisms:** could the new genes introduced into the cottons transfer to non-GM cotton crops, feral or native cottons, or to other organisms, with adverse consequences?
- **insecticide and herbicide resistance:** could target insects develop resistance to the insecticidal protein produced by the introduced insecticidal gene in Roundup Ready®/INGARD® cotton. Similarly, could weeds develop resistance to herbicide if the Roundup Ready® crop-herbicide combination is used inappropriately?

CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator considers that no risks to human health and safety, or to the Australian environment, will result from the continued commercial release of Roundup Ready® cotton and Roundup Ready®/INGARD® cotton, that are greater than the very low risks posed by non-GM cotton. The assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans

Roundup Ready® cotton and Roundup Ready®/INGARD® cotton are unlikely to prove more toxic or allergenic to humans than conventional cotton. As noted above, FSANZ has previously approved the use in food of oil and linters from Roundup Ready® cotton and INGARD® cotton, concluding that products from these GM cottons are as safe as are those from non-GM cotton. Therefore it is not considered necessary to impose any management conditions in relation to potential toxicity or allergenicity.

Toxicity to non-target organisms

Roundup Ready® cotton and Roundup Ready®/INGARD® cotton are unlikely to prove more toxic to non-target organisms than conventional cotton. The introduced proteins have been found to be non-toxic to non-target organisms, and these GM cottons have been used as stock feed with no

reports of adverse effects. Therefore it is not considered necessary to impose any management conditions in relation to potential non-target toxicity.

Weediness

The risk of Roundup Ready® cotton or Roundup Ready®/INGARD® cotton establishing as a weed in cotton-growing areas of Australia south of latitude 22º South is very low, and not likely to be greater than that of conventional cotton. The germination and/or persistence of non-GM and GM cotton in southern Australia are effectively limited by the prevailing conditions of soil moisture, soil nutrients, plant competition and frosts. Therefore it is not considered necessary to impose any conditions to manage the risk of weediness in southern Australia.

Although Monsanto only seeks approval to grow Roundup Ready® cotton and Roundup Ready®/INGARD® cotton south of latitude 22º South, cottonseed from these GM cottons may be transported north for use as stockfeed. Limited experimental data suggests that INGARD® cotton may have the potential to be more weedy than non-GM cotton in certain habitats in northern Australia. However INGARD® cottonseed has been used as stockfeed in northern Australia since its commercial release in 1996, and Roundup Ready® and Roundup Ready®/INGARD® cottonseed since 2000, with no indication that these cottons have become problematic weeds, or more prevalent than conventional cotton.

Although it is considered that there is a low risk of Roundup Ready®/INGARD® cotton becoming weedy in specific habitats in northern Australia, conditions have been imposed to limit and monitor for the spread and persistence of these GM cottons in northern Australia.

Transfer of introduced genes to other organisms

Some gene transfer from Roundup Ready® cotton and Roundup Ready®/INGARD® cotton to other cultivated cotton is likely but the overall frequency of out-crossing is very low. If this occurs, it would not pose any risks additional to those posed by the GM cotton itself. The conventional farming practice of using certified (pure) seed every season minimises the presence of the new genes in non-GM crops.

The potential for transfer of the introduced genes to native cotton species is negligible, because of genetic incompatibility with all native species and, for many, geographic isolation.

The potential for gene transfer to feral (naturalised) cotton is low because of geographic isolation of known feral populations in Western Australia and the Northern Territory from areas of NSW and Queensland in which Roundup Ready® cotton and Roundup Ready®/INGARD® cotton will be grown. Although herbarium records suggest that feral cotton populations may also occur in Queensland, there is a relative lack of detailed information on the location of such populations. Licence DIR 022/2002, issued for the continued commercial release of INGARD® cotton, requires Monsanto to document the location of these populations, if they exist, in Queensland and determine their distance from cotton production locations, to assess whether additional controls are required.

The likelihood of transfer of the introduced genes to other organisms is negligible, but even if such transfer occurred it would be unlikely to pose any hazard to human health and safety or the environment. Therefore it is not considered necessary to impose any management conditions in relation to gene transfer.

Insecticide and herbicide resistance

The risk of the targeted insects developing resistance to the insecticidal protein in the long term is high, however this risk is being managed by the APVMA through oversight of an insecticide resistance management plan in connection to the use of INGARD® cotton.

There is also potential for development of herbicide-resistant weeds if the Roundup Ready® crop-herbicide combination is used inappropriately. This risk is also being managed by the APVMA, through conditions placed on the use of Roundup Ready® herbicide on Roundup Ready® cotton crops. Therefore no additional management conditions have been imposed in relation to insecticide and herbicide resistance.

THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)

As part of the evaluation process for this licence application, a risk management plan has been developed to addresses the identified risks (refer to Conclusion of the Risk Assessment, above). This plan is given effect by the licence conditions imposed. The key licence conditions are outlined below.

Toxicity or allergenicity to humans

Based on the risk assessment, no management conditions have been imposed in relation to toxicity or allergenicity.

Toxicity to non-target organisms

Based on the risk assessment, no management conditions have been imposed in relation to non-target toxicity.

Weediness

Based on the risk assessment, no management conditions have been imposed in relation to weediness in southern Australia. However, in order to complete the environmental monitoring program required in relation to the original commercial release of Roundup Ready® and Roundup Ready®/INGARD® cotton in 2000, the licence requires:

- continuation of monitoring for volunteer cotton in non-agricultural situations within the cotton growing areas of New South Wales and Queensland for an additional growing season.

Also based on the risk assessment, conditions have been imposed to limit the spread and persistence of GM cotton in northern Australia, and to further assess the potential for weediness of these GM cottons in northern Australia. The licence includes conditions that require:

- the use of covered vehicles for transport of GM cotton seed north of latitude 22º South;
- the licence holder to develop, in consultation with the OGTR, a strategy to communicate the importance of control of cotton plants to recipients of GM cotton seed north of latitude 22º South; and

- the licence holder to conduct an annual survey of the incidence of volunteer cotton in areas where GM cotton seed is used as stock feed, and the of effectiveness of the communication strategy required above.

Transfer of introduced genes to other organisms

Based on the risk assessment, no management conditions have been imposed in relation to gene transfer.

Insecticide and herbicide resistance

No conditions have been imposed in relation to management of insecticide or herbicide resistance, as this is the responsibility of the APVMA. The applicant's obligation to comply with any such conditions imposed by the APVMA is noted in the licence.

General conditions

Any licence issued by the Regulator also contains a number of general conditions, which are also relevant to risk management. These include, for example, identification of the persons or classes of person covered by the licence and informing the Regulator if the applicant becomes aware of any additional information about risks to human health or safety or to the environment.

Monitoring and enforcement of compliance by the OGTR

As well as the legislative capacity to enforce compliance with licence conditions, the Regulator has additional options for risk management. The Regulator can direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment.

FURTHER INFORMATION

Detailed information on the evaluation of the application, including the licence conditions, is available in the risk assessment and risk management plan document for this application, which can be obtained from the website of the Office of the Gene Technology Regulator (www.ogtr.gov.au), or by calling 1800 181 030 (please quote application number DIR 023/2002).

CHAPTER 1 BACKGROUND

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

SECTION 1 THE APPLICATION

Project Title:	**Commercial release of herbicide tolerant (Roundup Ready®) and herbicide tolerant/insect resistant (Roundup Ready®/INGARD®) cotton**
Applicant:	Monsanto Australia Ltd PO Box 6051 Melbourne, VIC 8008
Common name of the parent organism:	Cotton
Scientific name of the parent organism:	*Gossypium hirsutum* L.
Modified trait(s):	Herbicide tolerance (Roundup® herbicide) Herbicide tolerance/Insecticidal (Roundup® herbicide/Cry1Ac protein) Antibiotic resistance
Identity of the gene(s) responsible for the modified trait(s):	• *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 (herbicide tolerance) • *cry1Ac* gene from the bacterium *Bacillus thuringiensis* (insecticidal) • *nptII* gene from bacterial Tn5 transposon (antibiotic resistance)
Proposed Release Location:	Cotton growing regions of New South Wales and Queensland south of latitude 22° South
Proposed Release Size:	As required by the Australian cotton industry. Planting of Roundup Ready®/INGARD® cotton is limited by the APVMA as part of the Insecticide Resistance Management Strategy for INGARD® cotton.
Proposed Release Date:	The proposed release is a continuation of the current commercial release, approved in 2000 under the former voluntary system. Roundup Ready®/INGARD® cotton is expected to be progressively replaced by Roundup Ready®/Bollgard II® cotton over two seasons.

2. The OGTR received an application (licence application number DIR 023/2002) from Monsanto Australia Ltd (Monsanto) for the intentional release of genetically modified (GM) herbicide tolerant (Roundup Ready®) cotton and herbicide tolerant in combination with insect resistant (Roundup Ready®/INGARD®) cotton into the environment in the cotton growing regions of New South Wales (NSW) and Queensland (Qld) south of latitude 22º South. Approval will enable the continued commercial release of the genetically modified cottons that was authorised to proceed under the

previous voluntary system administered by the Genetic Manipulation Advisory Committee (GMAC) (see Section 2.2 of this chapter).

3. Roundup Ready® cotton contains a gene that confers tolerance to glyphosate, the active ingredient of the herbicide Roundup®. Conventional cotton is susceptible to glyphosate. The use of Roundup Ready® cotton allows the application of glyphosate for the control of weeds that emerge early in the crop (up to the fourth true leaf stage of growth of the GM cotton). INGARD® cotton contains a gene, *cry1Ac*, that encodes a highly specific lepidopteran insect toxin derived from a common soil bacterium, *Bacillus thuringiensis*. INGARD® cotton was approved for commercial release under licence number DIR 022/2002, and is only considered here as far as it relates to Roundup Ready®/INGARD® cotton. Roundup Ready®/INGARD® cotton was produced by conventional breeding of Roundup Ready® cotton with INGARD® cotton, and has both the herbicide tolerance and insecticidal properties.

4. Monsanto proposes the phasing-out of Roundup Ready®/INGARD® cotton over the next two seasons while GM herbicide tolerant/insecticidal Roundup Ready®/Bollgard II® cotton is phased-in over the same period. Bollgard II® cotton contains the same *cry1Ac* gene as INGARD® cotton plus a second insect toxin gene from *Bacillus thuringiensis*. In September 2002 the Regulator approved the commercial release of Bollgard II® (insecticidal) cotton and Roundup Ready®/Bollgard II® (herbicide tolerant/insecticidal) cotton in the cotton growing regions of NSW and Qld south of latitude 22° South (DIR 012/2002).

Section 1.1 The proposed dealings

5. Monsanto sought approval for continued commercial release of Roundup Ready® and Roundup Ready®/INGARD® cotton, for planting in all Australian cotton growing regions south of latitude 22° South. It is also intended that GM cotton plants and their by-products, including cottonseed, be used in the same manner as non-GM cotton, including for human food and stockfeed.

6. Cottonseed is processed for oil that is used in a variety of food products and for cotton linters (a type of fibre that does not contain any genetic material) that are used as a cellulose base for several consumer food products. Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority, ANZFA) has approved the use of oil and linters from Roundup Ready® cotton and INGARD® cotton in human food.

7. The Australian Pesticides & Veterinary Medicines Authority (APVMA, formerly the National Registration Authority for Agricultural and Veterinary Chemicals, NRA) is responsible for determining the maximum allowable planting each season of cotton carrying the *cry1Ac* insecticidal gene (see Sections 2.2 and 2.3 of this chapter). The APVMA is also responsible for setting conditions on the use of Roundup Ready® herbicide on Roundup Ready® cotton crops, including conditions relating to herbicide resistance management.

Section 1.2 Parent organism

8. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia and is grown as an agricultural crop in NSW and Qld and on a trial basis in Western Australia and the Northern Territory. *G. hirsutum* is not a pathogenic organism. More detailed information on cotton can be found in a review document 'The Biology and Ecology of Cotton (*Gossypium*

hirsutum) in Australia' (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document is available at www.ogtr.gov.au.

Section 1.3 Genetic modification and its effect

9. Roundup Ready® cotton contains a herbicide tolerance gene, *cp4 epsps*, derived from a bacterium, *Agrobacterium* sp. strain CP4. This gene encodes an enzyme that naturally tolerates glyphosate, the active ingredient in Roundup® herbicide.

10. Roundup Ready® cotton plants also contain two bacterial antibiotic resistance genes, *nptII* and *aad*. The *nptII* gene encodes resistance to the antibiotics kanamycin and neomycin. It was used as a selectable marker during the initial laboratory stage of development to select for cotton plants containing the *cp4 epsps* gene. The *aad* gene encodes spectinomycin and streptomycin resistance. It was used in the laboratory prior to the production of the genetically modified plants to select for bacteria containing the modified DNA. The *aad* gene is not expressed in the GM cotton plants because the bacterial promoter controlling its expression is not active in plants.

11. Roundup Ready®/INGARD® cotton plants are produced through the conventional breeding of GM Roundup Ready® cotton with GM INGARD® cotton. Therefore, Roundup Ready®/INGARD® cotton plants also contain an insecticidal gene, *cry1Ac*, derived from a common soil bacterium, *Bacillus thuringiensis* (Bt). This gene produces a protein that is toxic to lepidopteran caterpillars, including the two key *Helicoverpa* pests of cotton.

12. Short regulatory sequences (promoters and terminators) that control expression of the introduced genes are also present in the genetically modified cotton. These sequences are derived from the cauliflower mosaic virus, figwort mosaic virus, *Agrobacterium tumefaciens* and soybean. Although a number of these organisms are plant pathogens, the regulatory sequences comprise only a small part of their total genome and are not in themselves capable of causing disease.

13. Detailed information on the *cp4 epsps*, *cry1Ac* and *nptII* genes, and characterisation of the GMO and the new proteins expressed by Roundup Ready® and Roundup Ready®/INGARD® cotton are provided in Appendix 1.

Section 1.4 Method of gene transfer

14. Roundup Ready® cotton was generated by inserting the *cp4 epsps*, *nptII* and *aad* genes into cotton from a plasmid vector carried by the bacterium *A. tumefaciens*. INGARD® cotton was developed by inserting the c*ry1Ac, nptII* and *aad* genes into cotton using the same method. The plasmid vectors used were 'disarmed', that is, lacking the genes that encode the tumorigenic functions of *A. tumefaciens*. (see Appendix 1, Section 4 for details). Roundup Ready®/INGARD® cotton was produced by conventional breeding of GM Roundup Ready® cotton with GM INGARD® cotton.

SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

Section 2.1 Previous limited releases in Australia

15. Prior to commercial release, numerous limited and controlled releases of Roundup Ready® and Roundup Ready®/INGARD® cotton were conducted under the voluntary system overseen by GMAC, as listed below:

Roundup Ready® - 23 limited and controlled releases undertaken by:

- Deltapine Australia Pty Ltd (PR-32, PR-52, PR-52X, PR-52X2, PR-52X3, PR-71, PR-71X, PR-71X2, PR-83, PR-83X, PR-83X3, PR-140, PR-140X and PR-143);
- CSIRO Division of Plant Industry (PR-55, PR-55X, PR-55X2, PR-55X3, PR-55X5 and PR-55X6);
- Cotton Seed Distributors Pty Ltd (PR 55-X4); and
- Monsanto (PR-83X2 and PR-83X4).

Roundup Ready®/INGARD® - 12 limited and controlled releases undertaken by:

- Deltapine Pty Ltd (PR-83, PR-83X, PR-83X3, PR-109, PR-109X, PR-140, PR-140X and PR-143);
- CSIRO Division of Plant Industry (PR-94); and
- Cotton Seed Distributors Pty Ltd (PR94X, PR-94X2 and PR-94X3).

16. These limited and controlled releases approved by GMAC ranged in size from 0.2 – 5000 hectares. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

Section 2.2 Approval for general (commercial) release via issuing of a deemed licence

17. Roundup Ready® cotton and Roundup Ready®/INGARD® cotton were approved for general (commercial) release on 14 September 2000, by the Minister for Health and Aged Care, on the basis of advice from GMAC and the Interim Office of the Gene Technology Regulator (IOGTR) (GR-9). INGARD® cotton had previously been approved for general release in 1996 by the APVMA (then the NRA), also on the basis of advice from GMAC (GR-3). The Commonwealth Government entered into a legally binding Deed of Agreement with Monsanto to ensure compliance with the agreed conditions.

18. Planting of Roundup Ready® cotton and Roundup Ready®/INGARD® cotton was restricted to the cotton growing regions of NSW and Qld south of latitude 22º South, due to concerns about the potential weediness of the cotton in tropical areas, as well as the potential for out-crossing to feral cotton in these areas. No restrictions on the planting area (hectares) for Roundup Ready® cotton were imposed. The maximum planting area of INGARD® cotton varieties, including Roundup Ready®/INGARD® cotton, is restricted by the APVMA, as part of an insecticide resistance management plan (see Appendix 6). Since June 2000, planting of cotton carrying the *cry1Ac* gene (including Roundup Ready®/INGARD®, Bollgard II® and Roundup Ready®/Bollgard II® cotton) has been limited to 30% of the cotton crop (by region) in any one season.

19. As noted in section 1.1 of this chapter, the APVMA is also responsible for setting conditions on the new use of Roundup Ready® herbicide on cotton crops, including conditions relating to herbicide resistance management.

20. Under transitional arrangements set out in Section 190 of the Act, GMAC's advice to proceed with the general release of Roundup Ready® and Roundup Ready®/INGARD® cotton, issued to Monsanto, was deemed as a licence for the purposes of the Act. The deemed licence took effect with the commencement of the new legislation on 21 June 2001. However, the transitional arrangements under the *Gene Technology Act 2000* meant that the approval for Roundup Ready® and Roundup Ready®/INGARD® cotton could only be for a maximum of two years after the legislation took effect, on 21 June 2001. Releases proposed to continue beyond that date require submission of a new application, which is evaluated under the provisions of the new legislative system.

Section 2.3 Approvals by Other Australian Government Agencies

21. The OGTR is responsible for assessing the biosafety risks to human health and the environment associated with development and use of GMOs. Other government regulatory requirements must also be met in respect of the release of the GMOs, and the use of products of the GMOs, including the requirements of the APVMA and FSANZ.

2.3.1 Australian Pesticides & Veterinary Medicines Authority (APVMA)

22. The *cry1Ac* gene in Roundup Ready®/INGARD® cotton falls under the *Agricultural and Veterinary Chemicals Code (1994)* definition of an agricultural chemical product, due to its production of an insecticidal substance and is, therefore, subject to regulation by the APVMA (formerly the NRA).

23. As INGARD® cotton is genetically modified to resist key insect pests of cotton, the APVMA has previously imposed conditions in connection with the insecticidal activity of this cotton to manage the development of insecticide resistance in the cotton industry as a whole. The APVMA continue to specify a maximum proportion of planting of INGARD® cotton, as noted in Section 2.2 of this chapter.

24. The APVMA has also imposed conditions on the use of the herbicide glyphosate (the active ingredient of Roundup®) used in connection with Roundup Ready® cotton as part of an overall strategy to limit the development of glyphosate resistant weeds.

25. Therefore no conditions have been imposed in licence DIR 023/2003 in relation to insecticide- or herbicide-resistance management, as these matters will be managed in the context of the APVMA regulatory scheme.

26. Further information about use patterns of glyphosate on glyphosate-tolerant crops, and about the management of herbicide and insecticide resistance, is available from the APVMA:

Australian Pesticides & Veterinary Medicines Authority
PO Box E240
KINGSTON ACT 2604

Phone: (02) 6272 5158
Fax: (02) 6272 4753
Email:contact@apvma.gov.au
http://www.apvma.gov.au

2.3.2 Food Standards Australia New Zealand (FSANZ)

27. The safety and labelling of foods derived from genetically modified plants are the responsibility of FSANZ, rather than the OGTR.

28. In previous risk assessments of genetically modified Roundup Ready® and INGARD® cotton, FSANZ found that refined oil and processed linters derived from these GM cottons are as safe for human consumption as are those derived from other commercial cotton varieties (see Appendix 2).

29. Further details of this risk analysis of Roundup Ready® and INGARD® cotton conducted by FSANZ, and information about food labelling, are available from FSANZ:

Food Standards Australia New Zealand
PO Box 7186
Canberra Mail Centre ACT 2610
Phone: (02) 6271 2222
Fax: (02) 6271 2278
E-mail: info@foodstandards.gov.au
http://www.foodstandards.gov.au

Section 2.4 International Approvals

30. Roundup Ready® cotton and Roundup Ready®/INGARD® cotton have been approved in a number of countries for environmental release, human food and animal feed, as indicated in Table 1.

Table 1 Approvals of Roundup Ready® and Roundup Ready®/INGARD® cotton in other countries

Country	GM cotton	Environment	Food	Stock feed
Argentina	Roundup Ready® INGARD®	1999 1998	2001 1998	2001 1998
Canada	Roundup Ready® INGARD®		1996 1996	 1996
China	INGARD®	1997	1997	1997
India	INGARD®	2002		
Japan	Roundup Ready® INGARD®	1997 1997	1997 1997	1998 1997
South Africa	INGARD®	1997	1997	1997
United States of America	Roundup Ready® INGARD®	1995 1995	1995 1995	1995 1995

31. Other countries where Roundup Ready® and INGARD® cotton varieties are pending approval include Israel and the European Union. No country has refused an application for the release of either Roundup Ready® or INGARD® cotton.

32. There have been no reports of adverse effects on human health or the environment resulting from any of the international or Australian releases of Roundup Ready® or Roundup Ready®/INGARD® cotton.

CHAPTER 2 SUMMARY OF THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN

33. The Act and the Regulations require that risks associated with dealings with GMOs are identified and assessed as to whether they can be managed to protect human health and safety and the environment (see Appendix 8).

SECTION 1 ISSUES RAISED IN SUBMISSIONS DURING CONSULTATION ON THE APPLICATION AND RARMP

34. Comments received in response to the consultation on the risk assessment and risk management plan undertaken, with expert groups and key stakeholders as required by Section 50 of the Act, and with the public as required by section 52 of the Act (see Appendix 8), were very important in shaping this risk assessment and risk management plan, which formed the basis of final decision on the application.

35. Written submissions received from the agencies prescribed by Section 50 of the Act, in relation to application DIR 023/2002, suggested that the following issues should be addressed in the risk assessment and the risk management plan:

- the possibility that the GM cotton may be harmful to humans, as a result of allergenic reactions in cotton plantation workers (Appendix 2, Section 2 refers);
- the possibility that food products from the GM cotton may be harmful to humans, as a result of toxicity or allergenicity (Appendix 2, Section 2 refers);
- the possibility that the GM cotton may be harmful to the environment because of inherent weediness or increased potential for weediness, particularly in favourable habitats such as adjacent to natural or artificial waterways (Appendix 4, Section 2 refers);
- potential for negative environmental impact of Roundup Ready® cotton volunteers in on- and off- farm situations (Appendix 4 refers);
- the extent of cross-pollination from GM cotton plantations to other cotton crops (Appendix 5, Section 2 refers);
- the possibility that the new genes introduced into the cotton can transfer to other organisms with adverse consequences (Appendix 5, Sections 3 and 4 refers);
- the emergence of insects resistant to the insecticidal protein in Roundup Ready®/INGARD® cotton, and the adequacy of the Insecticide Resistance Management plan (Appendix 6, Section 1 refers); and
- measures to limit the unintentional dispersal in the environment of GM cottonseed north of latitude 22º South (Appendix 7, Section 3 refers).

36. In accordance with Section 51 of the Act, the Regulator has taken into account all written submissions on matters relevant to human health and safety and the environment in preparing this document.

37. The key issues raised in submissions from the public that related to human health and safety and the environment were:

- potential for long term health effects from food use the GM cotton products (Appendix 2, Section 2 refers);
- potential weediness of cotton in the Australian environment, and appropriateness of relevant management conditions (Appendix 4, Section 2, and Appendix 7 refer);
- the emergence of insects resistant to the insecticidal protein of Roundup Ready®/INGARD® cotton, and the adequacy of the Insecticide Resistance Management plan (Appendix 6, Section 1 refers).

38. Public submissions also raised a number of issues, such as impacts on domestic markets and export expansion, that are outside the scope of the evaluations conducted under the Act and have therefore not been considered as part of the assessment process.

39. In total, the OGTR received 4 submissions from the public on this risk assessment and risk management plan. A summary of these written submissions, and where issues relating to human health and safety and the environment were taken into account in the RARMP, is provided in Appendix 9.

SECTION 2 FINALISATION OF THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN

40. In accordance with Section 51 of the Act, the Regulator has taken into account all issues raised in written submissions that related to human health and safety and to the environment in finalising the risk assessment and the risk management plan. These issues were considered carefully and weighed against the body of current scientific information in reaching the conclusions set out in this document.

41. The risk assessment process, detailed in Appendix 8, identified a number of potential hazards that may be posed by the proposed dealings. The risks posed by these hazards were assessed as being either 'negligible', 'very low', 'low', 'moderate', 'high' or 'very high', by considering:

- the likelihood of the hazard occurring;
- the likely consequences (impact) of the hazard, were it to be realised; and
- risk management options to mitigate any significant hazards.

42. The following table (Table 2) lists each of the potential hazards that were considered during the risk assessment process in the *Hazard Identification* column and summarises the assessment of each hazard under the column headed *Risk*. A comprehensive assessment of each identified hazard is provided in Appendices 2 - 6, as cross-referenced in the column headed *Summary of Risk Assessment*.

43. Where it is considered that risk management is necessary to protect the health and safety of humans and/or the environment, the table also summarises risk management options for each hazard (*Risk Management (RM) Options*) and identifies the *Preferred RM Method* and summarises the reason for selecting a particular method (*Reason(s) for Selecting RM Methods*). The risk management plan for the proposed dealing will be given effect by specific conditions within the

licence. These conditions are summarised in the final column, headed *Licence Conditions*, and detailed in Appendix 7.

44. Based on current knowledge, there remains a low risk that insecticidal cotton may, in certain circumstances, be more weedy in northern Australia (north of latitude 22º South) than non-GM cotton. A communication strategy, developed by the licence holder, in consultation with the OGTR, will inform transporters, retailers and endusers of GM whole cotton seed in northern Australia of the requirements associated with transporting GM cotton seed and of how to identify and effectively manage cotton volunteers if they occur. The licence holder is also required to conduct annual surveys of areas where GM cotton seed is used in northern Australia in order to regularly monitor the extent of volunteer populations and the effectiveness of the communication strategy.

45. In finalising the conditions for the licence, the Regulator has carefully considered the enforceability of the conditions and the ability for the licence holder and persons covered by the licence to comply with the conditions in practice.

SECTION 3 DECISION ON THE APPLICATION

46. Details of the matters that the Regulator must consider in making a decision are provided in Appendix 8. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings can be managed so as to protect human health and safety and the environment.

47. It is concluded that there are no risks to public health and safety or to the Australian environment arising from the proposed release of GM Roundup Ready® and Roundup Ready®/INGARD® cottons that are additional to those posed by the commercial production of conventional cotton. Detailed risk analyses based on the available scientific information are provided in Appendices 2 - 6 in support of this conclusion.

48. In accordance with the matters required to be considered under section 58 of the Act, the Regulator has determined that Monsanto Australia Ltd is suitable to hold a licence for a dealing involving intentional release of a GMO into the environment. Further information on the process of assessing the suitability of the applicant is contained in Appendix 8.

49. Therefore the Regulator has issued licence number DIR 023/2002.

Table 2 Summary of the risk assessment and the risk management plan (including summary of key licence conditions)

Roundup Ready® cotton — the GM herbicide tolerant cotton proposed for release.
Roundup Ready®/INGARD® cotton — the GM herbicide tolerant/ insecticidal cotton proposed for release.
INGARD® cotton: — GM insecticidal cotton (see DIR 022/2002).
CP4 EPSPS — protein conferring tolerance to glyphosate, encoded by the introduced *cp4 epsps* gene.
Cry1Ac: — insecticidal protein, encoded by the introduced *cry1Ac* gene, toxic to lepidopteran caterpillar pests of cotton.
NPTII: — protein providing antibiotic resistance that allowed identification of modified plants in the laboratory, encoded by the introduced *nptII* gene (marker gene).
Lepidoptera: — order of insects, including the caterpillar insect pests targeted by INGARD® cotton.

HAZARD IDENTIFICATION	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management?	Risk Management (RM) options [* Preferred RM 0ption(s)]	Reason(s) for selecting RM methods	Is risk managed?	Licence conditions (refer to Appendix 7 for detailed licence conditions)
TOXICITY AND ALLERGENICITY FOR HUMANS: **Food**	**Very Low**	**See Appendix 2** ▪ there have been no reported toxic or allergic effects from these GM cottons since their commercial release in Australia, in September 2000; ▪ cottonseed oil and linters used in food do not contain DNA or protein, and those from the GM cottons can not be distinguished from the non-GM products; ▪ FSANZ has concluded that oil and linters derived from Roundup Ready® and INGARD® cotton as safe for human consumption as those derived from non-GM cotton; ▪ the introduced proteins are already widespread in the environment and present in human food; ▪ evidence indicates that the introduced proteins are not allergenic, nor do they have properties characteristic of known allergenic proteins; ▪ feeding studies indicate that the GM cottons are not more toxic than non-GM cotton.	No	N/A	N/A	N/A	Not required
TOXICITY AND ALLERGENICITY FOR HUMANS: **Occupational exposure**	**Very Low**	**See Appendix 2** ▪ exposure to the introduced proteins through working with cotton plants is very low; ▪ cotton pollen is not wind dispersed and therefore not likely to be an airborne allergen; ▪ although dust and lint from cotton can be created at processing facilities, the use of protective equipment prevents respiratory irritation, and fibre characteristics of the GM cottons are the same as for non-GM cotton; ▪ the introduced proteins are already widespread in the environment; ▪ evidence indicates that the introduced proteins are not allergenic, nor do they have properties characteristic of known allergenic proteins; ▪ compositional and agronomic analyses have not indicated any differences between the GM and non-GM cottons, other than the presence of the introduced proteins and the intended traits.	No	N/A	N/A	N/A	Not required
TOXICITY AND ALLERGENICITY FOR HUMANS: **Wearing & using household items containing**	**Very low**	**See Appendix 2** ▪ cotton lint, linters and oil used in clothing and other household products do not contain DNA or protein, and those from the GM cottons can not be distinguished from the non-GM products; ▪ compositional and agronomic analyses have not indicated any differences between the GM and non-GM cottons, other than the presence of the introduced proteins and the intended traits.	No	N/A	N/A	N/A	Not required

cotton products.							
HAZARD IDENTIFICATION	**Risk** (combines 'likelihood' and 'impact')	**Summary of Risk Assessment (refer to appendices for details)**	**Does risk require management?**	**Risk Management (RM) options [* Preferred RM option(s)]**	**Reason for selecting RM option**	**Is risk managed?**	**Licence conditions (refer to Appendix 7 for detailed licence conditions)**
TOXICITY FOR OTHER ORGANISMS: **Mammals and wildlife including birds and fish,**	**Very low**	**See Appendix 3** ▪ the introduced proteins, CP4 EPSPS, Cry1Ac and NPTII, are present at low levels in Roundup Ready® and Roundup Ready®/INGARD® cotton; ▪ the nutritional and agronomic properties of these GM cottons are similar to those of conventional cotton; ▪ the introduced proteins are already widespread in the environment, through the presence of the bacteria to which they are native; ▪ exposure of stock and wild life to these GM cottons is low, except for stock fed cotton seed; ▪ there are no differences in performance of stock fed seed of these GM cottons, compared to seed of non-GM cotton; ▪ the toxicity of Cry1Ac is highly specific to Lepidopteran insect larvae; ▪ toxicity studies with Roundup Ready® and INGARD® cotton tissue indicate that they are not more toxic to mammals, birds or fish than non-GM cotton.	No	N/A	N/A	N/A	Not required
TOXICITY FOR OTHER ORGANISMS: **Non-target invertebrates, including soil insects**	**Very low**	**See Appendix 3** ▪ the toxicity of Cry1Ac is highly specific to Lepidopteran insect larvae; ▪ the CP4 EPSPS and NPTII enzymes are not known to be toxic to any organism; ▪ laboratory and field studies suggest that populations of key non-target invertebrates are unlikely to be affected by the Bt toxin. Indeed it is likely that their populations would be favoured by decreases in the use of broad-spectrum insecticides.	No	N/A	N/A	N/A	Not required
TOXICITY FOR OTHER ORGANISMS: **Microorganisms**	**Very low**	**See Appendix 3** ▪ laboratory studies indicate that Cry1Ac has no adverse affect on the growth of various bacteria, yeast, fungi, algae or protozoans; ▪ the presence of GM cotton (INGARD®) plant material in the soil produces only transient changes in soil microbial communities; ▪ natural degradation of Cry1Ac in the soil limits bioaccumulation.	No	N/A	N/A	N/A	Not required

HAZARD IDENTIFICATION	Risk Assessment (RA; combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management?	Risk Management (RM) options [* Preferred RM option(s)]	Reason for selecting RM option	Is risk managed?	Licence conditions (refer to Appendix 7 for detailed licence conditions)
WEEDINESS: **South of latitude 22º South**	**very low**	**See Appendix 4** ▪ cotton has a low potential for dispersal by natural means; ▪ the genetic modifications in these GM cottons have not affected these characteristics; ▪ surveys carried out since the commercial release of Roundup Ready® and Roundup Ready®/INGARD® cotton in 2000 indicate that these GM cottons have not become problematic weeds; ▪ major constraints on weediness of Roundup Ready®, Roundup Ready®/INGARD® and non-GM cotton are water availability, nutrient availability, plant competition, herbivory by non-lepidopteran species, frost and fire; ▪ cotton volunteers, whether Roundup Ready®, Roundup Ready®/INGARD® or non-GM, can establish on roadsides but do not persist or lead to spread into the wider environment; ▪ control of Roundup Ready® cotton volunteers can be achieved by cultivation or treatment with herbicides other than glyphosate; ▪ although the two traits (herbicide tolerance and insect resistance) may have an additive effect, neither of these traits individually has been found to be significant for weediness.	No However, in order to complete the environmental monitoring program required in relation to the original commercial release of Roundup Ready® and Roundup Ready®/INGARD® cotton in 2000, continuation of monitoring for volunteer cotton in non-agricultural situations will be required for an additional growing season.	N/A	N/A	N/A	Not required for management Research requirement: *1) incidence of Roundup Ready® and Roundup Ready®/INGARD® cotton volunteers in non-crop situations*: The licence holder must, in consultation with the OGTR, conduct an survey of roadsides for the incidence of Roundup Ready® and Roundup Ready®/INGARD® cotton volunteers.
WEEDINESS: **North of latitude 22º South**	**low**	**See Appendix 4** Although Monsanto seeks approval to grow INGARD® cotton only in southern Australia, the GMO may be transported north for use as stockfeed. INGARD® cottonseed has been used as stockfeed in northern Australia since 1996, and Roundup Ready®/INGARD® cotton since 2000, with no indication that they have become more weedy than non-GM cotton; however preliminary experimental data suggest that INGARD® cotton may be more weedy than non-GM cotton in certain nutrient-rich habitats such as artificial water ways and stock feeding areas.	Yes Persistence in northern Australia requires management, as risk of weediness is thought to be low but yet to be conclusively determined.	1) Limit scale of release. **2) *Limit location of release**. **3) *Secure cottonseed during transportation.** 4) ***Require the licence holder to communicate to recipients of cottonseed the need to adopt the removal of volunteers as a matter of good farm practice.**	*2) Limit location of release*: limits spread and persistence north of 22° South. *3) Secure cottonseed during transportation*: limits escape of seed into the environment. *4) Communicate the need to remove volunteers*: further limit the unlikely event of volunteer cotton populations establishing as a consequence of using cottonseed for stock feed.	Yes	*2) Limit location of release*: release limited to specified shires in NSW and QLD below 22° South. *3) Secure cottonseed during transportation*: cottonseed may only be transported in signed, covered vehicles. *4) Communicate the need to remove volunteers*: the licence holder must in consultation with the OGTR, develop a strategy to communicate the importance of monitoring for and removing cotton volunteers to all recipients of cotton seed. The licence holder must take all reasonable steps to implement the communication strategy.

				5) ***Require the licence holder to conduct an annual survey of areas where stock are fed to assess the extent of volunteer populations.** 6) disallow release.	*5) Conduct* an *annual survey*: enable the OGTR to determine whether the licence holder is successfully encouraging the adoption of volunteer removal by recipients of cottonseed.		*5) Conduct an annual survey*: the licence holder must, in consultation with the OGTR conduct an annual survey of areas where stock are fed and where stock graze.

HAZARD IDENTIFICATION	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management?	Risk Management (RM) options [* Preferred RM 0ption(s)]	Reason for selecting RM method	Is risk managed?	Licence conditions (refer to Appendix 7 for detailed licence conditions)
GENE TRANSFER: Plants • Other cotton crops	**Negligible**	**See Appendix 5** ▪ gene transfer would not pose any risks additional to the low risks posed by Roundup Ready® and Roundup Ready®/INGARD® cotton.	No	N/A	N/A	N/A	Not required
GENE TRANSFER: Plants • Volunteers and naturalised (feral) cotton	**Negligible**	**See Appendix 5** ▪ gene transfer would not pose any risks additional to the low risks posed by Roundup Ready® and Roundup Ready®/INGARD® cotton; ▪ volunteer cotton in the cotton growing regions of Australia already include Roundup Ready® and Roundup Ready®/INGARD® cotton, and there is no indication that these are more weedy than non-GM cotton; ▪ gene transfer to naturalised (feral) cotton populations is thought to be unlikely because of the geographic isolation.	No	N/A	N/A	Yes	Not required
GENE TRANSFER: Plants • Native cottons and other plant species	**Negligible**	**See Appendix 5** ▪ genetic incompatibility and geographical isolation prevent the production of fertile hybrids; ▪ well established genetic incompatibility prevents successful cross pollination with other plant species.	No	N/A	N/A	N/A	Not required
GENE TRANSFER: Microorganisms	**Negligible**	**See Appendix 5** ▪ all of the introduced genes in Roundup Ready® and Roundup Ready®/INGARD® cotton are already widespread in the environment, and are readily available for transfer from these sources via demonstrated natural mechanisms; ▪ gene transfer has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other sources of these genes.	No	N/A	N/A	N/A	Not required
GENE TRANSFER: Animals, including humans	**Negligible**	**See Appendix 5** ▪ the introduced genes of Roundup Ready® and Roundup Ready®/INGARD® cotton are not present in human food products; ▪ the likelihood of transfer is extremely low, and not greater than the likelihood of transfer from other sources of the introduced genes in the environment; ▪ transfer of the introduced genes of Roundup Ready® and Roundup Ready®/INGARD® cotton would not present a hazard to health and safety of human or animals.	No	N/A	N/A	N/A	Not required

HAZARD IDENTIFICATION	**Risk** (combines 'likelihood' and 'impact')	**Summary of Risk Assessment (refer to appendices for details)**	**Does risk require management?**	**Risk Management (RM) options [* Preferred RM 0ption(s)]**	**Reason for selecting RM method**	**Is risk managed?**	**Licence conditions (refer to Appendix 7 for detailed licence conditions)**
RESISTANCE **Insecticide**	**high**	**See Appendix 6** ▪ the likelihood of target lepidopteran pests developing resistance to the toxic effects of the Cry1Ac protein in INGARD® cotton is high; ▪ this risk is being managed by the APVMA.	Yes	***Continued use of insecticide resistance management strategies in accordance with APVMA requirements**	Decreases likelihood of insecticide resistance developing due to INGARD® cotton cultivation.	Yes	Not required Licence will note the requirement to adhere to the APVMA conditions, including their Insecticide Resistance Management Strategy.
RESISTANCE **herbicide**	**high**	**See Appendix 6** ▪ there is potential for development of herbicide-resistant weeds if the Roundup Ready® crop-herbicide combination is used inappropriately; ▪ this risk is being managed by the APVMA.	Yes	* **Continued use of herbicide resistance management strategies in accordance with APVMA requirements**	Decreases likelihood of herbicide resistance developing due the use of Roundup Ready® herbicide for weed control in Roundup Ready® cotton crops.	Yes	Not required Licence will note the requirement to adhere to the APVMA conditions on herbicide use, including any herbicide resistance management strategy.

APPENDIX 1 INFORMATION ABOUT THE GMO

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

51. This Appendix addresses these matters and provides detailed information about the GMOs for release, the parent organism, the genetic modification process, the genes that have been introduced and the new proteins that are expressed in the genetically modified cotton.

SECTION 1 SUMMARY INFORMATION ABOUT THE GMOS

52. Monsanto Australia Ltd (Monsanto) proposes to release two GMOs under application DIR 023/2002: herbicide tolerant Roundup Ready® cotton and herbicide tolerant/insect resistant Roundup Ready®/INGARD® cotton.

53. Roundup Ready® cotton is derived from an original genetic modification event referred to as Roundup Ready® cotton event 1445, which has subsequently been transferred into Australian cotton varieties by conventional breeding. Similarly, INGARD® cotton is derived from INGARD® cotton event 531. INGARD® cotton is the subject of a separate application (DIR 022/2002) and is only discussed in this document as far as it relates to Roundup Ready®/INGARD® cotton. Roundup Ready®/INGARD® cotton has been produced through conventional breeding of GM Roundup Ready® cotton with GM INGARD® cotton.

54. Roundup Ready® cotton contains the *epsps* gene from *Agrobacterium* species strain CP4. The *cp4 epsps* gene encodes an enzyme that is naturally tolerant to inhibition by glyphosate (Padgette et al. 1993), the active constituent of Roundup® herbicide, and has been introduced into the cotton plants to confer tolerance to the foliar application of glyphosate. Further details on the *cp4 epsps* gene and the CP4 EPSPS protein are provided in Sections 3 of this Appendix.

55. INGARD® cotton contains an insecticidal gene, *cry1Ac*, derived from the common soil bacterium *Bacillus thuringiensis* (Bt). This gene encodes a delta endotoxin protein (Bt toxin), Cry1Ac, which is a highly specific insecticidal protein that is toxic to lepidopteran caterpillar pests of cotton (Widner & Whiteley 1989; Van Rie et al. 1989; Dankocsik et al. 1990; Van Rie et al. 1990), including *Helicoverpa armigera* (cotton bollworm) and *H. punctigera* (native budworm). Further details on the *cry1Ac* gene and the Bt toxin are provided in Section 3 and in the Risk Assessment and Risk Management Plan for DIR 022/2002 (available at www.ogtr.gov.au).

56. The GM cotton plants also contain two bacterial antibiotic resistance genes. These genes were used as selectable marker genes in the early laboratory stages of development of the plants, to enable selection of bacteria or plant cells containing the desired genetic modification. The neomycin phosphotransferase type II (*nptII*) gene confers resistance to

the antibiotics kanamycin and neomycin. The aminoglycoside adenylyltransferase (*aad*) gene confers spectinomycin and streptomycin resistance but is linked to a bacterial promoter that does not function in plants, so the protein is not expressed in the GM cotton. The antibiotic resistance genes are discussed in more detail in Section 3 of this Appendix. Potential risks relating to transfer of these genes to bacteria are discussed in Appendix 5.

57. Roundup Ready®/INGARD® cotton lines contain the herbicide tolerance gene (*cp4 epsps*) in combination with the insecticidal gene (*cry1Ac*), as well as two copies of each of the antibiotic resistance genes (*nptII* and *aad*).

58. The GM cotton lines proposed for Australian release are backcross progeny of conventional crosses involving Roundup Ready® cotton GM event 1445 and/or INGARD® cotton GM event 531 and elite Australian cotton cultivars that are suitable for Australian cotton production areas. The methods used to introduce the genes into cotton are discussed in Section 4 of this Appendix.

SECTION 2 THE PARENT ORGANISM

59. A comprehensive review of the parent organism, *Gossypium hirsutum* L. (cultivated cotton), is provided in the document 'The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia' (OGTR 2002), available at www.ogtr.gov.au.

SECTION 3 THE INTRODUCED GENES AND THEIR PRODUCTS

Section 3.1 The *cp4 epsps* gene and encoded protein

60. The *cp4 epsps* gene, which confers tolerance to glyphosate (N-(phosphonomethyl) glycine), the active ingredient of Roundup® herbicide, was isolated from *Agrobacterium* sp. strain CP4.

61. EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) is a critical enzyme in aromatic amino acid biosynthesis, catalysing the addition of the enolpyruvyl moiety of phosphoenolpyruvate to shikimate-3-phosphate, and is the target of the herbicide glyphosate. EPSPS is essential for the synthesis of aromatic amino acid and almost all other aromatic compounds in plants, bacteria, algae and fungi but is absent from mammals (Bentley 1990; Eschenburg et al. 2002). Inhibition of EPSPS by glyphosate blocks these metabolic pathways which eventually leads to cell death (Steinrucken & Amrhein 1980). CP4 EPSPS is naturally insensitive to glyphosate (Padgette et al. 1993), as are a number of other microbial EPSPS enzymes (Schulz et al. 1985; Eschenburg et al. 2002).

62. A coding sequence of the *cp4 epsps* gene was modified by site-directed mutagenesis to achieve optimal expression in plants (Padgette et al. 1993). Although the gene sequence has been altered, the enzyme produced from the plant-optimised gene has exactly the same amino acid sequence as that of the native *Agrobacterium* enzyme, and its activity has been shown to be unaltered (information provided by the applicant).

63. The promoter (a region of DNA that determines whether or not a gene is expressed, and to what extent) controlling expression of the *cp4 epsps* gene in Roundup Ready® cotton

is the CMoVb promoter (34S promoter of the caulimovirus figwort mosaic virus) (Richins et al. 1987; Gowda et al. 1989; Sanger et al. 1990). This is linked to the chloroplast transit peptide (CTP) coding region from a plant *epsps* gene (from *Arabidopsis thaliana* (Klee et al 1987)). The CTP targets the EPSPS enzyme to the chloroplast, the site of aromatic amino acid biosynthesis. In plants, EPSPS is synthesised as a preprotein (ie. containing the CTP) by free cytoplasmic ribosomes. The precursor is transported into the chloroplast stroma and proteolytically processed to yield the mature enzyme (della-Cioppa et al. 1986). Once cleaved, chloroplast transit peptides are rapidly degraded (Bartlett et al. 1982; della-Cioppa et al. 1986).

64. Also required for gene expression in plants is a mRNA termination region, including a polyadenylation signal. For the *cp4 epsps* gene in Roundup Ready® cotton this is derived from the 3' non-translated region of the *nos* gene from *Agrobacterium tumefaciens*.

65. CP4 EPSPS was detected by Western blot analysis of protein extracts of Roundup Ready® cottonseed (Barry et al. 1993). An antibody specific for CP4 EPSPS reacted with a protein of 48 kD, the expected molecular weight for the protein minus the CTP, confirming that this peptide is cleaved during transport into the chloroplast. Potential hazards relating to toxicity and allergenicity of this enzyme are discussed in Appendices 2 and 3.

Section 3.2 The *cry1Ac* gene and encoded protein

66. The *cry1Ac* gene in INGARD® cotton is derived from *Bacillus thuringiensis*, a gram positive, spore-forming, soil bacterium that is ubiquitous in the environment. The Cry (for crystaline) proteins (also Bt proteins or Bt toxins) are a diverse family of insecticidal proteins produced by various subspecies of *Bacillus thuringiensis*. They are classified according to their target specificity and their degree of amino acid homology (Hofte & Whiteley 1989). For example the Cry1 toxins, including the Cry1Ac toxin from *B. thuringiensis* variety *kurstaki* (Btk), are highly specific to lepidopteran insects (moths and butterflies) (Widner & Whiteley 1989; Dankocsik et al. 1990; Macintosh et al. 1990).

67. The toxic effect of Bt proteins requires alkaline conditions (as provided in the larval insect gut) to dissolve the crystals, partial digestion by specific proteases to release the active core toxin and binding to specific receptors found on the insect midgut epithelium surface. Binding leads to formation of pores in the cell membrane and eventually cell death, gut paralyses and starvation. It is these steps that provide the high degree of target specificity of each Bt toxin (Hofmann et al. 1988; Van Rie et al. 1989; English & Slatin 1992; Knowles & Dow 1993).

68. The Cry1Ac protein expressed in INGARD® cotton is in fact a chimeric gene that combines parts of two genes isolated from Btk. Part of the *cry1Ab* gene (nucleotides 1 - 1398, corresponding to amino acids 1 – 466 (Fischhoff et al. 1987)) was linked to a portion of the *cry1Ac* gene (nucleotides 1399-3534, corresponding to amino acids 467 - 1178 (Adang et al. 1985)). The hypervariable region responsible for insecticidal specificity is from the Btk *cry1Ac* gene, hence the chimeric gene is referred to in this document as the *cry1Ac* gene.

69. The coding sequence of this gene has been further modified to achieve optimal expression in plants. The Cry1Ac protein expressed in INGARD® cotton is 99.4% identical to the native Btk Cry1Ac protein (Adang et al. 1985).

70. Expression of the *cry1Ac* gene in INGARD® cotton is driven by a modified (enhanced) 35S promoter from cauliflower mosaic virus (CaMV) (Odell et al. 1985; Kay et al. 1987). The mRNA termination region is provided by the 3' non-translated region of the soybean alpha subunit of the beta-conglycinin gene (referred to as the 7S 3' termination sequence) (Schuler et al. 1982).

71. Western blot analysis was used to compare the Cry1Ac protein expressed in INGARD® cotton with commercially available microbial pesticides containing numerous Bt protoxins (Berberich & Fuchs 1992). This study showed that the protein expressed by INGARD® cotton is of similar molecular weight and immunological reactivity to one or more proteins contained in the commercial Bt products Dipel® (Abbott Laboratories) and Thuricide® (Sandoz Inc.).

72. Sims (1994) demonstrated that the biological activity and species-specificity of the full-length Cry1Ac protoxin expressed in INGARD® cotton are equivalent to those of a Cry1Ac core toxin expressed in Btk.

73. More detailed information about the cry1Ac gene present in INGARD® cotton, and the Bt toxins, can be found in the Risk Assessment and Risk Management Plan for DIR 022/2002 (available at www.ogtr.gov.au).

Section 3.3 The *nptII* gene and encoded protein

74. The *nptII* gene was isolated from the bacterial Tn5 transposon (Beck et al. 1982). It encodes an enzyme, neomycin phosphotransferase type II (NPTII), which confers resistance to the aminoglycoside antibiotics kanamycin and neomycin. The NPTII enzyme uses ATP to phosphorylate neomycin, and the related kanamycin, thereby inactivating the antibiotic and preventing it from killing the NPTII producing cell.

75. The NPTII protein is widespread in the environment and in food chains, in naturally occurring kanamycin-resistant microorganisms found in soil and in mammalian digestive systems (Flavell et al. 1992).

76. Expression of the *nptII* gene present in both Roundup Ready and INGARD® cotton is controlled by the CaMV 35S promoter (Odell et al. 1985), hence the NPTII enzyme is produced in the GM cotton plants. The mRNA termination region of the gene is derived from the 3' non-translated region of the *nos* gene from *A. tumefaciens* (Depicker et al. 1982).

77. The *nptII* gene functioned as a selectable marker in the initial laboratory stages of cotton plant cell selection following genetic modification (De Block et al. 1984; Horsch et al. 1984), allowing modified cells to grow while inhibiting the growth of non-GM cells. The potential hazard of toxicity of NPTII is discussed in Appendices 2 and 3, and that of antibiotic resistance gene transfer in Appendix 5.

Section 3.4 The *aad* gene and encoded protein

78. The second antibiotic resistance gene, *aad*, was isolated from the bacterial Tn7 transposon and confers resistance to the antibiotics spectinomycin and streptomycin (Davies & Benveniste 1974). This gene encodes the enzyme 3'(9)-O-aminoglycoside adenylyltransferase (AAD) and is under control of its own bacterial promoter. This gene is not expressed in GM cotton plants because its native bacterial promoter is not active in plants and regulatory elements necessary for expression in plants have not been added to the gene.

79. The *aad* gene was used in the laboratory, prior to production of the genetically modified plants, to select for bacteria carrying the plasmid with the desired modified DNA.

SECTION 4 METHOD OF GENE TRANSFER

80. Roundup Ready® cotton and INGARD® cotton were each produced separately by *Agrobacterium*-mediated DNA transformation (Zambryski 1992). Each genetic modification was made in the Coker 312 cotton variety, because of its positive response to the tissue culture system used in producing GM plants. Coker 312 was grown commercially in the US but will not be commercially produced in Australia.

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

82. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains do not contain the genes (*iaaM*, *iaaH* and *ipt*) responsible for the overproduction of auxin and cytokinin, which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). A useful feature of the Ti plasmid is the flexibility of the *vir* region to act in either cis or trans configurations to the T-DNA. This has allowed the development of two types of transformation systems:

- co-integration vectors that join the T-DNA that is to be inserted into the plant and the *vir* region in a single plasmid (Stachel & Nester 1986); and
- binary vectors that have the T-DNA and *vir* regions segregated on two plasmids (Bevan 1984).

83. Both provide functionally equivalent transformation systems. *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants without causing any biosafety problems.

84. In this case binary, single-border plasmid transformation vectors were used for each genetic modification. These plasmids contain well characterised DNA segments required for their replication and selection in bacteria, and for transfer of DNA from *Agrobacterium* and its integration into the plant cell genome (Bevan 1984; Wang et al. 1984).

85. Roundup Ready® cotton was produced using the plasmid PV-GHGT07, carrying the *cp4 epsps*, *nptII* and *aad* genes. This plasmid also carried a copy of the *gox* gene (encoding the glyphosate oxidoreductase enzyme (GOX), which metabolises glyphosate) from the bacterium *Ochromobacterium anthropii*, although this gene was not transferred to the plant genome in Roundup Ready® cotton event 1445.

86. INGARD® cotton (GM event 531) was produced using the plasmid PV-GHBK04, carrying the *Cry1Ac*, *nptII* and *aad* genes. INGARD® cotton is only discussed in this document as far as it relates to Roundup Ready®/INGARD® cotton. More detail about the genetic modification in INGARD® cotton can be found in the Risk Assessment and Risk Management Plan for DIR 022/2002 (available at www.ogtr.gov.au).

87. Roundup Ready®/INGARD® cotton was generated by conventional cross breading of Roundup Ready® and INGARD® GM cotton varieties and contains all of the genes which had been introduced into each of the parental GM varieties.

SECTION 5 CHARACTERISATION AND STABILITY OF THE INSERTED GENETIC MATERIAL

88. Southern blot analysis was used to demonstrate that a single copy of the *cp4 epsps*, *nptII* and *aad* genes has been inserted at one location into the cotton genome in Roundup Ready® cotton GM event 1445. The *gox* gene (see Section 4), although present in the intermediate plasmid vector, was not transferred to the plant genome. The T-DNA region of the insert was truncated before the *gox* gene began (this is not uncommon, see for example Bakkeren et al. (1989) and De Block et al. (1984)). The insert was shown to be maintained in a stable condition within the cotton genome for three generations (data supplied by the applicant).

89. Detailed information on the characterisation of the inserted genetic material and stability of the genetic modification in INGARD® cotton GM event 531 is provided in the Risk Assessment and Risk Management Plan for DIR 022/2002 (available at www.ogtr.gov.au). This GMO contains one complete copy of each of the *cry1Ac*, *nptII* and *aad* genes stably integrated at one location in the cotton genome.

90. Confirmation of the stability of the introduced genetic material in Roundup Ready® and Roundup Ready®/INGARD® cotton lines is also provided by the continued expression of the desired traits over many generations of breeding and several years of trialing and commercialisation (see Section 7).

SECTION 6 EXPRESSION OF THE INTRODUCED PROTEINS

91. Expression levels of EPSPS, Cry1Ac and NPTII proteins in various plant tissues of Roundup Ready® cotton, INGARD® cotton and Roundup Ready®/INGARD® cotton, from plants grown under field conditions in different locations and over numerous seasons, have been determined. Some representative data from these analyses are presented in this section.

Section 6.1 Roundup Ready® cotton

92. The concentrations of the CP4 EPSPS and NPTII enzymes were measured by enzyme linked immunosorbent assay (ELISA) in leaf and seed samples of Roundup Ready® cotton from US field trials (Table 1). In 1993 and 1994 samples were taken from six sites across the US cotton growing regions (Nida et al. 1994; Nida et al. 1995; Nida et al. 1996) and in 1998 and 1999 from four sites (Kolwyck et al. 1999; Kolwyck et al. 2000).

93. The CP4 EPSPS enzyme was detected at low levels in Roundup Ready® cotton in both leaves (season averages of 41 – 53 μg/g [or ppm]) and seed (60-299 μg/g). The NPTII enzyme was also detected at low levels in leaves (season averages of 29 - 45 μg/g) and seeds (7 - 35 μg/g).

94. Treatment of the plants with glyphosate did not alter the levels of CP4 EPSPS or NPT II (Nida et al. 1995).

95. Neither of these proteins was detected in the parental non-GM cotton varieties. The samples from the 1993 field trials were also assayed for the AAD protein (see Section 3) and GOX protein (see Section 4). As expected, these proteins were not detected in any sample. The *aad* gene is not expressed as its bacterial promoter is not active in plants, while the *gox* gene was not transferred into the cotton genome in Roundup Ready® cotton GM event 1445.

Section 6.2 INGARD® cotton

96. The concentration of the Cry1Ac and NPTII proteins were measured in leaf and seed samples of INGARD® cotton from US field trials in 1998 and 1999 (Kolwyck et al. 1999; Kolwyck et al. 2000) (Table 1). The Cry1Ac protein was found at very low levels in both young leaves (season averages of 3.7 and 5.0 μg/g) and seeds (3.7 and 4.3 μg/g). NPTII was also found at very low levels in young leaves (season averages of 4.9 and 5.4 μg/g) and seeds (5.4 and 13.2 μg/g).

97. The level of Cry1Ac protein in pollen and nectar from greenhouse grown INGARD® cotton has also been determined, to provide information on the potential for non-target insect exposure to the toxin (Ream 1994a). The level of Cry1Ac protein measured in pollen was 0.0115 μg/g fresh weight, representing 0.000164% of total pollen protein. No protein was detected in nectar, including Cry1Ac (the limit of detection for Cry1Ac was 0.0016 μg/g fresh weight).

Section 6.3 Roundup Ready®/INGARD® cotton

98. Expression levels of the Cy1Ac, CP4 EPSPS and NPTII proteins were also estimated in leaves and seeds from Roundup Ready®/INGARD® cotton (Kolwyck et al. 1999; Kolwyck et al. 2000). The concentrations of the Cry1Ac and CP4 EPSPS proteins were similar in the Roundup Ready®/INGARD® cotton as in the respective single trait GM parental cotton lines (Table 1). The NPTII protein was expressed to similar levels in Roundup Ready® and Roundup Ready®/INGARD® cotton in both leaves and seed.

99. These proteins were not detected in the non-GM parental cotton variety (Kolwyck et al. 1999; Kolwyck et al. 2000).

Table 1 Expression of the introduced proteins in Roundup Ready®, INGARD â and Roundup Ready®/INGARD â cotton

Season	Tissue	Protein [1]	Roundup Ready® [2]	INGARD® [3]	Roundup Ready®/ INGARD® [4]
1993	**leaf**	**CP4 EPSPS**	52± 16	Not reported	Not reported
		NPTII	45 ± 14		
	seed	**CP4 EPSPS**	82 ± 17	Not reported	Not reported
		NPTII	6.7 ± 1.0		
1994	**seed**	**CP4 EPSPS**	60 ± 12	Not reported	Not reported
		NPTII	7.0 ± 3.0		
1998	**leaf**	**CP4 EPSPS**	41.8 ± 8.64	ND	53.2 ± 12.8
		NPTII	29.3 ± 7.15	5.44 ± 1.82	30.8 ± 6.3-4
		Cry1Ac	ND[3]	3.72 ± 1.86	3.34 ± 1.23
	seed	**CP4 EPSPS**	299 ± 44.2	ND	313 ± 79.5
		NPTII	35.1 ± 3.35	13.2 ± 2.48	34.0 ± 6.44
		Cry1Ac	ND	3.70 ± 0.66	3.09 ± 0.77
1999	**leaf**	**CP4 EPSPS**	53.2 ± 5.79	ND	79.7 ± 28.5
		NPTII	30.3 ± 7.98	4.94 ± 1.70	35.1 ± 10.3
		Cry1Ac	ND	5.00 ± 1.84	4.84 ± 173
	seed	**CP4 EPSPS**	138 ± 61.3	ND	146 ± 52.9
		NPTII	14.8 ± 2.57	5.48 ± 0.83	22.0 ± 4.39
		Cry1Ac	ND	4.3 ± 0.86	3.81 ± 0.71

[1] Protein levels are reported as µg/g [equivalent to ppm] fresh weight ± standard deviation, as determined by ELISA.
[2] Roundup Ready® event 1445 in Coker 312 cotton variety in 1993 and 1994, and in DP5415 cotton variety in 1998 and 1999.
[3] INGARD® event 531 in DP5415 cotton variety.
[4] INGARD®/Roundup Ready® in DP5415 cotton variety.
ND = Not Detectable; below the limit of detection of the assay.

SECTION 7 COMPARISON OF GM AND NON-GM COTTON CHARACTERISTICS

100. The demonstration of agronomic and compositional similarity of the GM cotton varieties and conventional cotton indicates that no significant pleiotropic or epistatic effects (that is, unintended effects of a genetic change on other, apparently unrelated, plant genes or plant characteristics) have occurred.

Section 7.1 Agronomic performance

101.Agronomic performance and varietal selection trials conducted during development of Roundup Ready® cotton, INGARD® cotton and Roundup Ready®/INGARD® cotton have been monitored closely for differences between the GM and parental non-GM cotton varieties. New elite varieties are only released if they are agronomically and commercially acceptable (for example see Monsanto Australia Limited (2001))

102.Field trials in the US in 1998 (Hamilton & Reed 1999) and 1999 (Hamilton et al. 2000) demonstrated the agronomic equivalence of the Roundup Ready®, INGARD®, Roundup Ready®/INGARD® and non-GM cotton lines in terms of germination, growth habit, plant morphology and disease susceptibility. Fibre characteristics (length, strength, diameter) of the GM cottons are also equivalent to those of non-GM cotton (Cotton Seed Distributors 2002).

103.Both above and below ground parts of trial plants have been examined for the presence of disease development. Because the cotton plants were genetically modified using a disarmed *A. tumefaciens* vector, plants were specifically examined for the development of crown gall disease throughout the growing season. The GM varieties have been found to be equivalent to their non-GM parental varieties (information provided by the applicant).

104.Roundup Ready® cotton varieties have been assessed for tolerance to Roundup® herbicide application. Vegetative tolerance is high throughout the growing season but reproductive tolerance is relatively low (Monsanto Australia Limited 2001). Consequently, label instructions limit foliar application of glyphosate to early growth, up to the four-leaf stage, to limit damage to developing floral tissue. Later applications must be made using precision post-directed or hooded/shielded sprayers to avoid potential yield loss.

105.Data from both US and Australian trials indicate that the application of glyphosate to Roundup Ready® cotton can lead to loss of fruit from early fruiting positions, even for application at the three or four leaf stage, although this is not correlated to yield loss due to compensatory growth by the plants (Monsanto Australia Limited 2001; Jones & Snipes 2002). Fibre quality is similar in Roundup Ready® and Roundup Ready®/INGARD® cotton (whether glyphosate treated or not) and non-GM cotton (Monsanto Australia Limited 2001).

106.Australian field evaluation of INGARD® cotton has determined that the Cry1Ac expression in INGARD® cotton provides a high level of control of *Helicoverpa* target species for most of the season, with control diminishing after early to peak flowering time (Fitt et al. 1998).

107.Yields of the processed cottonseed fractions (linters, delinted seed, hulls, kernels, toasted meal, crude oil and refined oil) were statistically similar for parental non-GM cotton lines and Roundup Ready® cotton (Nida et al. 1994) or INGARD® cotton (Fuchs 1994), and comparable to the means and ranges previously reported for processed fractions from other cotton varieties.

Section 7.2 Compositional analysis of cottonseed

108.The results of extensive compositional analyses of whole cottonseed and processed cottonseed fractions demonstrate that the levels of the important nutritional and anti-nutritional components in Roundup Ready®, INGARD® and Roundup Ready®/INGARD® cotton are comparable to the parental non-GM varieties and are within established ranges for commercial cotton varieties.

109.Data from US field trials in 1993 and 1994 indicate that the nutrient composition of Roundup Ready® cotton is within the normal range for cottonseed in terms of the concentration of protein, oils, carbohydrate and ash, and amino acid and fatty acid profiles (Nida et al. 1994; Nida et al. 1995; Nida et al. 1996). The presence of CP4 EPSPS, an enzyme of the aromatic amino acid biosynthetic pathway, has not increased the level of aromatic amino acids. The levels of known anti-nutritional or toxic factors in Roundup Ready® cottonseed and cottonseed oil, including gossypol and cyclopropenoid fatty acids, are within the range of non-GM cotton controls. Treatment of the cotton with glyphosate had no effect on the nutrient composition or the levels of anti-nutritional or toxic factors (Nida et al. 1994; Nida et al. 1995; ANZFA 2001).

110.Seed composition was analysed from field trials in 1998, including three cotton breeding varieties in the US and one variety in the EU, each variety combined with Roundup Ready®, INGARD® and Roundup Ready®/INGARD® traits (Hamilton 2001). Comparisons were made between the Roundup Ready®/INGARD® lines and their single trait (Roundup Ready® and INGARD®) parental lines. Constituents measured were protein (including 18 individual amino acids), fat (including 12 individual fatty acids), ash, carbohydrate, calories, moisture, 9 minerals and gossypol. Of all these constituents (47 in total), in only three comparisons was a Roundup Ready®/INGARD® cotton line different to both of its respective GM parental lines. Each of these differences was found in only one of the cotton varieties, indicating that they are unlikely to be the result of the Roundup Ready® or INGARD® traits, and the mean values were within the ranges of corresponding values from the non-GM varieties involved.

111.Further compositional analysis was conducted for one of these breeding varieties, from field trials planted at four sites in the US in 1999 (Pyla et al. 2001). Comparisons were made between the Roundup Ready®/INGARD® line and the single trait (Roundup Ready® and INGARD®) parental lines as well as the non-GM line. Again 47 constituents were analysed. Most nutrients and anti-nutritional factors were not statistically different between the GM cotton lines and the non-GM parental variety, at each of four trial sites. Some comparisons were statistically different at only one site, while a few were also different for across-site averages, however the range of all values were within the expected range for non-GM cotton, with the exception of a single iron value for INGARD® cotton from one sample at one location. This value (73.34 μg/g) was slightly above the expected range (33.68 – 67.41 μg/g).

112.Thus the nutritional value and seed composition, including levels of endogenous toxicants, of Roundup Ready® and Roundup Ready®/INGARD® cotton is equivalent to that of non-GM cotton.

APPENDIX 2 HUMAN HEALTH AND SAFETY

113.Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan. This Appendix considers potential hazards that may be posed to human health and safety as a result of any toxicity or allergenicity of the GMO or its novel proteins.

114.It should be noted that since the commercial release of INGARD® cotton in 1996 and of Roundup Ready® and Roundup Ready®/INGARD® cotton in 2000, there have been no reported adverse toxic or allergic effects on human health resulting from occupational exposure, from ingestion of foods derived from the oil or linters of these GM cottons or from the use of other products containing their oil, lint or linters.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

115.Toxicity is the cascade of reactions resulting from exposure to a dose of chemical sufficient to cause direct cellular or tissue injury or otherwise inhibit normal physiological processes (Felsot 2000b). Allergic responses are immune system reactions, resulting from stimulation of a specific group of antibodies (known as IgE) or sensitisation of specific tissue bound lymphocytes (Taylor & Lehrer 1996; FAO/WHO 2000). Allergy has a well defined etiology (ie. biochemical cause) that is quite different from toxicity.

116.Roundup Ready® and Roundup Ready®/INGARD® cotton differ from conventional cotton in the expression of two and three additional proteins, respectively. These are the CP4 EPSPS and NPTII enzymes (in both GMOs) and the Cry1Ac protein (in Roundup Ready®/INGARD® cotton only) (see Appendix 1 for details of protein expression in the GMOs). The potential for these cottons to be toxic or allergenic to humans due to either expression of the novel gene products or because of unforeseen, unintended effects of the genetic modification is considered here.

117.The use of Roundup Ready® herbicide (a formulation of glyphosate) on Roundup Ready® and Roundup Ready®/INGARD® cotton crops in Australia is registered by the Australian Pesticides & Veterinary Medicines Authority (APVMA, formerly National Registration Authority for Agricultural and Veterinary Chemicals, NRA). As part of their assessment of this use, the APVMA consider any potential human health effects, for example risk arising through occupational exposure or residues in food. Thus risks associated with the use of glyphosate are not considered in the risk assessment of these GM cottons.

SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

118.In assessing the likelihood of adverse impacts due to toxicity or allergenicity of Roundup Ready® and Roundup Ready®/INGARD® cotton on human health and safety, the following factors were considered:

- the inherent toxicity and allergenicity of conventionally bred cotton;

- the potential exposure to these GM cottons, to their products and to the new proteins which are expressed in the cottons, the CP4 EPSPS, Cry1Ac and NPTII proteins;
- the potential exposure to the CP4 EPSPS, Cry1Ac and NPTII proteins from other sources in the environment;
- the potential toxicity and allergenicity of the new proteins expressed in the GM cottons; and
- the potential toxicity and allergenicity of Roundup Ready® and Roundup Ready®/INGARD® cotton (the GMOs).

Section 2.1 Toxicity and allergenicity of conventionally bred cotton

119.Cotton is a well established field crop with a long history of safe use. A comprehensive review of conventional cotton, including information on its toxicity and allergenicity, is provided in the document 'The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia' (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at www.ogtr.gov.au. Information on non-GM cotton is included here to establish a base-line for comparison with the GM cottons being considered in this risk assessment.

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

121.Processed cotton fibre contains 99.8% cellulose and is widely used in pharmaceutical and medical applications because of its very low allergenicity. Cottonseed oil has been in common use since the middle of the nineteenth century and achieved GRAS (Generally Recognised As Safe) status under the United States Federal Food Drug and Cosmetic Act because of its common use prior to 1958 (ANZFA 2002).

122.Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002), therefore its potential to act as an airborne allergen is extremely low. However, inhalation of cotton dust by mill workers can cause byssinosis, an asthma-like condition, in sensitive individuals. Preventative measures such as the use of facemasks have been successful in lowering the incidence of this condition.

Section 2.2 Exposure of people to cotton

123.People come into contact with cotton plants and cotton products in a variety of circumstances. Potentially, harm could occur if the GM cotton was toxic or allergenic for people, through:

- eating foods containing cottonseed oil or cotton linters;
- wearing cotton clothing or using household items made from cotton lint, cotton linters or cottonseed oil;
- working with cotton (eg. on cotton farms, in cotton processing facilities); or

- living in or near the areas where cotton is grown.

124.As discussed in Appendix 1, the introduced proteins are expressed at low levels in Roundup Ready® and Roundup Ready®/INGARD® cotton tissues. Exposure to the new proteins expressed in the GM cottons will be limited further according to the routes of exposure.

2.2.1 Exposure to cotton products in food

125.Cottonseed meal is not used for human consumption in Australia but is approved for use in human food in the USA and other countries, when derived from gossypol-free varieties of cotton or after processing to remove the gossypol. Human consumption of cotton seed meal is reported mainly in central American countries and India where it is used as a low cost, high quality protein ingredient (Franck 1989; Ensminger et al. 1990).

126.Only cottonseed oil and linters (short fibres removed from the seed coat) are used for human consumption in Australia (OGTR 2002). Many food products eaten on a daily basis contain these ingredients. Examples of common food products that may contain cottonseed oil are blended vegetable oils, margarines and salad dressings. Cotton linters are used as a cellulose base in foods such as high fibre dietary products and smallgoods casings, as well as a viscosity enhancer (thickener) in ice creams and salad dressings.

127.Cottonseed oil and cotton linters are highly refined and processed, with no detectable DNA (genetic material) or proteins (Leffler & Tubertini 1976; Sims et al. 1996). Oil and linters derived from the GM cottons can not be distinguished from those derived from non-GM cotton. Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority, ANZFA) has responsibility for assessing the safety of food for human consumption. FSANZ considers that food products for human consumption that are derived from INGARD® cotton and Roundup Ready® cotton (oils and linters) are as safe as those derived from conventional cotton varieties (ANZFA 1999; ANZFA 2001).

2.2.2 Exposure to cotton products through wearing clothing and using household products made from cotton lint, cotton linters and cottonseed oil

128.Cotton fabrics, used in clothing, upholstery, towels and other household products, are made from the cotton lint (long fibres) which surrounds the cotton seed. Household products that may contain cotton linters include 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 and variety of plastics (Gregory et al. 1999). Cotton fibre is widely used in pharmaceutical and medical applications because of its very low allergenicity.

129.Processed cotton lint, linters and oil contain no detectable DNA or protein (Leffler & Tubertini 1976; Sims et al. 1996). Fibre characteristics (length, strength, fineness) of Roundup Ready® and Roundup Ready®/ INGARD® cotton are the same as for non-GM varieties (Cotton Seed Distributors 2002). Therefore the safety of wearing cotton clothing or using other products made from cotton is not likely to be effected by the genetic make-up of the

cotton plants from which these components have been derived, that is, whether Roundup Ready®, Roundup Ready®/INGARD® or non-GM.

2.2.3 Exposure to cotton through working with cotton and living near cotton plantations

130.Humans working with cotton plants would be exposed primarily to the outer waxy cuticle layer at the plant surface, to the seed coat or to the cotton fibres, all of which are essentially free of protein. Exposure to proteins (including the new proteins expressed in the GM cottons) or to other cellular components of the cotton plants will only occur if plant cells are ruptured. Even if the cells rupture, exposure to the new proteins expressed in Roundup Ready® and Roundup Ready®/INGARD® cottons will be very low, as these proteins are only present at low levels in the GM cotton tissues (see Appendix 1 for details).

131.Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002), therefore limiting possible exposure to cotton pollen as a potential airborne allergen. The introduced proteins are expected to be expressed at very low levels in the pollen of these GM cottons, based on expression of the Cry1Ac protein in INGARD® cotton pollen (relative to that in other plant tissues) and the similarity of the promoter elements controlling the expression of the introduced genes (see Appendix 1).

132.The primary processing of cotton at cotton gins, and the bulk handling of cottonseed and cotton fibre, can create and stir up fine dust and lint particles. Use of personal protective equipment by exposed workers is commonplace in such facilities, to prevent respiratory irritations. Fibre characteristics (length, strength, fineness) of Roundup Ready® and Roundup Ready®/ INGARD® cotton are the same as for non-GM cotton varieties (Cotton Seed Distributors 2002). Thus GM cotton lint is no more likely to induce adverse responses in workers than is conventional cotton.

Section 2.3 Other sources of CP4 EPSPS, Cry1Ac and NPTII in the environment

133.The CP4 EPSPS, Cry1Ac and NPTII proteins are widespread in the environment, through the presence of the bacteria to which they are native. All three proteins can be found in or on fresh food.

134.EPSPS enzymes are present in all plants, bacteria and fungi. The difference between the natural plant enzymes and the bacterially derived CP4 EPSPS is in the amino acid sequence, not in the physiological function (see Appendix 1 for details). Other EPSPS enzymes present in food, from both plant and microbial sources, also vary to a similar degree in amino acid sequence (Felsot 2000a; Padgette et al. 1996). The CP4 EPSPS enzyme is derived from the common soil bacterium *Agrobacterium* species strain CP4 (Padgette et al. 1996), which can be found on plants and plant produce.

135.The native Cry1Ac protein is naturally produced by the bacterium *Bacillus thuringiensis* variety *kurstaki* (Btk). Related Cry toxins are also produced by other varieties of *Bacillus thuringiensis* (see Appendix 1). Bt spores and their crystal toxins are found widely in soils, on plant leaves and in grain stores (Meadows 1993).

136.The presence of the Cry1Ac protein in food has increased over the past 30 years due to the commercial use of Btk microbial sprays to protect food crops, including 'organic' crops, from insect attack. Residues of Btk proteins, including Cry1Ac, are present on a wide variety of foods such as cabbage, lettuce and tomato with no reported toxic or allergic responses (ANZFA 1999). The World Health Organisation's (WHO) International Program on Chemical Safety report on environmental health criteria for Bt concluded that 'Bt has not been documented to cause any adverse effects on human health when present in drinking water or food' (International Programme on Chemical Safety 1999). The Cry1Ac protein produced by the INGARD® cotton lines is almost identical (greater then 99.4% identity) to that found in nature and in the Btk microbial insecticide formulations (Adang et al. 1985).

137.Humans continually ingest kanamycin-resistant microorganisms, some containing the NPTII enzyme. The diet, especially raw salad, is the major source: at a conservative estimate, each human ingests 1.2×10^6 kanamycin-resistant microorganisms daily (Flavell et al. 1992). Large numbers of kanamycin- or neomycin-resistant bacteria already inhabit the human digestive system (Levy et al. 1998), with Flavell et al. (1992) estimating about 10^{12} per person.

Section 2.4 Toxicity and allergenicity of the introduced proteins

2.4.1 Toxicity

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

2.4.1.1 CP4 EPSPS

139.Purified CP4 EPSPS enzyme, at acute doses of up to 572 mg/kg body weight, produced no adverse effects in mice. This is more than a thousand times the anticipated potential consumption of CP4 EPSPS in commercial foods derived from all GM food crops expressing this enzyme under development by Monsanto at that time (soybean, potato, tomato, corn) (Harrison et al. 1996).

2.4.1.2 CRY1AC

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

141.A two-year chronic rat feeding study was undertaken with Bt microbial products at doses of up to 8400 mg/kg of body weight/day. A decrease in weight gain was observed in female rats at this dose but, in the absence of any other adverse findings (eg survival, clinical

observations or pathology), this was not considered to indicate Cry protein toxicity (McClintock et al. 1995).

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

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

2.4.1.3 NPTII

144.An acute oral toxicity study in mice, in which the purified NPTII protein was fed at doses of up to 5000 mg/kg of body weight (2500 mg/kg administered twice, four hours apart), did not show any adverse effects (Berberich et al. 1993). There were no treatment-related differences in mortality, weight gain, food consumption, behaviour, clinical signs or gross pathology.

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

2.4.2 Allergenicity

146.Although there are no predictive assays available to assess the allergenic potential of proteins, much is known about the biochemical events associated with allergic reactions, as well as the kinds of proteins that cause problems (Metcalfe et al. 1996; Taylor & Lehrer 1996). For example, food allergens are usually present as a major component of the ingested food and are resistant to heat, protease digestion and to the acid conditions of the stomach (Astwood et al. 1996; Metcalfe et al. 1996; Taylor & Lehrer 1996; Kimber et al. 1999).

147.The allergenic proteins of many major sources of allergens, including food allergens, have been characterised by molecular means, allowing comparisons to be made as a useful step in assessment of allergenic potential (Metcalfe et al. 1996).

148.None of the introduced proteins in Roundup Ready® and Roundup Ready®/INGARD® cotton are derived from known allergens, nor are they present as major components of the GM cotton plants (see Appendix 1). Furthermore, there is no detectible protein in the oil and linters that may be used in food (see Section 2.2.1).

149.Taylor (1992) has shown in double-blind placebo-controlled food challenges that individuals who are allergic to peanuts or sunflower seeds are able to consume oil derived from these seeds without it eliciting an allergic response. Taylor (1992) also suggests that when a

protein is present in food at levels well below 1 mg per serving, the hazard for allergenic consumers is minimal. Thus consumers are highly unlikely to develop allergic responses as a result of the use of oil or linters derived from Roundup Ready® or Roundup Ready®/INGARD® cotton in food.

2.4.2.1 CP4 EPSPS

150.The CP4 EPSPS protein is rapidly denatured by heat and by enzymatic digestion and acid in simulated mammalian gastric fluid (Harrison et al. 1996; Canadian Food Inspection Agency 1997; ANZFA 2001). CP4 EPSPS shows no significant protein sequence homology to allergens assembled from the Genpept, Pir and SwissProt protein databases (Mitsky 1993).

2.4.2.2 CRY1AC

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

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

2.4.2.3 NPTII

153.The NPTII protein does not display characteristics common to known food allergen proteins (Fuchs et al. 1993b; FDA 1994; FDA 1998; ANZFA 2000; ANZFA 2001). The NPTII protein is heat labile and degrades rapidly in simulated human gastric fluid. Fuchs et al. (1993b) reported that no NPTII was detected 10 seconds after addition of simulated gastric fluid as measured by both Western blot and enzymatic activity. NPTII shows no significant DNA or protein sequence homology to known allergens in the EMBL, Genbank, Pir and SwissProt protein databases (Fuchs & Astwood 1996).

Section 2.5 Toxicity and allergenicity assessment of Roundup Ready â and Roundup Ready â /INGARD â cotton

154.The nutrient composition of Roundup Ready® and Roundup Ready®/INGARD® cottonseed is within the normal range for cottonseed in terms of the concentration of protein, oils, carbohydrate and ash, and amino acid and fatty acid profiles. The presence of CP4 EPSPS, an enzyme of the aromatic amino acid biosynthetic pathway, does not cause an increase in the levels of aromatic amino acids. The levels of known toxic and anti-nutritional

factors in Roundup Ready® and Roundup Ready®/INGARD® cottonseed, including gossypol and cyclopropenoid fatty acids, are also within the range of non-GM cotton controls (see Appendix 1). Treatment of Roundup Ready® cotton with glyphosate had no effect on the nutrient composition or the levels of anti-nutritional or toxic factors (Nida et al. 1994; Nida et al. 1995). This, along with agronomic equivalence to conventional cotton (see Appendix 1, Section 7.1), suggests that no unintended effects have occurred as a result of the genetic modifications in these GM cottons.

155. The risks of toxicity or allergenicity from Roundup Ready®/INGARD® cotton are likely to be the same as for the two parental GM varieties, ie. Roundup Ready® cotton and INGARD® cotton (for details on INGARD® cotton see 'Risk Assessment and Risk Management Plan for DIR 022/2002', available at www.ogtr.gov.au). The herbicide tolerance and the insecticidal genes operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the two genes, their proteins or their metabolic pathways, and no reason to expect that this is likely to occur. Seed composition, fibre characteristics and other agronomic qualities of each of these GM cottons are similar to those of non-GM cotton, apart from the intended herbicide tolerance and insect resistance characteristics (see Appendix 1).

156. Two studies feeding rats raw, ground cottonseed for 4 weeks were carried out to compare INGARD® cotton with the parental non-GM line (Naylor 1993a; Naylor & Folk 1994). There were no significant differences in food consumption and body weight gain in animals fed a diet containing 5% INGARD® cottonseed, compared to animals fed the same amount of cottonseed from the parental line. At a higher dose of 10% there was decreased weight gain in some animals in one of the studies. This may have been due to reduced palatability as a result of slightly higher levels of sterculic acid in the particular INGARD® cottonseed batch used compared to that in the parental line cottonseed batch. There was no other evidence for toxicity or other adverse clinical signs during these studies or in post mortem analysis of organs.

157. Rats, quails and catfish fed with 5 to 20 % Roundup Ready® cotton seed meal in the diet showed no significant differences in weight gain, feed conversion or gross pathology compared to those fed non-GM cotton seed meal (Canadian Food Inspection Agency 1997).

158. The *cry1Ac* gene in INGARD® cotton and Roundup Ready®/INGARD® cotton is registered for use as a pesticide in GM plants in Australia by the APVMA. The APVMA assesses evidence from various types of toxicological studies as part of the registration process for agricultural chemicals in Australia. No safety directions for handling are required on the product label, indicating that the APVMA does not consider precautions need be taken to protect the health and safety of humans when using this product (APVMA product no. 48296, registered since 05/08/95).

SECTION 3 CONCLUSIONS REGARDING TOXICITY OR ALLERGENICITY

159. It is considered that the risk of Roundup Ready® or Roundup Ready®/INGARD® cotton being toxic or allergenic for humans is very low because:

- there have been no reported toxic or allergic effects from these GM cottons since their commercial release in Australia, in September 2000;

- cottonseed oil and linters used in food do not contain DNA or protein, and those from the GM cottons can not be distinguished from the non-GM products;
- FSANZ has concluded that oil and linters derived from Roundup Ready® and INGARD® cotton as safe for human consumption as those derived from non-GM cotton;
- cotton lint, linters and oil used in clothing and other household products do not contain DNA or protein, and those from the GM cottons can not be distinguished from the non-GM products;
- exposure to the introduced proteins through working with cotton plants is very low;
- cotton pollen is not wind dispersed and therefore not likely to be an airborne allergen;
- although dust and lint from cotton can be created at processing facilities, the use of protective equipment prevents respiratory irritation, and fibre characteristics of the GM cottons are the same as for non-GM cotton;
- the introduced proteins are already widespread in the environment and present in human food;
- feeding studies indicate that the introduced proteins are not toxic;
- evidence indicates that the introduced proteins are not allergenic, nor do they have properties characteristic of known allergenic proteins;
- compositional and agronomic analyses have not indicated any differences between the GM and non-GM cottons, other than the presence of the introduced proteins and the intended traits; and
- feeding studies indicate that the GM cottons are not more toxic than non-GM cotton.

160. Therefore it is not considered necessary to impose any management conditions in relation to potential toxicity or allergenicity. The licence holder is required to report any adverse effects on human health and safety (for example allergic reactions as a result of occupational exposure to the cotton) or to the environment.

APPENDIX 3 TOXICITY TO NON-TARGET ORGANISMS

161.Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan. This Appendix considers potential hazards that may be posed through any potential toxicity of the GMO or its novel proteins to non-target organisms.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY HAZARD

162.Roundup Ready® and Roundup Ready®/INGARD® cotton differ from conventional cotton in the expression of two and three additional proteins, respectively. These are the CP4 EPSPS and NPTII enzymes (in both GMOs) and the Cry1Ac protein (in Roundup Ready®/INGARD® cotton only) (see Appendix 1 for details of protein expression in the GMOs). The potential for these cottons to be toxic to organisms, other than the target pests of INGARD® cotton (Lepidopteran caterpillars), is considered. This could occur either due to expression of the novel gene products or because of unforeseen, unintended effects of the genetic modifications.

163.If Roundup Ready® or Roundup Ready®/INGARD® cotton is toxic for non-target organisms, the potential hazards could include adverse impacts on:

- Safety of feed for livestock (for example, livestock fed cottonseed meal or hulls).
- Wildlife, including mammals, fish and birds;
- Invertebrates, including beneficial insects (pollinators, parasitoids or predators of insect pests);
- Microbial organisms, particularly soil microorganisms, with direct impact on growth of crops on farms,

164.Toxicity for the lepidopteran insects may also present indirect hazards, with potential to harm the natural environment (for example, adverse impacts on native biodiversity) through:

- secondary effects on populations of specialist parasitoids and predators that feed on lepidopteran insects; and
- secondary effects on populations of organisms that are preyed on by non-target lepidopteran insects.

165.The use of Roundup Ready® herbicide (a formulation of glyphosate) on Roundup Ready® and Roundup Ready®/INGARD® cotton crops in Australia is registered by the Australian Pesticides & Veterinary Medicines Authority (APVMA, formerly National Registration Authority for Agricultural and Veterinary Chemicals, NRA). As part of their assessment of this use, the APVMA consider any potential health and environmental effects, such as toxicity to non-target organisms and herbicide residues in crops. Thus risks associated with the use of glyphosate are not considered in the risk assessment of these GM cottons. It should be noted, however, that the cultivation of Roundup Ready® cotton in Australia has lead to a shift in herbicide use away from 'residual', or environmentally persistent, herbicides, rather than to an increase in herbicide use (Australian Cotton Cooperative Research Centre 2002a; data from Monsanto).

SECTION 2 LIKELIHOOD OF THE TOXICITY HAZARD OCCURRING

166.In assessing the likelihood of adverse impacts due to toxicity of Roundup Ready® and Roundup Ready®/INGARD® cotton, a number of factors were considered including:

- the potential toxicity of conventional cotton (OGTR 2002);
- the modified characteristics of Roundup Ready® or Roundup Ready®/INGARD® cotton;
- the potential exposure to the CP4 EPSPS, Cry1Ac and NPTII proteins from other sources in the environment;
- information about the likely routes of exposure to Roundup Ready® and Roundup Ready®/INGARD® cotton and to the introduced proteins, the CP4 EPSPS, Cry1Ac and NPTII proteins;
- the potential toxicity of the new proteins expressed in the cotton for particular species; and
- the potential toxicity of Roundup Ready® and Roundup Ready®/INGARD® cotton for particular species.

167.Potential non-target effects of INGARD® cotton have been considered in detail in the risk assessment for DIR022/2002, available at www.ogtr.gov.au, and are only presented here in summary.

Section 2.1 Toxicity of conventionally bred cotton

168.Cotton is a well established field crop with a long history of safe use. A comprehensive review of conventional cotton, including information on its toxicity and allergenicity, is provided in the document "The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia" (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at www.ogtr.gov.au. Information on non-GM cotton is included here to establish a base-line for comparison with the GM cottons being considered in this risk assessment.

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

170.Mammals avoid feeding on cotton plants due to both the gossypol content and the morphology of the plant. The presence of gossypol and cyclopropenoid fatty acids in cottonseed limits the use of whole cottonseed as a protein supplement in animal feed, except for cattle which are less affected by these components. Inactivation or removal of these components during processing enables the use of some cotton seed meal for catfish, poultry and swine. The meal and hulls of cottonseed can also be used for cattle feed. Its use as stockfeed is limited, nonetheless, to a relatively small proportion of the diet and it must be introduced gradually, to avoid potential toxic effects.

171.Best Management Practices for the Australian cotton industry prohibits the use of cotton trash and stubble as a feed for animals, due to residues of other pesticides that could be found in the cotton trash and stubble.

Section 2.2 Modified characteristics of Roundup Ready® and Roundup Ready®/INGARD® cotton

172.As discussed in Appendix 1, the CP4 EPSPS, Cry1Ac and NPTII proteins are present at low levels in Roundup Ready® and Roundup Ready®/INGARD® cotton. The concentrations of the Cry1Ac protein within different tissues varies significantly and may also vary seasonally, depending on climatic conditions. The highest levels of expression of the introduced proteins occurs in young leaves and in seed. Mature whole plants contain less of the introduced proteins and expression in pollen is even lower. The level of exposure to the novel proteins in the GM crop may be limited further depending on routes of exposure.

173.Also as detailed in Appendix 1, the nutrient composition of Roundup Ready® and Roundup Ready®/INGARD® cottonseed is within the normal range for cottonseed in terms of the concentration of protein, oils, carbohydrate and ash, and amino acid and fatty acid profiles. The presence of CP4 EPSPS, an enzyme of the aromatic amino acid biosynthetic pathway, does not cause an increase in the levels of aromatic amino acids. The levels of known toxic and anti-nutritional factors in Roundup Ready® and Roundup Ready®/INGARD® cottonseed, including gossypol and cyclopropenoid fatty acids, are also within the range of non-GM cotton controls (see Appendix 1). Treatment of Roundup Ready® cotton with glyphosate had no effect on the nutrient composition or the levels of anti-nutritional or toxic factors (Nida et al. 1994; Nida et al. 1995). This, along with agronomic equivalence to conventional cotton (see Appendix 1, Section 7.1), suggests that no unintended effects have occurred as a result of the genetic modifications in these GM cottons.

Section 2.3 Other sources of CP4 EPSPS, Cry1Ac and NPTII in the environment

174.As discussed in Appendix 2, the CP4 EPSPS, Cry1Ac and NPTII proteins are widespread in the environment. The genes for these proteins have been derived from common soil bacteria, and hence the CP4 EPSPS, Cry1Ac and NPTII proteins are already a natural component of the soil.

175.EPSPS enzymes are present in all plants, bacteria and fungi. The difference between the natural plant enzymes and the bacterially derived CP4 EPSPS is in the amino acid sequence, not in the physiological function (see Appendix 1 for details). Other EPSPS enzymes from both plant and microbial sources also vary to a similar degree in amino acid sequence (Felsot 2000a; Padgette et al. 1996). The CP4 EPSPS enzyme is derived from the common soil bacterium *Agrobacterium* species strain CP4 (Barry et al. 1992; Padgette et al. 1996), which is found in soil and on plants.

176.The native Cry1Ac protein is naturally produced by the bacterium *Bacillus thuringiensis* variety *kurstaki* (Btk). Related Cry toxins are also produced by other varieties of *Bacillus thuringiensis* (see Appendix 1). Bt spores and their crystal toxins are found widely in both the agricultural and natural environment, including in soils, on plant leaves, in grain stores and in dead insects (Meadows 1993).

177.The presence of the Cry1Ac protein in agricultural situations has increased due to the commercial use of Btk microbial sprays to protect crops from insect attack. Bt protein insecticides, produced by fermentation of the same strain of bacterium from which the *cry1Ac* gene was derived, have been used in traditional agriculture over several decades, especially by organic farmers (Cannon 1993). In fact, the first commercial microbial Bt product (Sporeine) was produced in 1938 in France (van Frankenhuyzen 1993). The Cry1Ac protein produced by the Roundup Ready®/INGARD® cotton lines is almost identical (greater then 99.4% identity) to that found in nature and in the Btk microbial insecticide formulations (Adang et al. 1985).

178.The NPTII protein is widespread in the environment and in food chains, in naturally occurring kanamycin-resistant microorganisms found in soil and in mammalian digestive systems (Flavell et al. 1992).

Section 2.4 Potential toxicity hazard for stock and wildlife, including mammals, birds and fish

2.4.1 Exposure of stock and wildlife to cotton

179.As discussed in Section 2.1, most mammals avoid feeding on cotton due to the presence of gossypol and other components of cotton tissues. Use of cotton products as stock feed is also limited for this reason.

180.In the field, seed cotton is present as large lint-covered seeds that are unattractive to avian species (OGTR 2002), so birds are not likely to be exposed to the introduced proteins in the seeds of Roundup Ready® and Roundup Ready®/INGARD® cotton. Some exposure could occur following planting or through use of delinted cottonseed as cattle feed in stockyards.

181.Cottonseed or pollen is not expected to enter aquatic habitats in any significant quantity, limiting exposure of aquatic organisms. Irrigation practices used by cotton growers in Australia retain irrigation water run-off, as well as the first 15mm of storm water run-off, on farm to minimise the entrance of pesticide residues into natural waterways. In New South Wales this is a legislative requirement, while in Queensland this is part of Good Management Practice of the cotton industry.

2.4.2 Toxicity of Roundup Ready® and Roundup Ready®/INGARD® cotton to stock and wildlife

182.As detailed in Appendix 2, acute oral toxicity studies in a range of mammalian species (mice, rats and rabbits) with each of the CP4 EPSPS, Cry1Ac and NPTII proteins have not demonstrated any adverse effects. Additional studies in other species are described below.

183.In trials with rats, quail or catfish fed cottonseed meal at 5 to 20% of the diet, no significant differences in weight gain, feed conversion or gross necroscopy were found for animals fed Roundup Ready® cottonseed meal compared to those fed non-GM cottonseed meal (Canadian Food Inspection Agency 1997).

184.The performance of cows fed controlled diets including cottonseed from either non-GM cotton, Roundup Ready®, Roundup Ready®/INGARD® or INGARD® cotton have been compared (Hartnell et al. 2001; Castillo et al. 2001a; Castillo et al. 2001b). There were no significant

differences in body condition, milk yield or milk composition between cows fed the alternative diets. Moreover, Western blot assays of the milk tested negative for the new proteins expressed in INGARD® cotton (Hartnell et al. 2001).

185.Northern Bobwhite Quail fed raw cottonseed meal at up to 10%w/w, equivalent to 100 seeds/bird/day, for five days showed no significant differences in feed consumption or body weight for birds fed INGARD® cottonseed meal compared to birds fed non-GM cottonseed meal (Gallagher et al. 2000).

Section 2.5 Potential toxicity hazard for invertebrates

2.5.1 Exposure of invertebrates to cotton

186.Non-target invertebrates may be exposed to Roundup Ready® and Roundup Ready®/ INGARD® cotton and to the introduced proteins directly, through feeding on the plants, or indirectly through eating other organisms, including the lepidopteran target organisms of INGARD® cotton, that feed on the plants.

187.Relative exposure will be greatest for herbivorous species feeding on the cotton plants. Sap feeders, such as aphids, will have minimal exposure to the introduced proteins as the sap is primarily composed of sugars and mineral salts dissolved in water.

188.Pollinator species and various insects that feed on pollen will also have low exposure to the introduced proteins, because of their expected much lower expression in pollen, relative to that in other plant tissues, based on expression of the Cry1Ac protein in INGARD® cotton pollen and the similarity of the promoter elements controlling expression of the introduced genes (see Appendix 1). Species feeding on lepidopteran larvae may be exposed not only to the full-length Cry1Ac protein but also the activated core toxin (see Appendix 1 for information on the introduced proteins and their expression).

2.5.2 Toxicity of Roundup Ready® and Roundup Ready®/INGARD® cotton for invertebrates

189.The direct effects of the CP4 EPSPS and NPTII enzymes have not been tested on invertebrates. EPSPS enzymes are present in all plants, bacteria, algae and fungi (Appendix 1). NPTII is a phosphorylating enzyme which does not possess any properties that distinguish it toxicologically from other phosphorylating enzymes present in microorganisms, plants and animals (FDA 1994). Both proteins are already widespread in the environment in the bacteria from which their genes were derived. Thus the expression of these bacterial enzymes in plants is not likely to have any adverse toxic effects on invertebrates.

190.Most interest in toxicity of these GM cottons for invertebrates has concentrated on the Cry1Ac protein, which is expressed in Roundup Ready®/INGARD® cotton. Potential non-target effects of the Cry1Ac protein have been considered in detail in the risk assessment for the continued commercial release on INGARD® cotton, DIR022/2002, available at www.ogtr.gov.au. A summary is presented below.

191.A series of studies examined the effects of purified active core Btk Cry1Ac toxin on 18 agronomically important insect species, representing five orders, and one species of mite (Macintosh et al. 1990). Seven insects, all lepidopterans, were susceptible to the toxin. None of the remaining 11 non-lepidopteran species were susceptible.

192.Other studies with the Cry1Ac protein expressed in INGARD® cotton (Sims 1994; Sims 1995) found that of 14 species tested (representing seven orders), only lepidopteran species were susceptible to Cry1Ac. Detailed studies of the effect of Cry1Ac on specific beneficial non-target insects have been carried out, including:

- the larval and adult honey bee (*Apis mellifera* L.), a beneficial insect pollinator (Maggi 1993a; Maggi 1993b);
- parasitic Hymenoptera (*Nasonia vitripennis*), a beneficial parasitoid of the housefly (*Musca domestica*) (Palmer & Beavers 1993c; Sims 1994);
- ladybird beetles (*Hippodamia convergens*), a beneficial predatory insect which feeds on aphids and other plant bugs commonly found on stems and foliage of weeds and cultivated plants (Palmer & Beavers 1993b; Sims 1994);
- green lacewing larvae (*Chrysopa carnea*), a beneficial predatory insect commonly found on cotton and other cultivated crops (Palmer & Beavers 1993a; Sims 1994); and
- springtails (*Folsomia candida* and *Xenylla grisea*), scavenging soil insects important in nutrient cycling (Sims & Martin 1996).

193.There were no adverse effects of Cry1Ac observed, even at levels well above those found in INGARD® cotton, for any of the species tested.

194.The effects of INGARD® cotton on non-target arthropod populations have also been studied in the field in Australia (Addison 2001a; Addison 2001b; Addison 2001c; Fitt & Wilson 2002) and America (Naranjo & Ellsworth 2002). Invertebrate faunal diversity, apart from the target lepidopteran species and in some samples also their specialist parasitoids, and abundance in INGARD® cotton fields was comparable to that in unsprayed non-GM plots, and significantly higher than in conventionally managed (ie. sprayed) non-GM fields. A reduction of host lepidopteran species is unlikely to threaten the persistence of specialist parasitoids or predators, since a significant proportion of the host population is always present on other crops and uncultivated areas (Fitt & Wilson 2002).

195.The commercial release of INGARD® cotton, including Roundup Ready®/INGARD® varieties, has reduced the use of broad-spectrum insecticides on cotton crops in Australia, with an average of around 50% fewer insecticide sprays than used on conventional cotton fields (Australian Cotton Cooperative Research Centre 2002b; data from Monsanto). Similarly, the use of INGARD® cotton in China, with the associated reduction in insecticide use, resulted in an average of 24% increase in the number of insect predators over that found in conventional cotton fields (Xia et al. 1999).

Section 2.6 Potential toxicity hazard for microorganisms

2.6.1 Exposure of microorganisms to cotton

196.Microorganisms may be exposed to the GM cotton plants during growth or during decomposition of plant material. Exposure of organisms in soil to the introduced proteins may also occur as a result of root exudations, as has been observed in Bt corn expressing Cry1Ab (Saxena et al. 1999; Stotzky 2000). Preliminary work by (Gupta et al. 2002) shows that roots of INGARD® cotton also express the Cry1Ac protein and have been found to release this protein into soil during growth, although this was not quantified nor is the mechanism clear. Root breakage increases the release of Cry1Ac protein into the soil. After harvest of lint and seed, the remaining cotton plant residues are typically tilled into the soil, so that soil biota may be exposed to the introduced proteins as the GM cotton residues are broken down.

197.The initial level of exposure to the introduced proteins is likely to decrease with time, as a result of soil biodegradation. The Cry1Ac protein in INGARD® cotton plant material has been found to degrade in soil with a half life in the order of 2 to 46 days, depending on soil type and other environmental factors (Ream 1994b; Palm et al. 1996; Tapp & Stotzky 1998). The Cry1Ac protein adsorbs to various soil components (eg. humic acids, clay minerals), rendering it resistant to microbial degradation. Generally there is an initial rapid decline in Cry1Ac levels over several days, followed by a more gradual rate of decline. In some soils Cry1Ac was still detectable after several months (Palm et al. 1996).

198.Head et al. (2002) assayed for the presence of the Cry1Ac protein in soils from fields in which INGARD® cotton had been cultivated, and plant material incorporated into the soil by post harvest tillage, for three to six consecutive seasons. Samples were collected three months after the last seasons tillage. Assay by both enzyme-linked immunosorbent assay (ELISA) and bioassay (ie feeding to susceptible insect larvae) detected no Cry1Ac protein in any of the samples. Generally in Australia cotton is grown in alkaline soil, with a pH ranging from 7.5 – 8.5 (Australian Cotton Cooperative Research Centre 2002c), in which Bt endotoxins would desorb from clay soils and be degraded by soil microorganisms (Tapp & Stotzky 1998). Thus the Cry1Ac protein is not likely to accumulate in agricultural soils as a result of successive seasons of cultivation of INGARD® or Roundup Ready®/INGARD® cotton.

2.6.2 Toxicity of Roundup Ready® and Roundup Ready®/INGARD® cotton for microorganisms

199.The direct effects of the CP4 EPSPS and NPTII enzymes have not been tested on microorganisms. EPSPS enzymes are present in all plants, bacteria, algae and fungi. Thus the expression of a bacterial EPSPS enzyme in plants is not likely to have any adverse effect on microorganisms.

200.NPTII is a phosphorylating enzyme which does not possess any properties that distinguish it toxicologically from other phosphorylating enzymes present in microorganisms, plants and animals (FDA 1994). The function of this enzyme is the phosphorylation (inactivation) of the antibiotic neomycin (and the related kanamycin). In the environment, this enzyme is not likely to be active outside of living cells, as it requires specific chemical conditions for activity, including the availability

of specific co-factors. Although antibiotic production by non-pathogenic bacteria has been implicated in suppression of some plant diseases (Brimecombe et al. 2001), no evidence for the involvement of neomycin or kanamycin has been found in a search of the scientific literature. Neither are these antibiotics used in agriculture for controlling soil borne disease. Thus the presence of NPTII in soil is not expected to impact on microbial populations or plant disease susceptibility. Furthermore, expression of NPTII in a variety of crop plants (for example, canola, corn, cotton, tomato), over several years of agronomic performance testing and commercial cultivation, has not been linked to any increased occurrence of disease.

201. Potential effects of the Cry1Ac protein on microorganisms have been considered in detail in the risk assessment for the continued commercial release on INGARD® cotton, DIR022/2002, available at www.ogtr.gov.au. In a preliminary experiment, Gupta et al. (2002) examined the decomposition of INGARD® cotton and non-GM cotton plant residues, finding that the GM material decomposed more slowly. Fungal colonisation and total microbial activity was greater on INGARD® cotton residues, however total carbon usage by rhizoshere microorganisms (as measured on added ^{14}C-substrate) was reduced.

202. The effect on soil microorganisms of a truncated Cry 1Ac toxin, either purified or in GM cotton tissue (but not INGARD® cotton), have been examined (Donegan et al. 1995; Donegan & Seidler 1998). Numbers and types of protozoans, bacteria and fungi, as well as substrate utilisation tests and DNA fingerprinting of eubacterial ribosomal sequences, were used to analyse the composition of microbial soil community. The addition of purified Cry1Ac toxin to soil did not cause any detectable effects. Similarly, there was no difference between soils to which non-GM leaves were added with or without Cry1Ac toxin. Leaves of GM cotton produced a short-term stimulatory effect on soil bacterial and fungal populations, relative to leaves from the non-GM cotton line. It was speculated that this may reflect faster decomposition and nutrient release from the transgenic leaves compared to the non-GM leaves, possibly due to some unknown and unintended effect of the genetic modification (or the tissue culture process involved) on the plant characteristics, separate from expression of the truncated Cry1Ac protein.

SECTION 3 CONCLUSIONS REGARDING TOXICITY TO NON-TARGET ORGANISMS

203. It is considered that the risk of Roundup Ready® or Roundup Ready®/INGARD® cotton being toxic to non-target organisms (other than Lepidoptera) is very low because:

- the introduced proteins, CP4 EPSPS, Cry1Ac and NPTII, are present at low levels in Roundup Ready® or Roundup Ready®/INGARD® cotton;
- the nutritional and agronomic properties of these GM cottons are similar to those of conventional cotton;
- the introduced proteins are already widespread in the environment, through the presence of the bacteria to which they are native;
- exposure of stock and wild life to these GM cottons is low, except for stock fed cotton seed;
- there are no differences in performance of stock fed these GM cottons, compared to non-GM cotton;

- the toxicity of Cry1Ac is highly specific to Lepidopteran insect larvae;
- the CP4 EPSPS and NPTII enzymes are not known to be toxic to any organism;
- toxicity studies Roundup Ready® and INGARD® cotton tissue indicate that they are not more toxic to mammals, birds or fish than non-GM cotton;
- laboratory and field studies suggest that populations of key non-target invertebrates are unlikely to be affected by the Bt toxin. Indeed it is likely that their populations would be favoured by decreases in the use of broad-spectrum insecticides;
- laboratory studies indicate that Cry1Ac has no adverse affect on the growth of various bacteria, yeast, fungi, algae or protozoans;
- the presence of GM cotton (INGARD®) plant material in the soil produces only transient changes in soil microbial communities; and
- natural degradation of Cry1Ac in the soil limits bioaccumulation.

204. Therefore it is not considered necessary to impose any management conditions in relation to potential toxicity to non-target organisms. The licence holder is required to report adverse effects on human health and safety or the environment (for example, any indication of toxicity of the GM cottons for non-target organisms).

APPENDIX 4 WEEDINESS

205.Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. In this Appendix, risks posed by the proposed dealing to the environment are considered in relation to the potential for the GMO to become a problematic weed.

206.There are numerous definitions of weeds including 'a plant growing where it should not be'. Weeds become a problem to the community when their presence or abundance interferes with the intended use of the land they occupy. Weeds may also represent a source of food to various organisms, hence the introduction of weeds to an environment may also bring about ecological change by altering the structure of food webs.

SECTION 1 NATURE OF THE WEEDINESS HAZARD

207.Roundup Ready® and Roundup Ready®/INGARD® cotton differ from conventional cotton in the expression of two and three additional proteins, respectively. These are the CP4 EPSPS and NPTII enzymes (in both GMOs) and the Cry1Ac protein (in Roundup Ready®/INGARD® cotton only) (see Appendix 1 for details of protein expression in the GMOs).

208.The possibility was considered that Roundup Ready® or Roundup Ready®/INGARD® cotton might have the potential to be harmful to the environment, because of inherent weediness or increased potential for weediness, either due to expression of the novel gene products or as a result of unforeseen, unintended effects of the genetic modification.

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

210.The use of Roundup Ready® herbicide (a formulation of glyphosate) on Roundup Ready® and Roundup Ready®/INGARD® cotton crops in Australia is registered by the Australian Pesticides & Veterinary Medicines Authority (APVMA, formerly National Registration Authority for Agricultural & Veterinary Chemicals, NRA). As part of their assessment of this use, the APVMA consider any potential environmental effects, such as development of glyphosate-resistant weeds, levels of glyphosate in the environment and drift from spray applications. Therefore, risks associated with the use of glyphosate are not generally considered in the risk assessment of these GM cottons, except in the context of the environmental monitoring program required as part of the Dead of Agreement between the Commonwealth Government and Monsanto for the original commercial release of these GM cottons in 2000 (see Section 2.4). The risk of development of glyphosate-resistant weeds is also addressed briefly in Appendix 5.

SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

Section 2.1 Inherent weediness of conventional cotton

211.Attributes of non-GM cotton associated with potential weediness are discussed in the document 'The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia' (OGTR 2002)

that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at www.ogtr.gov.au. In summary, the document concludes that non-GM cotton is not a problematic weed in Australia, because factors including soil moisture, nutrient limitation, and roadside management practices limit the establishment and/or persistence of cotton seedlings. Further information on the weediness of non-GM cotton is included here to establish a base-line for comparison with the GM cottons being considered.

212.Cotton is not considered to possess the characteristics commonly associated with successful weeds, such as seed dormancy, long persistence in the soil, germination under a broad range of environmental conditions, rapid vegetative growth, short lifecycle, very high seed output, high seed dispersal and long-distance seed dispersal (Keeler 1985; Keeler 1989).

213.Another important element in prediction of weediness is taxonomic relationship, considering weediness within a taxon, including its history of weediness in any part of the world (Bergelson et al. 1998; Panetta 1993; Pheloung 1995). Cotton has been grown for centuries throughout the world without any reports that it is a serious weed pest. Cotton is not considered to be a problematic weed in Australia (Groves et al. 2000; Groves et al. 2002). There are about 50 species of *Gossypium* (Fryxell 1992; Craven et al. 1994) of which only one (*G. tomentosum*) is listed as a weed in the USA (Holm et al. 1997).

Section 2.2 Dispersal of cottonseed in the environment

214.The proposed dealing includes cultivation of Roundup Ready® and Roundup Ready®/ INGARD® cotton in the cotton growing regions of NSW and Qld south of 22° South, and entry of cotton by-products into general commerce after harvest.

215.Surveys (Eastick 2002; Farrell & Roberts 2002) have found that seed cotton (ie. cotton seed in its natural form with lint attached) is the most likely form of seed to be dispersed in the environment, as apposed to fuzzy (ginned) or black (delinted) seed. Seed cotton, as well as being the natural form of the seed produced on the plant, can be dispersed by falling from cotton modules during transport to cotton gins after harvest. There is only limited potential for movement of seed cotton in waterways (Eastick 2002). Seed cotton generally does not germinate until the season after its production, when climatic conditions become favourable.

216.Other forms of seed are present only as a result of human activity. Some uses of cotton seed may move seed beyond regions where cotton is grown, for example, cottonseed is used as a stockfeed north of 22° South. As well as the potential for seed dispersal during transport and feeding, a very small percentage of cottonseed consumed by stock can pass through their digestive system intact and able to germinate (Eastick 2002).

217.A survey of the transport routes between Emerald (in the Queensland cotton growing region) and the Atherton Tablelands (north of 22° South) indicated that cotton plants had established in roadside environment only infrequently, despite 12 years of use of these routes for transporting fuzzy seed for stock feed (Farrell & Roberts 2002)(see Section 2.4 for details). It was also noted that the adoption of 'roll-over tarp' systems for trailers transporting cottonseed to the Atherton Tablelands since 2001 had substantially reduced the opportunity for fuzzy seed to escape on route.

Section 2.3 Potential weediness of Roundup Ready â and Roundup Ready â / INGARD â cotton

218.Many of the characteristics associated with weediness are also important agronomic characteristics. Consequently these are assessed as part of the agronomic evaluations during the development of new cotton varieties, including GM varieties. Seed survival, germination, vigour, yield and disease susceptibility of Roundup Ready® and Roundup Ready®/INGARD® cotton varieties currently being grown have been evaluated in both controlled environments and field releases and are within the range of current non-GM cotton varieties (see Appendix 1, Section 7) (Monsanto Australia Limited 2001; Cotton Seed Distributors 2002). These data suggest that the genetic modifications in Roundup Ready® and Roundup Ready®/ INGARD® cotton have not lead to any unintended effects on characteristics typically associated with weediness.

219.As mentioned previously, INGARD® cotton has been in commercial release since 1996 and Roundup Ready® and Roundup Ready®/INGARD® cotton since 2000. The potential weediness of INGARD® cotton has been considered in the risk assessment for application DIR 022/2002, available at www.ogtr.gov.au, and is only presented here in summary. Since their commercial release, cottonseed from these GM cottons has been used as stockfeed in northern Australia. Over this period there has been no evidence that GM cotton has become more weedy than non-GM cotton (see Section 2.4). Surveys of volunteer cotton in Australia and experimental research on the weedy potential of GM cotton in Australia consistently suggest that major factors limiting cotton establishment and survival include water and nutrient availability, herbivory by non-lepidopteran species (vertebrate and invertebrate), plant competition, frost and fire (Eastick 2002; Farrell & Roberts 2002).

220.The results of this research strongly indicate that the likelihood of GM cotton establishing and persisting as a weed in southern Australia at higher levels than the very low rate of establishment of non-GM cotton is negligible, because environmental variables that are unaffected by the genetic modifications are the key limitations on cotton populations. For these reasons, it is also expected that the likelihood of GM cotton establishing as a weed in northern Australia is low, however this is yet to be determined conclusively (see Section 2.3.1.2).

2.3.1 Potential selective advantage conferred by the introduced proteins

2.3.1.1 CP4 EPSPS

221.The CP4 EPSPS protein could only confer a selective advantage in the presence of glyphosate herbicide application. In an agricultural setting, Roundup Ready® and Roundup Ready®/INGARD® cotton will have increased fitness where glyphosate is applied for weed control. However, glyphosate is not generally used to control established volunteers cotton plants in agricultural systems, as it has limited effectiveness on established cotton (ie. beyond the seedling stage). Cultivation or alternative herbicides are the main control strategies employed (Australian Cotton Cooperative Research Centre 2002a). It should be noted that beyond the four-leaf stage, exposure of Roundup Ready® or Roundup Ready®/INGARD® cotton to glyphosate leads to damage to reproductive development and hence reduced flower and seed production (see Appendix 1, Section 7.1).

222.Glyphosate may be used to control weeds on roadsides. In this situation, spraying is often limited to around fixtures such as signs and guide-posts, while slashing is used in accessible areas. Evidence suggests that cotton is not a significant weed in these situations and that resistance to glyphosate is not likely to change this (see Section 2.4).

2.3.1.2 CRY1AC

223.A detailed discussion of the potential of the Cry1Ac protein to influence weediness is provided in the risk assessment document for DIR022/2002, available at www.ogtr.gov.au. The Cry1Ac protein could confer a selective advantage in areas where lepidopteran insect predation limits one or more of the key life stages of cotton.

224.An investigation into the weediness potential of GM insecticidal cottons, including INGARD® cotton, has been conducted over two growing seasons in northern Australia (Eastick 2002). Habitats examined were bushland, roadsides, cattle areas and waterways. Data gathered demonstrate that for each of cotton's life stages, the performance of INGARD® cotton is not significantly different to that of conventional cotton. There was no evidence that insecticidal genes enhanced survival. Only limited data was obtained on reproductive capacity, due to low germination, growth and survival in most situations, however INGARD® cotton produced significantly higher numbers of bolls at one of the 13 sites studied (an artificial waterway site) in one of the two seasons.

225.Eastick (2002) concluded that cotton is rarely invasive, irrespective of insecticidal genes, and that that lepidopteran herbivores were not a major constraint on the weedy potential of GM insecticidal cottons in northern Australia. Rather, it was concluded that water and nutrient availability, herbivory by non-lepidopteran species (vertebrate and invertebrate), plant competition and fire were the most significant limitations on the establishment and persistence of cotton populations.

2.3.1.3 NPTII

226.The NPTII protein could confer a selective advantage to Roundup Ready® or Roundup Ready®/INGARD® cotton plants in the presence of a high concentration of neomycin or kanamycin. However, antibiotics are not applied to cotton crops and are not likely to be present in any environment where cotton grows. Thus the expression of NPTII is highly unlikely to confer any selective on these GM cottons.

2.3.1.4 CP4 EPSPS AND CRY1AC COMBINATION

227.The potential for weediness of Roundup Ready®/INGARD® cotton is not likely to be greater than that for the two parental GM varieties, ie. Roundup Ready® cotton and INGARD® cotton. The herbicide tolerance and the insecticidal genes operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the two genes, their proteins or their metabolic pathways, and no reason to expect that this is likely to occur.

228.Seed composition, fibre characteristics and other agronomic qualities of each of these GM cottons are similar to those of non-GM cotton, apart from the intended herbicide tolerance and insect resistance characteristics. Expression of the introduced proteins is similar in the combined

trait cotton plants to that in the respective single trait cottons (see Appendix 1). This, along with agronomic equivalence to conventional cotton (see Appendix 1, Section 7.1), suggest that no unintended effects have occurred as a result of the combined genetic modifications in these GM cottons.

229.There is the possibility of an additive effect in situations where growth or reproduction of cotton is limited by both lepidopteran insects and by glyphosate use, however neither of these factors is identified as significant in limiting weediness of cotton.

Section 2.4 Environmental monitoring program for Roundup Ready â and Roundup Ready â /INGARD â cotton

230.As a part of the Deed of Agreement between the Commonwealth Government and Monsanto for the commercial release of Roundup Ready® and Roundup Ready®/INGARD® cotton in September 2000, Monsanto was required to conduct an environmental monitoring program. This program, developed through consultation with the Genetic Manipulation Advisory Committee (GMAC), the cotton industry, scientists, cotton growers and other interested parties, was designed to assess the potential for these GM cottons to spread and establish populations outside the agricultural environment. This program was for a three year period starting in 2001. It comprises three core components:

- monitoring for the incidence of volunteer GM cotton in non-agricultural situations (eg. roadsides and non-crop areas);
- monitoring for the incidence of volunteer GM cotton in non-GM crops; and
- monitoring for any shifts in weeds resistant to glyphosate associated with the use of these GM crops.

231.The data gathered to date were reviewed as part of this evaluation.

2.4.1 Roundup Ready® cotton in non-agricultural situations (roadsides and non-crop areas)

232.Roadside surveys have been conducted to determine the incidence of volunteer cotton, including analysis for the Roundup Ready® trait, along roadsides in the Lower Namoi Valley (NSW) and Darling Downs (QLD). Roadside volunteers arise primarily from seed spilt during transport of the previous seasons harvest. Volunteers also arise as a result of the feeding of whole cotton seed to stock. Roundup Ready® and Roundup Ready®/INGARD® cotton was grown on approximately 12000 ha in the 2000/2001 season, and 84000 ha in the 2001/2002.

233.In March 2002, 700 km of the major cotton transport routes in the Lower Namoi were surveyed (examining 200 m of roadside every 10 km), revealing an average of 4.2 volunteers/km (Farrell & Roberts 2002). Volunteers were all well established (ie. not recently germinated seedlings) but were all less than one year old. These were clumped in a few favourable habitats, such as ditches and roadside drains, in six of the 72 sites examined. Roundup Ready® cotton volunteers were only identified at one of these sites, representing 85% of cotton plants at that site.

234.In March 2003 a second survey included the same routes in the Lower Namoi Valley plus 400 km in the Darling Downs (data provided by Monsanto). This survey was conducted after

substantial rains, creating ideal conditions for the germination of new cotton volunteers. In the Lower Namoi Valley, cotton volunteers were found at 33 of 77 sites, although at only 2 sites were these established (ie. not recently germinated and past the 4 leaf stage). Of the established cotton volunteers, Roundup Ready® cotton was identified at one of these sites (33% of plants at that site). No mature volunteers were found at sites where volunteers were identified in the previous season survey, indicating that these had not survived beyond their first year.

235.In the Darling Downs, cotton volunteers were found at five of 40 sites, although at only one site were these established (beyond the 4 leaf stage). Of the established cotton volunteers, 8% were found to be Roundup Ready®.

236.Of the seedling (ie. recently germinated and not past the 4 leaf stage) volunteer cotton plants found, Roundup Ready® cotton represented 33% in the Lower Namoi Valley and 3% in the Darling Downs. Plantings of Roundup Ready® cotton varieties in the previous season (from which volunteers would have arisen) in these two valleys were 19% and 11%, respectively, of the total cotton plantings. The distribution of frequencies of Roundup Ready® volunteers found at individual sites matched well with the distribution of frequencies of Roundup Ready® cotton planted on individual farms in each region. Thus volunteer cotton, arising from seed spilt from cotton modules after harvest, may reflect the proportion of GM and non-GM cotton grown and harvested on individual farms.

237.Farrell and Roberts (2002) also surveyed cotton volunteers on transport routes between the Queensland cotton growing region and the Atherton Tablelands, and at dairy farms in this region where fuzzy seed (ie. cottonseed that has been ginned to remove most of the lint) has been used as a stock feed supplement. Volunteer cotton plants were found at a low frequency on roadsides (0.26/km), with none being GM varieties (only four plants found, examining 1.28% of roadsides over 1200 km). Volunteers were found at seven of nine dairy farms surveyed, 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 apparently do not complete an entire reproductive cycle to produce new seedlings, being limited by physical damage, 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.

238.Cotton was not a significant roadside weed in any of the regions surveyed. Slashing and grazing appear to be common methods of roadside weed control, with herbicide use limited to around fixtures such as signs and guide posts, suggesting that glyphosate resistance is not likely to provide a significant selective advantage. There was no evidence of plants surviving for more than one season. Frost is likely to limit volunteer survival in some regions.

239.On roadsides, volunteers seem to originate from seed cotton (ie cotton seed in its natural form with lint attached) that can escape from cotton modules during post-harvest transport. However, the frequency of established volunteers was not related to the amount of seed cotton, which was seen at high densities in some areas (up to 1000's of seed per 200m surveyed). Much of the seed does not germinate and germination does not often lead to establishment.

240.Observations in these surveys suggest that cotton volunteers tend to establish in disturbed environments with increased water availability and limited competition from other vegetation, irrespective of whether, or not, they are genetically modified. Eastick (2002) made similar observations in the Northern Territory and Western Australia. Eastick (2002) also 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, and no cotton volunteers were found in undisturbed bush habitats.

2.4.2 Farm survey for Roundup Ready® cotton volunteers

241.A survey of volunteer cotton was undertaken during the 2001/2002 season in fields where Roundup Ready® cotton had been grown in the previous season (2000/2001), as well as in a smaller number of fields in which non-GM cotton had been grown (Perry 2002). The survey included 17 growers in four valleys (six of these growers, in two valleys, for non-GM previous season fields). These valleys accounted for half of the 12000 ha of Roundup Ready® cotton grown in the 2000/2001 season.

242.All fields were surveyed both in November 2001 and February/March 2002, to assess both volunteer emergence and effectiveness of volunteer control. Large differences between valleys in the prevalence of volunteer cotton were noted. Some Roundup Ready® cotton volunteers were identified in fields previously grown to non-GM cotton, as well as non-GM cotton in fields previously GM. The prevalence of volunteer cotton was consistently reduced in the second survey (Feb/Mar), whether the previous crop had been Roundup Ready® or conventional cotton.

243.Slightly higher numbers of volunteers were found in fields previously grown to Roundup Ready® cotton than in those of the same valley previously grown to non-GM cotton. However, there was no apparent difference in the effectiveness of control of non-GM and Roundup Ready® cotton volunteers, as the proportion of volunteers that were Roundup Ready® did not increase between the first and second surveys, regardless of the previous crop type.

2.4.3 Survey for development of weeds resistant to glyphosate

244.As part of the Deed of Agreement for the commercial release of Roundup Ready® and Roundup Ready®/INGARD® cotton, Monsanto were required to develop a crop management plan designed to minimise the potential for development of weeds resistant to glyphosate (Monsanto Australia Limited 2001). This plan has been endorsed by the herbicide tolerant cotton subcommittee of the Transgenic and Insect Management Strategy (TIMS) committee of the Australian Cotton Growers Research Association and is enforced by Monsanto under its' Technology User Agreement. Included is a requirement to prevent seed set of weeds that have survived exposure to Roundup Ready® herbicide.

245.Data collected by Monsanto from growers of Roundup Ready® cotton varieties indicates general compliance with this plan. Over three seasons of cultivation of these GM cottons, there has been no indication of a shift in weeds resistant to glyphosate and no change in the level of grower satisfaction with this technology. This suggests that the potential for development of weeds resistant to glyphosate is being managed effectively in Roundup Ready® (including Roundup Ready®/INGARD®) cotton crops.

Key findings of the environmental monitoring program

246.The environmental monitoring program for Roundup Ready® and Roundup Ready®/ INGARD® cotton demonstrates that, for regions of Australia south of latitude 22º South, the establishment and persistence of these GM cottons occurs infrequently and does not occur at levels disproportionate to the amount of cottonseed entering the environment. That is, for southern Australia, there is no indication that either Roundup Ready® or Roundup Ready®/ INGARD® cotton is more weedy than the negligible weediness of non-GM cotton. Moreover, where these volunteers have persisted, they can be managed easily either mechanically or with herbicides other than the glyphosate.

247.Similarly, results of the environmental monitoring program suggest that the weediness of GM cottons in northern Australia is also likely to be very low. If the Regulator decides to issue a licence, conditions would be imposed to restrict dealings with the GM cottons in northern Australia until the risk of weediness north of latitude 22º South can be determined conclusively.

Continuation of the environmental monitoring program

248.The original environmental monitoring program was proposed for a period of three years. The first opportunity to gather meaningful data on the spread and establishment of Roundup Ready® or Roundup Ready®/INGARD® cotton populations outside the agricultural environment was not until the 2001/2002 season, following their first commercial release in the 2000/2001 season. Thus data is currently only available for two seasons. Therefore, if the Regulator decides to issue a licence, conditions would require continuation of monitoring for volunteer cotton in non-agricultural situations for an additional growing season, complete the original program.

249.The removal of on-farm cotton volunteers has been adopted as good farm practice by cotton growers to aid in disease management for all cotton crops, and as part of the Insecticide Resistance Management Strategy for INGARD® and Bollgard II® cotton. Cultivation or alternative herbicides are the main control strategies employed (Australian Cotton Cooperative Research Centre 2002a). Additional alternative herbicides for control of Roundup Ready® cotton volunteers are also being investigated by the Australian Cotton Cooperative Research Centre, with the aim of further diversifying management options. Therefore no conditions are imposed in relation to monitoring of on-farm cotton volunteers.

250.The APVMA is responsible for the management of the development of herbicide resistant weeds resulting from the use of herbicides, under conditions of registration for the use of agricultural chemicals in Australia (see Appendix 6 Section 2). Therefore the OGTR would not continue to require data to be collected in relation to development of herbicide resistant weeds.

SECTION 3 CONCLUSIONS REGARDING WEEDINESS

251.It is concluded that the risk of Roundup Ready® or Roundup Ready®/INGARD® cotton establishing as environmental weeds in cotton growing regions of NSW and Queensland south of latitude 22º South, the region in which Monsanto proposes to grow the GM cottons, is very low because:

- cotton does not possess characteristics commonly associated with weediness, and is not known to be a problematic weed in any environment;
- cotton has a low potential for dispersal by natural means;
- the genetic modifications in these GM cottons have not affected these characteristics;
- surveys carried out since the commercial release of Roundup Ready® and Roundup Ready®/INGARD® cotton in 2000 indicate that these GM cottons have not become problematic weeds;
- major constraints on weediness of Roundup Ready®, Roundup Ready®/INGARD® and non-GM cotton are water availability, nutrient availability, plant competition, herbivory by non-lepidopteran species, frost and fire;
- cotton volunteers, whether Roundup Ready®, Roundup Ready®/INGARD® or non-GM, can establish on roadsides but do not persist or lead to spread into the wider environment;
- control of Roundup Ready® cotton volunteers can be achieved by cultivation or treatment with herbicides other than glyphosate; and
- although the two traits (herbicide tolerance and insect resistance) may have an additive effect, neither of these traits individually has been found to be significant for weediness.

252.It is also concluded that the risk of Roundup Ready® or Roundup Ready®/INGARD® cotton establishing as a weed north of latitude 22º South, associated with the use of cottonseed as stockfeed, is also likely to be low for the same reasons. However this is yet to be determined conclusively for cotton carrying the *cry1Ac* gene (including Roundup Ready®/INGARD® cotton) because:

- INGARD® cottonseed has been used as stockfeed in northern Australia since 1996, and Roundup Ready®/INGARD® cotton since 2000, with no indication that they have become more weedy than non-GM cotton; however
- preliminary experimental data suggest that INGARD® cotton may be more weedy than non-GM cotton in certain nutrient-rich habitats such as artificial water ways and stock feeding areas.

253.If Roundup Ready® or Roundup Ready®/INGARD® cotton is transported north of latitude 22º South for use as stockfeed, it is considered that the risks of cotton establishing as a weed could be managed to an acceptable level by implementing various strategies to minimise the spread and persistence of the cotton in the northern environment. The licence requires the licence holder to provide information about cotton volunteers and there control to endusers of cotton seed north of 22º South, and to conduct annual surveys of areas where seed is fed to stock. Refer to Chapter 2 and Appendix 7 for details of management condition.

254.In order to complete the environmental monitoring program required in relation to the original commercial release of Roundup Ready® and Roundup Ready®/ INGARD® cotton in 2000, the licence requires continuation of monitoring for volunteer cotton in non-agricultural situations within the cotton growing areas of New South Wales and Queensland for an additional growing season.

The licence holder is also required to report any adverse effects on human health and safety or the environment.

APPENDIX 5 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

255. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan. This Appendix considers potential hazards that may be posed through the transfer of the introduced genes from Roundup Ready® or Roundup Ready®/INGARD® cotton to other organisms.

256. Gene transfer is the movement of genes between individuals. Within a species genes are routinely exchanged between individuals of successive generations through sexual reproduction. Hybrids can be produced between closely related species through sexual reproduction. For example, in plants cross pollination of wheat and rye produces triticale, in animals fertilisation of a mare by a donkey produces a mule. Hybrid progeny may be fertile or sterile, meaning hybridisation may or may not lead to the introgression of a gene or genes into a population. Without the application of gene technology, gene transfer is not readily observed between distantly related species, except among bacteria. However gene transfer between sexually incompatible organisms can occur. Detailed examination of DNA sequence similarities reveals that ancestral plants have occasionally exchanged small DNA fragments with distantly related organisms. In general there seems to have been only very limited transfer of genes from plants to other types of organisms.

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

- Section 1 details the nature and likelihood of genes introduced to Roundup Ready® and Roundup Ready®/INGARD® cotton transferring to other plants, including other cotton crops;
- Section 2 details the nature and likelihood of genes introduced to Roundup Ready® and Roundup Ready®/INGARD® cotton transferring to microorganisms; and
- Section 3 details the nature and likelihood of genes introduced to Roundup Ready® and Roundup Ready®/INGARD® cotton transferring to animals, including humans.
- Section 4 draws together the conclusions from these Sections.

SECTION 1 GENE TRANSFER FROM ROUNDUP READY® AND ROUNDUP READY®/INGARD® COTTON TO OTHER PLANTS

Section 1.1 Nature of the gene transfer hazard

258. The regularity and extent of gene transfer in a plant population often relates to its breeding system. Plants are either self- or cross-pollinated, or a mixture of the two mechanisms may operate in a single plant or species, as is the case in cotton (*Gossypium hirsutum*). Plants with high levels of cross pollination show greater genetic variation than those which are self pollinated, as there is more opportunity for gene flow (transfer) and recombination within a population.

259. Cross pollination may occur between individuals of closely related species as well as between individuals within a species. Termed 'hybridisation', crossing of closely related species occurs in nature and is also a tool used in plant breeding. While plant evolution shows hybridisation to occur

frequently, it is not ubiquitous (Ellstrand et al. 1999). The incidence of natural hybridisation varies substantially among plant genera and families. Failure of hybridisation may relate to the taxonomic distance between species or to simple physical differences, such as timing of flowering.

260.In situations where gene transfer to other plants can occur, the hazards to the environment associated with any such transfers could be highly varied, broadly depending upon the resulting phenotype of the progeny, such as any alteration in survival or reproductive capacity.

Section 1.2 Likelihood of a hazard arising through gene transfer from Roundup Ready® and Roundup Ready®/INGARD® cotton to other plants

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

Section 1.2.1 The introduced genes

cp4 epsps (HERBICIDE TOLERANCE) GENE

262.Plants expressing this gene could become toxic to tolerant to the herbicide glyphosate. This could confer a selective advantage on the plant in the presence of glyphosate use.

cry1Ac (INSECTICIDAL) GENE

263.Plants expressing this gene could become toxic to lepidopteran insects. This could confer a selective advantage on the plants or adversely affect survival of lepidopteran insects and consequently also specialist predators and parasites of the lepidopteran insects.

nptII AND *aad* (ANTIBIOTIC RESISTANCE) GENES

264.Plants expressing these genes could become resistant to the antibiotics. This would only have an impact on plant survival if the antibiotics were used on the plants, or otherwise present in the environment of the plant, and were limiting its growth. Antibiotics are not generally applied to crops and would not limit their growth except at very high concentrations not found in the natural or agricultural environment. Expression of the *nptII* gene enabled selection of plant cells containing the genetic modification in the laboratory. The *aad* gene is not expressed in plant cells. It was used in the laboratory to allow selection of bacteria containing the desired genes prior to generation of GM plant cells.

CaMV 35S PROMOTER AND OTHER REGULATORY SEQUENCES

265.If these sequences were to be transferred to other plants without the associated genes of Roundup Ready® and Roundup Ready®/INGARD® cotton, the expression of endogenous plant genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

266.Some of the introduced regulatory sequences are derived from plant pathogens (cauliflower mosaic virus, figwort mosaic virus, *Agrobacterium tumefaciens*). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

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

1.2.2 Transfer to cultivated cotton

268.Cotton is primarily self pollinating, however in a cropping situation a low level of pollen transfer, by insect pollinators, to other nearby vegetation would be likely. For a detailed consideration of the likelihood of this occurring, including an overview of the pollination biology of cotton, see the document "The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia" (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton.

269.*Gossypium barbadense* (pima cotton) is also used for commercial cotton production, but only to a very minor extent in Australia (Lake Tandou and Bourke, NSW). *G. hirsutum* and *G. barbadense* are closely related and hybridisation between the two species can occur, yielding fertile progeny. Hybrid progeny exhibit characteristics intermediate to the parents but typically with lower capacity to produce fruit. After several generations, progeny of the hybrids revert to the characteristics of one or other of the parents. *G. barbadense* and hybrids are not more weedy or difficult to control than is *G. hirsutum* (personal communication, Warwick Stiller & Greg Constable, CSIRO). Thus the transfer of the novel genes from Roundup Ready® or Roundup Ready®/INGARD® cotton to *G. barbadense* crop plants would not present any hazards additional to those posed by the Roundup Ready® and Roundup Ready®/INGARD® cotton (see Appendices 2 - 6).

270.Currently for commercially released INGARD® cotton (and Bollgard II® cotton), no measures are taken to limit outcrossing and no specific segregation measures are used, other than the standard measures used in the industry for the production of certified (pure) seed. The use of pure seed by all growers every season prevents the accumulation of outcrossed progeny in planting seed from one season to the next and protects varietal integrity.

271.On farm, there is a requirement for cotton volunteers to be controlled as part of the INGARD® Technology User Agreement (TUA). While this requirement is motivated by the Insecticide Resistance Management Strategy, it indirectly limits gene transfer from INGARD® cotton crops to other cultivated cotton plants.

272.Transfer of the introduced genes or regulatory sequences to other cotton plants growing in cultivation would present the same hazards as the presence of the genes in Roundup Ready® and Roundup Ready®/INGARD® cotton (see Appendices 2 - 6).

1.2.3 Transfer to volunteer and naturalised (feral) cotton

273.Off farm, cotton volunteers may establish along roadsides in cotton growing areas (see Appendix 4), the majority of which are in pollinating distance of cotton crops, primarily due to transport of harvested seed cotton. Within and between cotton growing regions, transport of Roundup Ready® and Roundup Ready®/INGARD® cotton is unrestricted. Surveys indicate that

Roundup Ready® and Roundup Ready®/INGARD® cotton volunteers are not more weedy than non-GM cotton in these regions (Appendix 4, Section 2).

274.Transfer of the introduced genes to naturalised cotton may increase the likelihood that the genes could spread and/or persist in the environment (away from cotton farming systems). Gene transfer to naturalised (feral) cotton populations is thought to be unlikely because of the geographic distances between these naturalised populations and the cotton growing regions of NSW and QLD. However herbarium records of *G. hirsutum* and *G. barbadense* suggest that naturalised populations may occur, or may have occurred in the past, in central and south eastern Queensland. The remnants of these populations, which may be within pollinating distance of cotton crops, has not been confirmed. As part of the licence conditions for DIR 022/2002 (INGARD® cotton), Monsanto is required to conduct a survey of feral cotton populations in Queensland, based on herbarium records.

275.Transfer of the introduced genes or regulatory sequences to non-cultivated cotton plants would present the same hazards as the presence of the genes in Roundup Ready® and Roundup Ready®/INGARD® cotton (see Appendices 2 - 6).

1.2.4 Transfer to native cottons and other plant species

276.Australian flora contains 17 native *Gossypium* species. All of the Australian *Gossypium* species are diploids (C, G or K genomes), while the cultivated cottons are tetraploids (AD-genomes). The native species with highest potential for hybridising with *G. hirsutum* is *G. sturtianum*. Hybrids have been produced without application of plant hormones, when plants were planted in close proximity of each other. However these hybrids were sterile, effectively eliminating any potential for introgression of *G. hirsutum* genes into *G. sturtianum* populations.

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

278.The failure of cross-pollination due to well established genetic incompatibility also prevents gene transfer from Roundup Ready® and Roundup Ready®/INGARD® cotton to other plant species.

SECTION 2 GENE TRANSFER FROM ROUNDUP READY® AND ROUNDUP READY®/INGARD® COTTON TO MICROORGANISMS

Section 2.1 Nature of the gene transfer hazard

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

280.The potential hazards associated with the introduced genes of Roundup Ready® and Roundup Ready®/INGARD® cotton transferring to microorganisms could be highly varied, broadly depending upon the phenotype of the recipient and any changes to its survival or reproductive capacity.

Section 2.2 Likelihood of hazard arising through gene transfer from Roundup Ready® and Roundup Ready®/INGARD® cotton to microorganisms

281.The likelihood of gene transfer creating a hazard for human health and safety or the environment depends on the characteristics of introduced gene sequences, as well as on the likelihood of the transfer itself and on the likelihood of transfer from other sources of these genes in the environment, discussed in following sub-sections.

Section 2.2.1 The introduced genes

cp4 epsps (HERBICIDE TOLERANCE) GENE

282.Microorganisms expressing this gene could gain the capacity to synthesise aromatic amino acid and other aromatic compounds in the presence of glyphosate. However this would not have a significant effect on microbial communities, since the ability to rapidly degrade glyphosate is widespread among microorganisms, and soil microorganism populations are not significantly effected by glyphosate application (Malik et al. 1989).

cry1Ac (INSECTICIDAL) GENE

283.Microorganisms expressing this gene could become toxic to lepidopteran insects. This could impact on survival of lepidopteran insects if the recipient microorganisms were ingested inadvertently at high levels. Microorganism populations could also be affected if toxicity to lepidopteran insects gave the recipient a survival or reproductive advantage.

nptII AND *aad* (ANTIBIOTIC RESISTANCE) GENES

284.Microorganisms could become resistant to the antibiotics. The consequences of this for human health and safety and the environment would depend on other characteristics of the microorganism (for example pathogenicity), the use and significance of the antibiotic(s) in clinical and/or veterinary practice and whether these antibiotics limit growth or survival of the microorganism in other circumstances.

285.Some microorganisms may be limited by antibiotics, either due to the use of antibiotic medicines or in some limited environmental situations where competing microorganisms produce antibiotics. Viruses are not limited by antibiotics.

CaMV 35S PROMOTER AND OTHER REGULATORY SEQUENCES

286.If these sequences were to be transferred to microorganisms without the associated genes of INGARD® cotton, the expression of endogenous genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

287.Some of the introduced regulatory sequences are derived from plant pathogens (cauliflower mosaic virus, figwort mosaic virus, *Agrobacterium tumefaciens*). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants. There is a possibility that, due to sequence similarity, the virally derived regulatory sequences could recombine with the genome of another virus infecting the plants to create a novel recombinant virus. While the likelihood of recombination increases with increasing sequence relatedness, the amount of sequence change in the recipient resulting from the recombination falls. Also the genes linked to these elements in the GM cottons will not offer any selective advantage to a virus, if transferred along with the homologous sequences.

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

2.2.2 Other sources of the introduced genes in the environment, and their potential for horizontal transfer

289.Information on other sources of the introduced genes in the environment is discussed here to provide base line information on the prevalence and transfer of these genes that would happen naturally, irrespective of the GM cottons.

290.All of the introduced genes in Roundup Ready® and Roundup Ready®/INGARD® cotton are already widespread in the environment, being derived from common soil bacteria. Some of the regulatory sequences are also derived from common plant viruses.

cp4 epsps (HERBICIDE TOLERANCE) GENE

291.All plants, bacteria and fungi carry *epsps* genes. The difference between the *cp4 epsps* gene in Roundup Ready® and Roundup Ready®/INGARD® cotton and other *epsps* genes, and the encoded enzymes, is in their DNA and amino acid sequences, not in their physiological functions (see Appendix 1 for details). The *cp4 epsps* gene is derived from the common soil bacterium *Agrobacterium* species strain CP4 (Barry et al. 1992; Padgette et al. 1996), which is found in soil and on plants. The encoded CP4 EPSPS enzyme is naturally insensitive to glyphosate (Padgette et al. 1993), as are a number of other microbial EPSPS enzymes (Schulz et al. 1985; Eschenburg et al. 2002). Thus insensitivity to glyphosate is already widespread in microbial populations.

cry1Ac (INSECTICIDAL) GENE

292.The *cry1Ac* insecticidal gene expressed in Roundup Ready®/INGARD® cotton occurs naturally in a common soil bacterium, *Bacillus thuringiensis* (Bt). Bt has been isolated from a wide range of sources such as forest, soil, grain dust, bat dung, sea water and dead insects (Martin & Travers 1989).

293.Many Bt toxin genes are not carried in chromosomal DNA, but are encoded on extra-chromosomal DNA, known as plasmids. Plasmids are known to be exchanged between bacterial species in nature by conjugation and transformation. The native *cry1Ac* gene has been identified on a plasmid of Bt *kurstaki* strain HD-73 (Lereclus et al. 1993). It has been demonstrated in the

laboratory that Bt strains can interchange toxin-encoding plasmids with other Bt strains and with other bacterial species (Glare & O'Callaghan 2000). Horizontal gene transfer may also occur by transduction mediated by bacteriophages (Glare & O'Callaghan 2000).

nptII AND *aad* (ANTIBIOTIC RESISTANCE) GENES

294.The *nptII* and *aad* genes were originally isolated from mobile genetic elements (transposons) found in the plasmids and chromosomes of common bacteria. Transposons are readily transferable between bacterial species in nature. The *nptII* gene is associated with transposon Tn5 and is observed in gram negative bacteria and *Pseudomonas sp*. While it is widely dispersed in the environment, other genes that also confer resistance to neomycin and kanamycin are more common, and also readily transferable (Smalla et al. 1994; Belgian Biosafety Server 1999). The *aad* gene is found in several transposons (eg. Tn7 and Tn21) and occurs at high frequency among gram-negative bacteria (Belgian Biosafety Server 1999).

Section 2.2.3 Likelihood of gene transfer from Roundup Ready® and Roundup Ready®/INGARD® cotton to microorganisms occurring

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

296.In contrast, transfers of plant genes to other organisms such as bacteria, fungi or viruses are exceedingly rare (Mayo & Jolly 1991; Nielsen et al. 1998; Nielsen et al. 2000; Harper et al. 1999; Schoelz & Wintermantel 1993; Greene & Allison 1994; Pittard 1997; Aoki & Syono 1999; Worobey & Holmes 1999). The transfer of plant genes to bacteria and viruses has been observed in laboratory and glasshouse experiments (Nielsen et al. 1998; Nielsen et al. 2000; Schoelz & Wintermantel 1993; Greene & Allison 1994; Pittard 1997; Worobey & Holmes 1999). However, in all cases this was achieved only under controlled conditions with the presence of related gene sequences (homologous recombination), and using powerful selection methods to detect extremely rare gene transfer events.

2.2.3.1 BACTERIA

297.Natural transformation is a mechanism by which transfer of DNA from plants to microorganisms could have occurred during evolution (Bertolla & Simonet 1999) and is the mechanism that is most likely to contribute to a horizontal gene transfer from transgenic plants to bacteria (Smalla et al. 2000). Natural transformation enables competent bacteria to generate genetic variability by taking up and integrating free DNA that is present in their surroundings. This uptake of DNA does not necessarily depend on DNA sequence, thus indicating the potential of gene transfer from divergent donor organisms (Nielsen 1998).

298.A number of steps and conditions would need be fulfilled for functional natural transformation to occur (Bertolla & Simonet 1999), many of which are highly unlikely, making the overall likelihood of gene transfer, and of resulting hazard, extremely low:

- Release of the DNA molecules from plant cells into the environment;

- Persistence of the free DNA in the environment;
- Presence of bacterial genotypes capable of developing competence for natural transformation;
- Appropriate biotic and abiotic conditions for the development of the competent stage.
- uptake of DNA fragments;
- Chromosomal integration via recombination or autonomous replication of the transforming DNA;
- Expression of the genes by the recipient bacterium;
- Selective advantage to fix the transformation into the gene pool.

299. Thus horizontal gene transfer from plants to bacteria has not been demonstrated under natural conditions (Syvanen 1999) and deliberate attempts to induce such transfers have so far failed (eg. Schlüter et al. 1995; Coghlan 2000). Transfer of plant DNA to bacteria has been demonstrated only under highly artificial laboratory conditions, between homologous sequences and under conditions of selective pressure (Mercer et al. 1999; Gebhard & Smalla 1998; De Vries & Wackernagel 1998; De Vries et al. 2001) and even then only, at a very low frequency.

300. Using antibiotic selection to detect extremely rare events, *Acinobacter sp*. cells containing a defective copy of the neomycin resistance (*nptII*) gene (with 10 bp or 317 bp of DNA deleted) were observed to incorporate DNA from GM plants (sugarbeet, tomato, potato or oilseed rape) carrying the intact *nptII* gene, leading to restoration of neomycin resistance. Without the artificially introduced homology in the recipient strain, no uptake of DNA could be detected in *Acinobacter* sp. (Nielsen et al. 2000; De Vries et al. 2001) or in *Pseudomonas stutzeri* (De Vries et al. 2001).

RELEASE AND PERSISTENCE

301. Several studies have demonstrated the persistence of plant DNA in the soil (Gebhard & Smalla 1999; Paget & Simonet 1994; Widmer et al. 1996; Paget & Simonet 1997; Widmer et al. 1997). Bacteria residing on the plant surface can access nutrients leaking from the leaf or exuded from the root and they often aggregate in biofilms that can facilitate cell-to-cell contact and thereby possibly DNA transfer. Several studies have also demonstrated the persistence of plant DNA in the gastrointestinal tract of animals, in contact with the microorganisms that colonise the whole length of the gastrointestinal tract and aid in the digestive process. However, the proportion of DNA which may derive from the introduced genes of GM plants in the animal diet is extremely low (see Section 3.2.3).

BACTERIAL COMPETENCE AND DNA UPTAKE

302. The major limiting factor for natural transformation remains the presence of potentially competent bacterial species and the development of competence (Smalla et al. 2000). Competence in bacteria is not usually constitutively expressed: bacterial species that are transformable need to enter a physiologically regulated state of competence for the uptake of exogenous DNA (Lorenz & Wackernagel 1994). Few bacteria induced to express competence in the laboratory have subsequently been shown to be able to express competence under natural conditions (Nielsen, 1998).

303.Electrical fields and current are also known to be capable of permeabilising bacterial cell membranes under laboratory conditions, facilitating experimental transformation. Given that the environment is subjected to regular thunderstorms and lightning discharges that induce enormous electrical perturbations, the possibility of natural electro-transformation of bacteria has been investigated. Bacteria added to soil have been transformed via simulated lightning in the laboratory (Demaneche et al. 2001), however there is no direct evidence that this is occurring in nature.

DNA INTEGRATION

304.Integration of genes into the genome of recipient bacteria is known to be dependent on sequence homology between the captured DNA and that of the recipient bacteria. It seems that heterology between these sequences is the main barrier to the stable introduction of diverged DNA in bacteria (Baron et al. 1968; Rayssiguier et al. 1989; Matic et al. 1995; Vulic et al. 1997). In enterobacteria there is an exponential relationship between recombination frequencies and sequence similarity of introduced DNA (Vulic et al. 1997). Although there is a higher probability of recombination when the sequences are more similar, the consequent risk of adverse effect is reduced because with highly similar sequences the likelihood of any recombinants expressing novel properties is low.

EXPRESSION AND SELECTION

305.Even if the barriers to uptake and integration are overcome, there are also barriers to expression of the exogenous genes. For example:

- many plant promoters will not be active in bacteria;
- processing of the intermediate RNA may be required for protein expression (eg. removal of introns to generate functional mRNA for translation), which will not occur in bacteria;
- coding sequences of plant genes may not be efficiently translated in bacteria due to differences in codon usage (note that the coding sequences of the bacterially derived *cry1Ac* and *cp4 epsps* genes were modified to enhance expression in plants); and
- processing of an encoded 'pro-protein' may be required fro production of a functional product (eg. cleavage of the Chloroplast Transit Peptide from plant EPSPS pro-proteins, see Appendix 1).

306.Prokaryotes have efficient genomes and generally do not contain extraneous DNA sequences. If the genes are not useful to the organism then there will be no selective advantage in maintaining them in the genome, and they are not likely be persist. Thus the risk of gene transfer leading to hazardous consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.2.2).

2.2.3.2 VIRUSES

307.There is a theoretical possibility of recombination between sequences that have been introduced into the genome of GM plants and the genome of viruses that infect the plants (Hodgson 2000a; Ho et al. 2000; Hodgson 2000b). Recombination between viral genomes and plant DNA has only been observed at very low levels, and only between homologous sequences under conditions of

selective pressure, eg regeneration of infectious virus by complementation of a defective virus by viral sequences introduced into a GM plant genome (Greene & Allison 1994; Teycheney & Tepfer 1999). With homologous sequences the consequent risks of adverse effects arising from gene transfer is reduced because with highly similar sequences the likelihood of any recombinants expressing novel properties low.

308.Thus the risk of gene transfer leading to hazardous consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.2.2).

2.2.3.3 FUNGI

309.Fungi are known to be transformable, and horizontal gene transfer from plants to plant-associated fungi has been claimed. Uptake of DNA from the host plant by *Plasmodiophora brassicae* (Bryngelsson et al. 1988; Buhariwalla & Mithen 1995) and uptake of the hygromycin gene from a GM plant by *Aspergillus niger* (Hoffman et al. 1994) have been reported. However, stable integration and inheritance of the plant DNA in the genome of these fungi has not been substantiated by experimental evidence (Nielsen 1998).

310.Thus the risk of gene transfer leading to hazardous consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.2.2).

SECTION 3 GENE TRANSFER FROM ROUNDUP READY® AND ROUNDUP READY®/INGARD® COTTON TO ANIMALS

Section 3.1 Nature of the gene transfer hazard

311.The potential hazards associated with the genes introduced in Roundup Ready® and Roundup Ready®/INGARD® cotton transferring to animals, including humans, could be highly varied, broadly depending upon the phenotype of the recipient and any changes to the survival or reproductive capacity of it or its progeny.

Section 3.2 Likelihood of hazard arising through gene transfer from INGARD â cotton to animals (including humans)

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

Section 3.2.1 The introduced genes

cp4 epsps (HERBICIDE TOLERANCE) GENE

313.The expression of this gene in animals would not be expected to lead to any significant effects, since this gene encodes an enzyme in a biosynthesis pathway that is not present in animals, including humans.

cry1Ac (INSECTICIDAL) GENE

314.Animals could become toxic to lepidopteran insects. This is not likely to pose any consequences for lepidopteran insects, nor would such a transfer confer a selective advantage to the animal.

nptII AND *aad* (ANTIBIOTIC RESISTANCE) GENES:

315.Animals cells could gain the ability to degrade the corresponding antibiotics. If the transfer occurred to humans or other animals treated with these antibiotics, this may affect antibiotic treatment. However the gene products, the NPTII and AAD enzymes, would only be active within the transformed animal cells, where appropriate conditions and co-factors for activity exist, therefore interference with any antibiotic treatment is unlikely. Animals are not controlled by antibiotics, so no selective advantage would result.

CaMV 35S PROMOTER AND OTHER REGULATORY SEQUENCES

316.If these sequences were to be transferred to animals without the associated genes of Roundup Ready® and Roundup Ready®/INGARD® cotton, the expression of endogenous genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on the resulting phenotypic change induced. However the same is true of any plant gene regulatory sequences, if transferred into a new genetic context. Thus the potential hazard is generally not increased relative to that of transfer from non-GM plants.

317.If these sequences were to be transferred to animals without the associated genes of INGARD® cotton, the expression of endogenous genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

318.Some of the introduced regulatory sequences are derived from plant pathogens (cauliflower mosaic virus, figwort mosaic virus, *Agrobacterium tumefaciens*). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

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

3.2.2 Humans

320.The most significant route for entry of foreign DNA into humans is through food, as it passes through the gastrointestinal tract. The epithelial lining of the gastrointestinal tract is exposed to foreign DNA released from food. Microorganisms colonise the whole length of the gastrointestinal tract, aiding the digestive process.

321.Cotton oil and linters are the only fraction of the GM cotton plants used in human food. Since these products are free of DNA (see appendix 2), humans will not be exposed to GM cotton DNA via the digestive system, excluding the possibility of gene transfer to human cells in the gut.

3.2.3 Animals

322.GM cotton seed may be fed to farm animals, exposing their gastrointestinal tract to the introduced genes. The fate of DNA in the digestive tract of various animals has been studied. A review of the safety issues associated with the DNA in animal feed derived from GM crops (Beever & Kemp 2000) indicated exposure to introduced DNA from GM crop material is negligible compared with normal exposure to non-GM DNA. They calculated that in a diet containing 40% GM maize, the introduced genes would represent 0.00042% of total dietary DNA intake.

323.Alexander et al. (2002) investigated the digestive fate of DNA from GM glyphoste-tolerant (Roundup Ready®) canola. They used PCR to detect the presence of two genes in various canola feed fractions following *in vitro* incubated in bovine ruminal fluid. The genes analysed were the *cp4 epsps* gene introduced by genetic modification and an endogenous nuclear-encoded *rbcS* gene (encoding the small subunit of the photosynthetic enzyme Rubisco).

324.Whole seed, cracked seed, canola meal or a prepared diet (containing 6.5% canola meal) were examined. Processing of canola seed to meal was found to significantly reduce the amount of DNA detected. There were no significant differences in the detection of the introduced or endogenous gene. These feeds were incubated in batch cultures of ruminal fluid. Both genes could be detected in the cultures of whole and cracked seed for up to 48 hours, but only up to eight hours for meal and four hours for the prepared diet. The genes were detected in the plant debris but not in the aqueous phase of the ruminal cultures. The authors concluded that the plant DNA was rapidly degraded by rumen fluid and that the persistence of DNA was inversely related to plant cell digestion (Alexander et al. 2002). These results support the conclusion that the rapid degradation of DNA following release from plant cells during ruminant digestion represents a considerable barrier to transfer of plant DNA, GM and non-GM, to rumen bacteria or to ruminant animals.

325.Einspanier et al. (2001) investigated the fate of DNA from GM maize fed to cattle and chickens, using PCR to detect the introduced *cry1Ab* gene (which confers resistance to insects) and an endogenous plant chloroplast gene. Since multiple chloroplasts are present in plant cells, more copies of the chloroplast gene are present in the GM maize than of the *cry1Ab* gene.

326.For cattle fed GM maize silage, both the *cry1Ab* gene and the chloroplast marker were detected in chyme (duodenal juice). The chloroplast marker was detected in lymphocytes and faint signals were occasionally detected in milk, but it was not detected in faeces, whole blood, muscle, liver or spleen. The *cry1Ab* gene was not detected in any of these samples (Einspanier et al. 2001).

327.In chickens fed a diet containing GM maize, the chloroplast marker was detected in muscle, liver, spleen and kidney, but not in faeces or eggs. In contrast, the *cry1Ab* gene was not detected in any tissue sample or eggs (Einspanier et al. 2001).

328.The possibility of DNA transfer in the gut has also been investigated by feeding mice large quantities of purified bacteriophage DNA (Schubbert et al. 1997). Bacteriophage DNA was detected in the faeces and the livers of mice as well as in rarely in newborn mice (Schubbert et al. 1997). However the relevance of this work to gene transfer from GM plants was questioned by Beever and Kemp (2000), who concluded that the bacteriophage DNA was in a form which would

stimulate a response by cells of the immune system, and that the cells containing this DNA in various organs and newborns were macrophages involved in scavenging and removing foreign DNA.

329.In the rare event of plant DNA uptake by animals cells, a further step of chromosomal integration has not been demonstrated. Furthermore, any uptake of plant DNA is likely to occur in non-reproductive (somatic) cells such as immune system or gut epithelium cells, and the introduced gene would not be transmitted to the cells of any progeny. Thus the likelihood of transfer is extremely low, and not greater than the likelihood of transfer from other sources of the introduced genes in the environment (Section 2.2.2).

SECTION 4 CONCLUSIONS REGARDING GENE TRANSFER TO OTHER ORGANISMS

Section 4.1 Conclusions regarding gene transfer to other plants

330.It is considered that although some gene transfer from Roundup Ready® and Roundup Ready®/INGARD® cotton to cultivated cotton (of both *G. hirsutum* and *G. barbadense*) is likely, the risks posed are negligible because:

- gene transfer would not pose any risks additional to the low risks posed by Roundup Ready® and Roundup Ready®/INGARD® cotton.

331.Although transfer of the introduced genes from Roundup Ready® or Roundup Ready®/INGARD® cotton to naturalised (feral) cotton (both *G. hirsutum* and *G. barbadense*) may increase the likelihood that the genes could spread and/or persist in the environment, it is considered that the likelihood of a hazard arising through gene transfer to volunteer or naturalised cotton is negligible, because:

- gene transfer would not pose any risks additional to the low risks posed by Roundup Ready® and Roundup Ready®/INGARD® cotton;
- volunteer cotton in the cotton growing regions of Australia already include Roundup Ready® and Roundup Ready®/INGARD® cotton, and surveys indicate that these are not more weedy than non-GM cotton; and
- gene transfer to naturalised (feral) cotton populations is thought to be unlikely because of the geographic isolation.

332.It is considered that the risk of gene transfer from Roundup Ready® or Roundup Ready®/INGARD® cotton to native cotton species is negligible, because:

- genetic incompatibility and geographical isolation prevent the production of fertile hybrids.

333.It is considered that the risk of gene transfer from Roundup Ready® or Roundup Ready®/INGARD® cotton to other plant genera is negligible, because:

- well established genetic incompatibility prevents successful cross pollination with other plant species.

334.Therefore it is not considered necessary to impose any management conditions in relation to gene transfer to other plants. The licence holder is required to report adverse effects on human health and safety or the environment.

Section 4.2 Conclusions regarding gene transfer to microorganisms

335.It is considered that the risk of a hazard arising through transfer of the introduced genes from Roundup Ready® or Roundup Ready®/INGARD® cotton to microorganisms is negligible, because:

- all of the introduced genes in Roundup Ready® and Roundup Ready®/INGARD® cotton are already widespread in the environment, and are readily available for transfer from these sources via demonstrated natural mechanisms; and
- gene transfer has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other sources of these genes.

336.Therefore it is not considered necessary to impose any management conditions in relation to gene transfer to microorganisms. The licence holder is required to report adverse effects on human health and safety or the environment.

Section 4.3 Conclusions regarding gene transfer to animals, including humans

337.The most significant route of entry of foreign DNA into animals and humans is through food. The gastrointestinal tract may be exposed to free DNA during digestion. It is considered that the risk of a hazard arising through transfer of the introduced genes from Roundup Ready® or Roundup Ready®/INGARD® cotton to animals, including humans, is negligible because:

- the introduced genes of Roundup Ready® and Roundup Ready®/INGARD® cotton are not present in human food products;
- the likelihood of transfer is extremely low, and not greater than the likelihood of transfer from other sources of the introduced genes in the environment; and
- transfer of the introduced genes of Roundup Ready® and Roundup Ready®/INGARD® cotton would not present a hazard to health and safety of human or animals.

338.Therefore it is not considered necessary to impose any management conditions in relation to gene transfer to animals, including humans. The licence holder is required to report adverse effects on human health and safety or the environment.

APPENDIX 6 INSECTICIDE AND HERBICIDE RESISTANCE

339.Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. In this part of the document, risks posed by the proposed dealing to the environment are considered in relation to the potential for the development of insecticide resistance among the targeted pests and herbicide resistance among weeds.

SECTION 1 INSECTICIDE RESISTANCE HAZARD

340.Extensive cultivation of INGARD® cotton varieties, including Roundup Ready®/ INGARD® cotton, could potentially result in the emergence of resistance to the Cry1Ac protein in the target species (*Helicoverpa armigera* and *H. puntigera*) and other susceptible lepidopteran species feeding on cotton. This would result in a reduction in the efficacy of these cottons for the control of insect pests, and could also have impacts on the use of Bt microbial sprays to control insects in other agricultural systems. Potential adverse effects include attenuation of the benefits of growing Roundup Ready®/INGARD® cotton to the environment and human health.

341.The possibility of target pests developing resistance to the toxic effects of the Cry1Ac protein present in INGARD® cotton is discussed in detail in Appendix 6 of the risk assessment for DIR022/2002, available at www.ogtr.gov.au. A conclusion of this assessment is that the likelihood of this risk being realised is high.

342.The Australian Pesticides & Veterinary Medicines Authority (APVMA, formerly National Registration Authority for Agricultural and Veterinary Chemicals, NRA) have registered the use of cotton containing the *cry1Ac* gene as an agricultural chemical (product number 48296), due to its production of an insecticidal protein. The APVMA oversees the Insecticide Resistance Management Strategy for INGARD® cotton (including Roundup Ready®/INGARD® cotton) and ensures that the cotton industry continues to refine and implement the Strategy as required to minimise the risk of resistance development.

343.It should also be noted that INGARD® cotton is being phased out in favour of Bollgard II® cotton, which is expected to significantly delay the development of insecticide resistance in target pests (see Chapter 1 and the risk assessments for DIR012/2002 and DIR022/2002, available at www.ogtr.gov.au).

SECTION 2 HERBICIDE RESITANCE HAZARD

344.There is some potential for development of herbicide-resistant weeds if the Roundup Ready® crop-herbicide combination is used inappropriately. As part of the Deed of Agreement between the Commonwealth Government and Monsanto for the original commercial release of these GM cottons in 2000, Monsanto were required to develop a crop management plan designed to minimise the potential for development of weeds resistant to glyphosate (Monsanto Australia Limited 2001).

345.This plan has been endorsed by the herbicide tolerant cotton subcommittee of the Transgenic and Insect Management Strategy (TIMS) committee of the Australian Cotton Growers Research Association and is enforced by Monsanto under its' Technology User Agreement. The use of

Roundup Ready® herbicide (a formulation of glyphosate) on Roundup Ready® and Roundup Ready®/INGARD® cotton crops in Australia is registered by the APVMA. The APVMA has responsibility for setting registration conditions for the use of glyphosate on cotton crops, including implementation of herbicide resistance management programs.

346.Data collected by Monsanto from growers of Roundup Ready® cotton varieties indicates general compliance with this plan. Over three seasons of cultivation of these GM cottons, there has been no indication of a shift in weeds resistant to glyphosate and no change in the level of grower satisfaction with this technology. This suggests that the potential for development of weeds resistant to glyphosate is being managed effectively in Roundup Ready® (including Roundup Ready®/INGARD®) cotton crops.

SECTION 3 CONCLUSIONS REGARDING INSECTICIDE AND HERBICIDE RESISTANCE

Section 3.1 Insecticide resistance

347.Given the large-scale of the INGARD® cotton release, including Roundup Ready®/ INGARD® cotton, it is considered that the risk of insects developing resistance to the insecticidal protein is high. This risk is managed by APVMA, under conditions of registration for the use of agricultural chemicals in Australia. Therefore no specific conditions are imposed in the licence in relation to management of insecticide resistance, however the requirement to comply with conditions imposed by the APVMA is noted.

Section 3.2 Herbicide resistance

348.There is potential for development of herbicide-resistant weeds if the Roundup Ready® crop-herbicide combination is used inappropriately. This risk is managed by the APVMA, under conditions of registration for the use of agricultural chemicals in Australia. Therefore no specific conditions are imposed in the licence in relation to management of herbicide resistance, however the requirement to comply with conditions imposed by the APVMA is noted.

APPENDIX 7 LICENCE CONDITIONS

Note in relation to Herbicide and Insecticide Resistance Management

One of the genetically modified organisms referred to in this licence falls into *the Agricultural and Veterinary Chemicals Code (1994)* definition of an agricultural chemical product, due to its production of an insecticidal substance, and therefore is subject to regulation by the APVMA (the Australian Pesticides and Veterinary Medicines Authority, formerly the National Registration Authority for Agricultural and Veterinary Chemicals).

The APVMA has imposed conditions in connection with the insecticidal activity of this genetically modified organism, including specifying maximum areas for release, for the purpose of managing the development of insecticide resistance in the target pest species. Conditions of this licence do not relate to management of insecticide resistance, and do not replace any conditions set by the APVMA. The licence holder must comply with any conditions imposed by the APVMA in relation to dealings with this GMO.

The genetically modified organisms referred to in this licence have been modified to be tolerant to a herbicide. The APVMA has responsibility for setting registration conditions for the use of herbicides in Australia, including implementation of herbicide resistance management programs. Conditions of this licence do not relate to use of herbicide, and do not replace any conditions set by the APVMA. The licence holder must comply with any conditions imposed by the APVMA in relation to the use of herbicides in connection with these GMOs.

PART 1

Holder of licence

1. The holder of this licence ('the licence holder') is Monsanto Australia Limited.

Persons covered by licence

3. The persons covered by this licence are the licence holder and other persons who undertake any dealing in connection with the GMOs authorised by Clause 5 of Part 1 of this licence.

(Explanatory Note: Each person covered by this licence is a 'person covered by a GMO licence' for the purposes of the Gene Technology Act 2000 (Cth)).

Dealings authorised by licence

5. This licence authorises the licence holder and persons covered by the licence to conduct dealings with the GMOs subject to the limitations on dealing with the GMOs that are contained elsewhere in the conditions in this licence.

Period covered by licence

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

(Note: Although the applicant has stated an intention to phase-out dealings with Roundup Ready®/INGARD® cotton over the next 2 growing seasons, while phasing-in Roundup Ready®/Bollgard II® cotton (under licence number DIR 012/2002), the proposed licence conditions for Roundup Ready®/INGARD® cotton do not include a specific termination clause. Once the phase-out is complete, the applicant can apply to the Regulator to vary the licence.)

PART 2

Interpretation and Definitions

Words and phrases used in this licence have the same meanings as they do in the *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001*.

Words importing a gender include any other gender.

Words in the singular include the plural and words in the plural include the singular.

Words importing persons include a partnership and a body whether corporate or otherwise.

References to any statute or other legislation (whether primary or subordinate) is to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time unless the contrary intention appears.

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:

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

'Covered Vehicles' means vehicles that use tight fitting covers to prevent spillage of the load during transporting (for example a trailer with sides moulded to the base fitted with a roll-over tarp).

'Deal with' has the same meaning as under the *Gene Technology Act 2000* in which 'deal with', in relation to the GMO, means the following:

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

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

'Feral cotton' means naturalised, self-perpetuating populations of unmodified *Gossypium hirsutum* L. and/or *Gossypium barbadense* L.

'GM' means genetically modified.

'GMO' means genetically modified organism authorised for release by this licence.

'OGTR' means the Office of the Gene Technology Regulator.

'Restricted Zone' means north of latitude 22º South in NT, QLD and WA.

'Technology User's Agreement' means the licence issued by the licence holder for use of the GMO.

'Volunteer plant' means progeny of the GMO.

PART 3 CONDITIONS OF LICENCE

The licence holder and persons covered by this licence must comply with the conditions of this licence. The reasons for the specific conditions are set out in the Summary Table presented in Chapter 2 of the risk assessment and risk management plan.

Section 1: General Conditions

Informing people of their obligations

1. The licence holder must inform each person covered by this licence of the obligations imposed on them as a result of the conditions in this licence.

Reporting

2. The licence holder must immediately notify the Regulator in writing if the licence holder becomes aware of:

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

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

3. The licence holder must provide the Regulator with a written report within 90 days of each anniversary of this licence, in accordance with any Guidelines issued by the Regulator in relation to annual reporting. This report must include notification of any adverse impacts on human health and safety or the environment, caused as a result of the GMOs.

Material changes in circumstances

4. The licence holder must immediately notify the Regulator in writing 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 Commonwealth, 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.

Remaining an accredited organisation

5. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and comply with any conditions of accreditation set out in the OGTR Guidelines for Accreditation of Organisations.

Changes to details

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

Section 2: Specific Conditions

Restrictions on growing of the GMOs

7. The licence holder must not enter into a Technology User's Agreement or any other agreement which would permit the GMOs to be grown *outside* of the shires of New South Wales and Queensland specified in Attachment.

Transport of GM whole cotton seed *into* the Restricted Zone

8. The licence holder must provide written notification to cotton gins from which GM whole cotton seed will be transported into the Restricted Zone stating the requirements for transportation *into* the Restricted Zone required by Condition 11. The licence holder must also maintain a record of this action.

9. The licence holder must prepare and distribute to cotton gins from which GM whole cotton seed will be transported *into* the Restricted Zone sufficient copies of the sign required by Condition 11(b). This sign must accompany each shipment of cotton seed into the restricted Zone, as required by condition 11.

10. Cotton gins from which GM whole cotton seed is transported *into* the Restricted Zone must convey the information in the notification prepared by the licence holder under Condition 8 to transporters of GM whole cotton seed *into* the Restricted Zone, provide transporters with a sign to accompany *every* shipment of cottonseed into the restricted Zone, and must maintain a record of this action.

11. Transporters of GM whole cotton seed to destinations within the Restricted Zone must:

(a) only transport the GM whole cotton seed in Covered Vehicles;

(b) sign Covered Vehicles to indicate that they contain GM whole cotton seed, and with instructions to contact the licence holder in the event that the GM whole cotton seed is spilt or misdirected, including telephone contact numbers.

Use of GM whole cotton seed *within* the Restricted Zone

12. The licence holder must, in consultation with the OGTR, develop a communication strategy, including a document for distribution, to convey the importance of appropriate control of cotton volunteers, to all recipients of GM whole cotton seed.

13. The licence holder must provide a written request to cotton gins from which GM whole cottonseed is transported *into* the Restricted Zone that the cotton gins attach the document required by Condition 12 to bill of loading/invoice/weighbridge certificate, such that recipients of GM whole cotton seed in the Restricted Zone will receive a copy of the document.

14. The licence holder must take all reasonable steps to distribute the document specified in condition 12 to:

    (a) the cotton gins from which GM whole cotton seed is sourced for transport *into* the Restricted Zone;

    (b) the transporters of GM whole cotton seed *into* the Restricted Zone; and

    (c) all recipients of GM whole cotton seed *within* the Restricted Zone, including retailers and the end users of the GM whole cotton seed.

Research

15. The licence holder must, in consultation with the OGTR, conduct a survey of non-crop areas for the incidence of Roundup Ready® and Roundup Ready®/INGARD® cotton volunteers.

16. The licence holder must, in consultation with the OGTR, conduct an annual survey within the Restricted Zone of:

    (a) the incidence of volunteer cotton in areas where stock are fed GM whole cotton seed;

    (b) the incidence of volunteer cotton in areas where stock graze after being fed GM whole cotton seed; and

    (c) the extent to which the communication strategy, required in conditions 12 – 14, has been effective.

17. Each of the key geographic regions where GM whole cotton seed is used as stock feed in northern Australia (eg. Atherton Tablelands, Eungella, Katherine, Broome) must be represented in the annual survey required in Condition 16.

18. The findings of research, required in Condition 15 – 17, must be included in the licence holder's annual report to the OGTR.

Compliance management plan

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

Testing Methodology

20. 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 any transferred genetically modified material that might be present in a recipient organism. The instrument must be provided within 30 days of planting the GMOs.

ATTACHMENT

to the Licence for dealings involving an intentional release of GMOs (Roundup Ready® cotton event 1445 and Roundup Ready®/INGARD® cotton event 531) into the environment

DIR No: 023/2002

Shires of New South Wales and Queensland in which the licence holder may permit the GMOs to be grown:

NSW	QLD
Balranald	Aramac
Barraba	Balonne
Berrigan	Banana
Bingara	Bauhinia
Bland	Belyando
Bogan	Broadsound
Bourke	Bungil
Brewarrina	Cambooya
Broken Hill	Chinchilla
Carrathool	Clifton
Central Darling	Dalby
Cobar	Duaringa
Conargo	Emerald
Coolah	Fitzroy
Coonabarabran	Flinders
Coonamble	Gatton
Deniliquin	Inglewood
Dubbo	Jondaryan
Forbes	Kingaroy
Griffith	Milmerran
Gunnedah	Monto
Hay	Murilla
Jerilderie	Murweh
Lachlan	Peak Downs
Manilla	Pittsworth
Moree Plains	Quilpie
Murray	Richmond
Murrumbidgee	Rosalie
Narrabri	Tara
Narromine	Taroom
Parkes	Toowoomba
Parry	Waggamba
Quirindi	Wambo
Tamworth	Warroo

Urana	Warwick
Wakool	Wondai
Walgett	
Warren	
Wellington	
Wentworth	
Yallaroi	

APPENDIX 8 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING THE INTENTIONAL RELEASE OF GENETICALLY MODIFIED ORGANISMS

SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

349.The *Gene Technology Act 2000* (the Act) took effect on 21 June 2001. The Act, supported by the *Gene Technology Regulations 2001*, an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, underpins Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs). The regulatory system replaces the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC).

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

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

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

353.The requirements under the legislation for consultation and for considering and assessing licence applications and preparing risk assessment and risk management plans are discussed in detail in Division 4, Part 5 of the Act and summarised below.

354.Detailed information about the national regulatory system and the gene technology legislation is also available from the OGTR website (www.ogtr.gov.au).

SECTION 2 THE LICENCE APPLICATION

355.Licence applications for dealings involving the intentional release (DIR) of a genetically modified organism into the environment must be submitted in accordance with the requirements of Section 40 of the Act. As required by Schedule 4, Part 2 of the Regulations, the application must include information about:

- the parent organism;
- the GMOs;
- the proposed dealing with the GMOs;
- interaction between the GMOs and the environment;
- risks the GMOs may pose to the health and safety of people;

- risk management;
- previous assessments of approvals; and
- the suitability of the applicant.

356. The application must also contain:

- additional information required for a GMO that is:
  - a plant;
  - a micro-organism (not living in or on animals and not a live vaccine);
  - a micro-organism that lives in or on animals;
  - a live vaccine for use in animals;
  - a vertebrate animal;
  - an aquatic organism;
  - an invertebrate animal;
  - to be used for biological control;
  - to be used for bioremediation; and
  - intended to be used as food for human or vertebrate animal consumption;
- supporting information from the Institutional Biosafety Committee.

357. A preliminary screening of an application is undertaken by OGTR staff to determine whether it complies with the Act and the Regulations, by containing the required information. If this information is provided in the application, the Regulator may then accept the application for formal consideration. Section 43 of the Act provides that the Regulator is not required to consider an application if the application does not contain the required information.

358. After accepting an application for consideration, the Regulator must decide to issue, or refuse to issue, a licence. The decision must be taken following an extensive consultation and evaluation process, as detailed in Sections 3-6 of this Appendix. Regulation 8 of the Regulations prescribe a period of 170 working days within which this decision must be taken. This period does not include weekends or public holidays in the Australian Capital Territory. Also, this period does not include any days in which the Regulator is unable to progress the application because information sought from the applicant in relation to the application has not been received.

SECTION 3 THE INITIAL CONSULTATION PROCESSES

359. In accordance with Section 50 of the Act, the Regulator must seek advice in preparing a risk assessment and risk management plan from prescribed agencies:

- State and Territory Governments;
- the Gene Technology Technical Advisory Committee (GTTAC);
- prescribed Commonwealth agencies (Regulation 9 of the *Gene Technology Regulations 2001* refers);

- the Environment Minister; and
- relevant local council(s) where the release is proposed.

360.Section 49 of the Act requires that if the Regulator is satisfied that at least one of the dealings proposed to be authorised by the licence may pose significant risks to the health and safety of people or to the environment, the Regulator must publish a notice (in national and regional news papers, in the *Gazette* and on the OGTR website) in respect of the application, inviting written submissions on whether the licence should be issued.

361.As a measure over and above those required under the Act, in order to promote the openness and transparency of the regulatory system, the Regulator may take other steps. For example, receipt of applications is notified to the public by posting a notice of each application's receipt on the OGTR website and directly advising those on the OGTR mailing list. Copies of applications are available on request from the OGTR.

SECTION 4 THE EVALUATION PROCESSES

362.The risk assessment process is carried out in accordance with the *Act* and *Regulations*, using the Risk Analysis Framework (the Framework) developed by the Regulator (available on the OGTR website). It also takes into account the guidelines and risk assessment strategies used by related agencies both in Australia and overseas. The Framework was developed in consultation with the States and Territories, Commonwealth government agencies, GTTAC and the public. Its purpose is to provide general guidance to applicants and evaluators and other stakeholders in identifying and assessing the risks posed by GMOs and in determining the measures necessary to manage any such risks.

363.In undertaking a risk assessment, the following are considered and analysed:

- the data presented in the proponent's application;
- data provided previously to GMAC, the interim OGTR or the OGTR in respect of previous releases of relevant GMOs;
- submissions or advice from States and Territories, Commonwealth agencies and the Environment Minister and the public;
- advice from GTTAC;
- information from other national regulatory agencies; and
- current scientific knowledge and the scientific literature.

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

- the risks posed to human health and safety or risks to the environment;
- the properties of the organism to which the dealings relate before it became a GMO;
- the effect, or the expected effect, of the genetic modification that has occurred on the properties of the organism;

- provisions for limiting the dissemination or persistence of the GMO or its genetic material in the environment;
- the potential for spread or persistence of the GMO or its genetic material in the environment;
- the extent or scale of the proposed dealings;
- any likely impacts of the proposed dealings on the health and safety of people.

365.In accordance with Regulation 10 of the Regulations, the following are also taken into account:

- any previous assessment, in Australia or overseas, in relation to allowing or approving dealings with the GMO;
- the potential of the GMO concerned to:
  - be harmful to other organisms;
  - adversely affect any ecosystems;
  - transfer genetic material to another organism;
  - spread, or persist, in the environment;
  - have, in comparison to related organisms, a selective advantage in the environment; and
  - be toxic, allergenic or pathogenic to other organisms.
- the short and long term when taking these factors into account.

SECTION 5 FURTHER CONSULTATION

366.Having prepared a risk assessment and a risk management plan, the Regulator must, under Section 52 of the Act, seek comment from stakeholders, including those outlined in Section 3 and the public.

367.All issues relating to the protection of human health and safety and the environment raised in written submissions on an application or a risk assessment and a risk management plan are considered carefully, and weighed against the body of current scientific information, in reaching the conclusions set out in a final risk assessment and risk management plan. Section 56 of the Act requires that these be taken into account in making a decision on whether or not to issue a licence for the proposed release.

368.Comments received in written submissions on this risk assessment and risk management plan are very important in shaping the final risk assessment and risk management plan and in informing the Regulator's decision on an application. A summary of public submissions and an indication of where such issues have been taken into account are provided in an Appendix to the final risk assessment and risk management plan.

369.It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings are able to be managed so as to **protect human health and safety and the environment**. Matters in submissions that do not address these issues and/or

concern broader issues outside the objective of the legislation will not be considered in the assessment process. In most instances, as determined in the extensive consultation process that led to the development of the legislation, they fall within the responsibilities of other authorities.

SECTION 6 DECISION ON LICENCE

370.Having taken the required steps for assessment of a licence application, the Regulator must decide whether to issue or refuse a licence (Section 55 of the Act). The Regulator must not issue the licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in such a way as to protect the health and safety of people and the environment.

371.The Regulator must also be satisfied, under section 57 of the Act, that the applicant is a suitable person to hold the licence. Section 58 outlines matters the Regulator must consider in deciding whether a person or company is suitable to hold a licence eg.:

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

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

373.The Monitoring and Compliance Section of the OGTR compiles compliance histories of applicants, considering all previous approvals to deal with GMOs under the Act and the previous voluntary system. These histories as well as other information such as follow-up actions from audits may be taken into account. The ability of an organisation to provide resources to adequately meet monitoring and compliance requirements may also be taken into account.

374.If a licence is issued, the Regulator may impose licence conditions (Section 62 of the Act). For example, conditions may be imposed to:

- limit the scope of the dealings;
- require documentation and record-keeping;
- require a level of containment;
- specify waste disposal methods;
- manage risks posed to the health and safety of people, or to the environment;
- require data collection, including studies to be conducted;
- limit the geographic area in which the dealings may occur;
- require contingency planning in respect of unintended effects of the dealings; and
- limit the dissemination or persistence of the GMO or its genetic material in the environment.

375.It is also required as a condition of a licence that the licence holder inform any person covered by the licence of any condition of the licence which applies to them (Section 63 of the Act). Access to the site of a dealing must also be provided to persons authorised by the Regulator for the purpose of auditing and monitoring the dealing and compliance with other licence conditions (Section 64 of the Act). It is a condition of any licence that the licence holder inform the Regulator of:

- any new information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence;
- any contraventions of the licence by a person covered by the licence; and
- any unintended effects of the dealings authorised by the licence.

376.It should be noted that, as well as imposing licence conditions, the Regulator has additional options for risk management. The Regulator has the legislative capacity to enforce compliance with licence conditions, and indeed, to direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment. The OGTR also independently monitors trial sites to determine whether the licence holder is complying with the licence conditions, or whether there are any unforseen problems.

APPENDIX 9 SUMMARY OF PUBLIC SUBMISSIONS

ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

Submission from: **A**: agricultural organisation; **I**: individual.

Issues raised/consideration: **APVMA**: issues dealt with by APVMA; **C**: contamination; **D**: insufficient data/evidence; **FC**: food chain; **FSANZ**: food safety and labelling; **G**: gene transfer; **H**: human health and safety; **IR**: insecticide resistance; **L**: labeling; **MA**: markets; **OSA**: outside scope of the assessment; **RA**: risk assessment; **RM**: risk management; **SEG**: segregation; **W**: weediness.

Sub. No:	Type	Summary of issues raised	Issue	Consideration of issue
1	A	Demand for clean and natural produce is reflected in the rapid growth of the global organic market, currently valued at $40 billion.	FC, MA	FSANZ, OSA
		Uptake of GMOs and their use in the food chain must be accountable, with appropriate labelling of all GMO products and accurate identification to ensure product integrity and consumer confidence.	FC, L	FSANZ, OSA
		Cottonseed meal comprises up to 5% of pig's diet in Australia, and could safely be raised to 20% during feed shortage. Thus, the introduction of GM cotton into the food chain holds strong implications for the pork industry.	FC, MA	FSANZ, OSA
		There are risks through partial or complete rejection of animals fed GM products. Australian pork exporters are now required to provide written assurance to Japanese customers that the diets of their pigs are GMO free.	FC, MA	FSANZ, OSA
		Introduction of GM cotton is likely to increase the amount of cotton seed meal for use in pig diets provided any changes result in cheaper prices. This will offer improvements in ingredient supply and could enhance competitiveness in markets where GMOs are not an issue. However in our major Asian markets … unlikely to provide a competitive advantage.	FC, MA	FSANZ, OSA
		Imperative that tracking, tracing an identity preservation issues are addressed to avoid contamination and ensure that the introduction of GMOs would not impede our export expansion internationally nor negatively impact on our domestic markets.	FC, L, MA, SEG	FSANZ, OSA
		While it is beyond the scope of the current RARMP, further research needs to be conducted aimed at the prospects of improving and expanding on-farm food safety systems and to develop effective tracking and tracing and identity preservation systems. If the benefits, if any, are to be realised, … produce must be traced from its point of origin through to its final destination … through to the end of the production process.	D, FC, L, SEG, MA	FSANZ, OSA

		Need for the cotton growers and policy makers to consider identifying appropriate risk management, tracking and tracing, communication strategies and appropriate labelling, for not just the cotton fibre end product but also for the GM inputs to other industries.	FC, L, SEG, MA	FSANZ, OSA

2	I	These GMOs pose unknown and unknowable risks to the environment and so to human health. However slight these risks are you have no right to take them.	D	App 2 -7
3	I	Can it be said with certainty that GM cotton seed oil or linters are not likely to have adverse effects over a long time frame of 20 years or more, when used in food consumption? Have any longitudinal studies, over generations been done, independently of the companies patenting the GM product? Are any studies being done on human subjects?	D, H,FC	App 2, FSANZ
		What measures are to be taken if the GM cotton does establish as a weed? Can it be controlled easily? Is it possible to manage the problem quickly before it gets out of hand?	W, RM	App 4, 7
		If there is gene transfer of GM cotton to non-GM cotton in neighbouring areas, then how will this be managed and prevented from happening at all, so as not to disadvantage the non GM farmer?	G, C, RM	App 5, 7
		What will be done when insect resistance develops? Will this resistance be able to transfer to other agricultural pests? Can it be said with certainty that this will not happen and how will the applicant be sure it will not?	IR, RM	App 6, OSA, APVMA
		Do not think commercial release is a good idea until all the above can be answered with certainty. If trials have to go on for many years, then that is surely safer than hoping but not really being sure. I oppose the commercial release.	D	Ch 1, App 2 - 6
4	A	Various research projects in the Australian Cotton Cooperative Research Centre (Cotton CRC) have focussed on the role of herbicide tolerant cottons in sustainable farming systems. Research demonstrates the potential for Roundup Ready cotton varieties to greatly assist with the management of hard-to-control weeds and to significantly reduce the use of long-residual herbicides as a component of integrated weed management (IWM) strategy [www address for CRC WEEDpak provided, see http://www.cotton.crc.org.au/Publicat/Weeds/WPConten.htm]		Noted
		Need for additional management strategies to guard against the emergence of Roundup resistant weeds and to counter changes in the weed spectrum. Such strategies are clearly set out in the WEEDpak manual. Adherence to recommendations is clearly important for the long-term sustainability of this technology.	RM	App 7
		Strongly supports the further registration of Roundup Ready cotton technology and the need for robust management strategies to protect against the emergence of new weed issues.	RM	App 7

		Overall concur with the conclusions of the risk assessment. However have concerns about one point in the RA and one statement in the "Summary of the RMP" [page iv], relating to risk of weediness on INGARD/Roundup Ready cottons in northern Australia: "However, data suggest that INGARD cotton may have the potential to be more weedy than non-GM cotton in certain habitats in northern Australia." This is at odds with the conclusions of the report on 'The Potential Weediness of Transgenic Cotton in Northern Australia' by Rowena Eastick (CSIRO Plant Industry and Cotton CRC), submitted to the OGTR. Eastick notes: ➢ "data clearly demonstrate that for each of cottons life stages, the response on INGARD cotton is not significantly different to that of conventional cotton." ➢ no conclusive evidence that the Bt gene conferred additional fitness on cotton plants in non-cropping habitats in northern Australia. ➢ data did show that cotton itself was more invasive when in a niche such as an irrigation channel or cattleyard than in other environments such as roadsides, open woodlands and ephemeral creeks. ➢ however, no difference in invasiveness between conventional and Bt cotton.	RA, W	App 4, 7
		Volunteer cotton in these environments should be managed. There are insect and disease management issues that are addressed by the removal of volunteer cotton, therefore it is in the growers interest to control and remove them, regardless of whether they are Bt, Roundup Ready or conventional plants.	RA, W	App 4
		Believe that dotpoint 4 under "Conclusions of the Risk Assessment" should read: *there is a low risk of Roundup Ready/INGARD cotton becoming weedy in specific habitats in northern Australia*, however this risk is no greater than for conventional genotypes *and can be managed*.	RA, W	App 4
		Eastick also notes: ➢ existing feral populations of *Gossypium hirsutum* in northern Australia "do not exhibit weedy characteristics" and none of the long established feral populations was increasing in extent. Moreover at 7 sites studied in detail the feral cotton was shown to be old cultivars unlike any grown in Australia in the last 30 years. ➢ "no [cotton] plants survived after the collapse of the cotton industry [in Western Australia] in the 1970's, even though farmers simply "walked off" their farms, leaving the cotton crop in the paddocks." Modern *G hirsutum* varieties were cultivated in the Ord River region for at least 15 years from 1957 to 1972.	RA, W	App 4
		These observations strongly question the capacity for current cultivated cotton varieties to establish and survive in northern environments.	RA, W	App 4

APPENDIX 9 REFERENCES

Adang, M.J., Staver, M.J., Rocheleau, T.A., Leighton, J., Barker, R.F., Thompson, D.V. (1985). Characterised full-length and truncated plasmid clones of the crystal protein of *Bacillus thuringiensis* subsp. *kurstaki* HD-73 and toxicity to *Manduca sexta. Gene* **36**: 289-300.

Addison, S. (2001a). Bollgard II - Non-target arthropod East 1999/2000. Report No. MAL-200124, Monsanto Australia, Melbourne.

Addison, S. (2001b). Bollgard II - Non-target arthropod East 2000/2001. Report No. MAL-200126, Monsanto Australia, Melbourne.

Addison, S. (2001c). Bollgard II - Non-target arthropod West 2000. Report No. MAL-200125, Monsanto Australia, Melbourne.

Alexander, T.W., Sharma, R., Okine, E.K., Dixon, W.T., Forster, R.J., Stanford, K., McAllister, T.A. (2002). Impact of feed processing and mixed ruminal culture on the fate of recombinant EPSP synthase and endogenous canola plant DNA. *FEMS Microbiology Letters* **214**: 263-269.

ANZFA (1999). Full assessment report and regulatory impact statement, A341: Oil and linters derived from insect resistant cotton. Report No. A341, Australia New Zealand Food Authority, Canberra, Australia.

ANZFA (2000). Draft risk analysis report, application A355: Food produced from glyphosate-tolerant cotton line 1445. Australia New Zealand Food Authority, Canberra, Australia.

ANZFA (2001). Food derived from glyphosate-tolerant cotton line 1445 - A safety assessment. Australia New Zealand Food Authority, Canberra, Australia.

ANZFA (2002). Draft assessment report (Full assessment - S.15) Application A436: Oil and linters derived from insect-protected cotton containing event 15985. Report No. Full assessment - S.15 Application A436, Australia New Zealand Food Authority, Canberra, Australia.

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

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

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

Australian Cotton Cooperative Research Centre (2002b). Australian dryland cotton: production guide. Cotton Research & Development Corporation, Narrabri, Australia.

Australian Cotton Cooperative Research Centre (2002c). NUTRIpak - A practical guide to cotton nutrition. Australian Cotton Cooperative Research Centre, Narrabri, NSW.

Bakkeren, G., Koukolikova-Nicola, Z., Grimsley, N., Hohn, B. (1989). Recovery of *Agrobacterium tumefaciens* T-DNA molecules from whole plants early after transfer. *Cell* **57**: 847-857.

Barbera, P.W. (1995). Toxicity/pathogenecity testing of *Bacillus thuringiensis* strain EG 7826 following acute oral challenge in rats. Report No. IITRI Project Number L08574, IIT Research Institute, Chicago IL.

Baron, L.S., Gemski, P., Johnson, E.M., Wohlhieter, J.A. (1968). Intergeneric bacterial matings. *Bacteriological Reviews* **32**: 362-369.

Barry, G., Kishore, G., Padgette, S., Taylor, M.L., Kolacz, K., Weldon, M., Re, D., Eichholtz, D., Fincher, K., Hallas, L.E.(1992). Inhibitors of amino acid biosynthesis: Strategies for imparting glyphosate tolerance to crop plants. *In* BK Singh, HE Flores, JC Shannon, eds "Biosynthesis and Molecular regulation of Amino acids in Plants" Vol 7. American Society of Plant Physiologists, Rockville, USA, pp 139-145.

Barry, G.F., Taylor, M.L., Lakemeyer, L.L., Smith, C.E., Bailey, M., and Nida, D.L. (1993). Characterisation of 5-enol-pyruvyl-shikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) produced by glyphosate-tolerant cotton and assessment of equivalence relative to CP4 EPSPS produced by *E. coli*. Report No. MSL-13166, Monsanto Company, USA.

Bartlett, S.G., Grossman, A.R., Chua, N.H.Edelman, M., Hallick, R.B., Chua, N.H. (1982). Methods in chloroplast molecular biology. Elsevier, Amsterdam.

Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B., Schaller, H. (1982). Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. *Gene* **19**: 327-336.

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

Belgian Biosafety Server (1999). Types of antibiotics and related resistance genes. (http://www.antibioresistance.be/ARmenu.html).

Bentley, R. (1990). The shikimate pathway - a metabolic tree with many branches. *Critical Reviews in Biochemistry and Molecular Biology* **25**: 307-384.

Berberich, S.A., Leimgruber, R.M., and Regan, G.J. (1993). Preparation and verification of dose for a mouse acute oral toxicity study with neomycin phosphotransferase II protein (NPTII), study ML-91-409. Report No. MSL 13277, Monsanto Company.

Berberich, S.H. and Fuchs, R.L. (1992). Comparison of the B.t.k HD-73 protein expressed by insect resistant cotton with commercially available microbial pesticides containing the B.t. proteins. Report No. MSL12946, Monsanto Company, USA.

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

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

Bertolla, F., Simonet, P.(1999). Horizontal gene transfers in the environment: natural transformation as a putative process for gene transfers between transgenic plants and microorganisms abstract no. 6). Res Microbiol 150: 375-384

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

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

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

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

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

Canadian Food Inspection Agency (1997). Decision Document 97-21: Detemination of the safety of cotton lines with Roundup Ready genes (*Gossypium hirsutum* L.). Report No. 97-21 , Plant health and Produciton Division, Plant Biotechnology Office,

Cannon, R.J.C. (1993). Prospects and progress for *Bacillus thuringiensis*-based pesticides. *Pesticide Sciences* **37**: 331-335.

Carter, J.N. and Ligget, M.P. (1994). Acute oral toxicity and infectivity/pathogenicity to rats of EG 7841. Report No. HRC Study Report number ECO 6/942538, Huntingdon Research Centre Ltd., Huntington Cambridgeshire England.

Castillo, A.R., Gallardo, M.R., Maciel, M., Giordano, J.M., Conti, G.A., Gaggiotti, M.C., Quaino, O., Gianni, C., Hartnell, G.F. (2001a). Effect of feeding dairy cows with cottonseeds containing Bollgard(R) and Roundup Ready(R) genes or control non-transgenic cottonseeds on feed intake, milk yield and milk composition. *Journal of Dairy Science* **84**: 413.

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

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

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

Cotton Seed Distributors (2002). Wee Waa, NSW, Australia,2002 -2003 variety guide. Cotton Seed Distributors Ltd.

Craven, L. A., Stewart, J. M., Brown, A. H. D., and Grace, J. P. (1994). Challenging the future; the Australian wild species of *Gossypium*. pp. 278-281.

Dankocsik, C., Donovan, W.P., Jany, C.S. (1990). Activation of a cryptic crystal protein gene of *Bacillus thuringiensis* subspecies *kurstaki* by gene fusion and determination of the crystal protein insecticidal specificity. *Molecular Microbiology* **4**: 2087-2094.

Davies, J.E., Benveniste, R.E. (1974). Enzymes that inactivate antibiotics in transit to their targets. *Annals of the New York Academy of Sciences* **235**: 130-136.

De Block, M., Herrera-Estrella, L., Van Montagu, M., Schell, J., Zambryski, P. (1984). Expression of foreign genes in regenerated plants and in their progeny. *EMBO Journal* **3**: 1681-1689.

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

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

della-Cioppa, G., Bauer, S.C., Klein, B.K., Shah, D.M., Fraley, R.T., Kishore, G.M. (1986). Translocation of the precursor of 5-*enol*pyruvylshikimate-3-phiosphate synthase into chloroplasts of higher plants *in vitro*. *Proceedings of the National Academy of Sciences of the United States of America* **83**: 6873-6977.

Demaneche, S., Bertolla, F., Buret, F., Nalin, R., Sailland, A., Auriol, P., Vogel, T.M., Simonet, P. (2001). Laboratory-Scale Evidence for Lightning-Mediated Gene Transfer in Soil. *Applied and Environmental Microbiology* **67**: 3440-3444.

Depicker, A., Stachel, S., Dhaese, P., Zambryski, P., Goodman, H.M. (1982). Nopaline synthase: transcript mapping and DNA sequence. *Journal of Molecular and Applied Genetics* **1**: 561-573.

Donegan, K.K., Palm, C.J., Fieland, V.J., Porteous, L.A., Ganio, L.M., Schaller, D.L., Bucao, L.Q., Seidler, R.J. (1995). Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin. *Applied Soil Ecology* **2**: 111-124.

Donegan, K.K., Seidler, R.J.(1998). Effect of transgenic cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin on soil microorganisms- Risk assessment studies. *In* YPS Bajaj, ed "Biotechnology in Australia,Volume 42:Cotton". pp 300-312.

Eastick, R. (2002). Evaluation of the potential weediness of transgenic cotton in northern Australia. Report No. Technical Bulletin no. 305, Northern Territory Government and Australian Cotton Cooperative Research Centre,

Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser, R., Schwagele, F., Janreis, G., Flachowsky, G. (2001). The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. *European Food Research and Technology* **212**: 129-134.

Ellstrand, N.C., Prentice, H.C., Hancock, J.F. (1999). Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* **30**: 539-563.

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

Ensminger, M.E., Oldfield, J.E., Heinemann, W.W.(1990). Excerpts with reference to cottonseed and cottonseed components. *In* ME Ensminger, ed "Feeds and Nutrition". Ensminger Publishing Company, Clovis, California, pp 252-474.

EPA (2001). Biopesticides registration action document: *Bacillus thuringiensis* plant-incorporated protectants. US EPA,

EPA (2000). Biopesticide fact sheet: *Bacillus thuringiensis* subsp. *kurstaki* Cry1Ac delta-endotoxin and its controlling sequences as expressed in cotton (006445) Issued: 4/00.

EPA (1994). Neomycin phosphotransferase II; tolerance exemption. *Federal Register* **59**: 49351-49353.

Eschenburg, S., Healy, M.L., Priestman, M.A., Lushington, G.H., Schonbrunn, E. (2002). How the mutation glycine96 to alanine confers glyphosate insensitivity to 5-enolpyruvyl shikimate-3-phosphate synthase from *Escherichia coli*. *Planta* **216**: 129-135.

FAO/WHO (2000). Safety aspects of genetically modified foods of plant origin. World Health Organization,

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

FDA (1998). Guidance for Industry: Use of antibiotic resistance marker genes in transgenic plants. U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Premarket Approval, http://vm.cfsan.fda.gov/~dms/opa-armg.html.

FDA (1994). Secondary food additives permitted in food for human consumption; food additives permitted in feed and drinking water of animals; aminoglycoside 3'-phosphotransferase II; final rule. Report No. 59, United Staes Food and Drug Administration, Washington, USA.

Felsot, A.S. (2000a). Herbicide tolerant genes. Part 1: squaring up Roundup Ready crops. *Agrichemical and environmental news* **173**:

Felsot, A.S. (2000b). Insecticidal genes. Part 2: Human health hoopla. *Agrichemical and environmental news* **168**: 1-7.

Fischhoff, D.A., Bowdish, K.S., Perlak, F.J., Marrone, P.G., McCormick, S.M., Niedermeyer, J.G., Dean, D.A., Kusano-Kretzmer, K., Mayer, E.J., Rochester, D.E., Rogers, S.G., Fraley, R.T. (1987). Insect tolerant transgenic tomato plants. *Biotechnology* **5**: 807-813.

Fitt, G. P., Daly, J. C., Mares, C. L., and Olsen, K. (1998). Changing efficacy of transgenic Bt cotton - patterns and consequences. In "*Proceedings of the Sixth Australasian Applied Entomological Research Conference, University of Queensland"*, Brisbane, Australia. pp. 189-196.

Fitt, G.P., Wilson, L.J.(2002). Non-target effects of Bt-cotton: a case study from Australia. *In* RJ Akhurst, CE Beard, PA Hughes, eds "Biotechnology of *Bacillus thuringiensis* and its environmental impact". CSIRO Entomology, Canberra,

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

Franck, A.W.(1989). Food uses of cottonseed protein. *In* "Development in Food proteins". New York, pp 31-80.

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

Fuchs, R.L. (1994). Gene expression and compositional analysis from field-grown insect resistant cotton tissues. Report No. MSL-13315, Monsanto Company, St. Louis, MO, USA.

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

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

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

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

Gebhard, F., Smalla, K.(1999). Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer abstract no. 3). Fems Microbiology Ecology 28: 261-272

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

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

Gowda, S., Wu, F.C., Shepard, R.J. (1989). Identification of promoter sequences for the major RNA transcripts of figwort mosaic and peanut chlorotic streak viruses (caulimovirus group). *Journal of Cellular Biochemistry* **13D (supplement)**: 301.

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

Gregory, S.R., Hernandez, E., Savoy, B.R.(1999). Cottonseed processing. *In* WC Smith, JT Cothren, eds "Cotton: Origin, History, Technology and Production". pp 793-819.

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

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

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

Hamilton, K.A. (2001). Amended report for compositional analyses of seed from cotton expressing both Roundup Ready and Bollgard traits grown in 1998 U.S. and E.U. field trials. Report No. MSL-17118, Monsanto Company,

Hamilton, K.A., Coyler, J., and Fabellar, A. (2000). Production of tissue samples from cotton plants expressing both Bollgard insect protected and Roundup Ready traits grown in 1999 U.S. field trials. Report No. MSL-16702, Monsanto Company, USA.

Hamilton, K.A. and Reed, A. (1999). Production of tissue samples from cotton plants expressing both Bollgard insect protected and Roundup Ready traits grown in 1998 U.S. field trials. Report No. MSL-16418, Monsanto Company,

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

Harrison, L.A., Bailey, M.R., Naylor, M.W., Ream.J.E., Hammond, B.G., Nida, D.L., Burnette, B.L., Nickson, T.E., Mitsky, T.A., Taylor, M.L., Fuchs, R.L., Padgette, S.R. (1996). The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phospate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested *in vitro* and is not toxic to actutely gavaged mice. *Journal of Nutrition* **126**: 728-740.

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

Head, G., Surber, J.B., Watson, J.A., Martin, J.W., Duan, J.J. (2002). No detection of Cry1Ac protein in soil after multiple years of transgenic cotton (Bollgard®) use. *Environmental Entomology* **31**: 30-36.

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

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

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

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

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

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

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

Horsch, R.B., Fraley, R.T., Rogers, S.G., Sanders, S.G., Lloyd, R.P.R., Hoffmann, N. (1984). Inheritance of functional foreign genes in plants. *Science* **223**: 496-498.

International Programme on Chemical Safety (1999). Environmental Health Criteria 217: *Bacilus thuringiensis*. Report No. E, United Nations Environment Programme; International Labour Organisation; World Health Organization,

Jones, M.A., Snipes, C.E. (2002). Tolerance of transgenic cotton to topical applications of glyphosate. *Journal of Cotton Science* **3**: 19-26.

Kay, R., Chan, A., Daly, M., McPherson, J. (1987). Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. *Science* **236**: 1299-1302.

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

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

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

Klee, H.J., Muskopf, Y.M., Gasser, C.S. (1987). Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: sequence analysis and manipulation to obtain glyphosate-tolerant plants. *Molecular and General Genetics* **210**: 437-442.

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

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

Kolwyck, D., Hamilton, K.A., and Lirette, R. (2000). Cry1Ac, CP4 EPSPS and NPTII protein levels in cotton samples produced in the 1999 U.S. field trials. Report No. MSL-16614, Monsanto Company, USA.

Kolwyck, D., Hamilton, K.A., and Reed, A. (1999). Cry1Ac, CP4 EPSPS and NPTII protein levels in cotton samples produced in the 1998 U.S. field trials. Report No. MSL-1624, Monsanto Company, USA.

Lai, M.M.C.(1992). RNA recombination in animal and plant viruses abstract no. 1). Microbiol Rev 56: 61-79

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

Leffler, H.R., Tubertini, B.S. (1976). Development of cotton fruit: accumulation and distribution of mineral nutrients. *Agronomy Journal* **68**: 858-861.

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

Levy, S.B., Marshall, B., Schluederberg, S., Rowse, D., Davis, J. (1998). High frequency of antimicrobial resistance in human fecal flora. *Antimicrobial Agents and Chemotherapy* **32**: 1801-1806.

Lorenz, M.G., Wackernagel, W.(1994). Bacterial gene transfer by natural genetic transformation in the environment abstract no. 3). Microbiol Rev 58: 563-602

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

Maggi, V.L. (1993a). Evaluation of dietary effects of purified B.t.k. endotoxin proteins on honey bee adults. Report No. CAR 181-92, Monsanto Australia Limited.

Maggi, V.L. (1993b). Evaluation of dietary effects of purified B.t.k. endotoxin proteins on honey bee larvae. Report No. CAR 180-92, Monsanto Australia Limited.

Malik, J., Barry, G., Kishore, G. (1989). The herbicide glyphosate. *Biofactors* **2**: 17-25.

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

Matic, I., Rayssiguier, C., Radman, M. (1995). Interspecies gene exchange in bacteria: the role of SIS and mismatch repair systems in evolution of species. *Cell* **80**: 507-515.

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

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

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

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

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

Mitsky, T.A. (1993). Comparative alignment of CP4 EPSPS to known allergenic and toxic proteins using Fasta algorithm. Unpublished. Report No. Monsanto Technical Report No. MSL: 12820, Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198.

Monsanto Australia Limited (2001). Roundup Ready® Cotton Technical Manual. 2. Monsanto Australia Ltd.

Naranjo, S. E. and Ellsworth, P. C. (2002). Arthropod communities and the transgenic cotton in the western United States: implications for biological control. In "*Proceedings of the First International Symposium For Biological Control"*, Van Driesche, R. G. eds, US Forestry Service, Honolulu, Hawaii.

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

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

Naylor, M.W. and Folk, R.M. (1994). One month feeding study with MON46001 and MON46002 in Sprague Dawley rats. Report No. EHL 94007, Monsanto Company, The Agricultural Group, St. Louis, USA.

Nida, D.L., Halsey, M., Jackson, T., Taylor, M.L., Ebert, C., Taylor, N., and Sims, S. (1994). Evaluation of cotton with Roundup Ready genes generated in 1993 US field lest locations. Report No. MSL-13613, Monsanto Company, St Louis MO USA.

Nida, D.L., Kolacz, K.H., Buehler, R.E., Deaton, W.R., Schuler, W.R., Armstrong, T.A., Taylor, M.L., Ebert, C.C., Rogan, G.J., Padgette, S.R., Fuchs, R.L. (1996). Glyphosate tolerant cotton: genetic characterisation and protein expression. *Journal of Agricultural and Food Chemistry* **44**: 1960-1966.

Nida, D.L., Rogan, G.J., and Taylor, M.L. (1995). Evaluation of cotton with Roundup Ready genes generated in 1994 US field test locations. Report No. MSL-140463, Monsanto Company, St Loius MO USA.

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

Nielsen, K.M., Bones, A.M., Smalla, K., van Elsas, J.D.(1998). Horizontal gene transfer from transgenic plants to terrestrial bacteria - a rare event? abstract no. 2). FEMS Microbiol Rev 22: 79-103

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

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

Odell, J.T., Nagy, F., Chua, N.H. (1985). Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. *Nature* **313**: 810-812.

OGTR (2002). The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia.

Padgette, S.R., Barry, G.F., Re, D.B., Eichholtz, D.A., Weldon, M., Kolacz, K., and Kishore, G.M. (1993). Purification, cloning and characterisation of a highly glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp.strain CP4. Report No. MSL-12738, Monsanto Company, USA.

Padgette, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M., Fraley, R.T.(1996). New weed control opportunities: development of soybeans with a Roundup Ready gene. *In* SO Duke, ed "Herbicide-resistant crops: agricultural, environmental, economic, regulatory and technical aspects". CRC Press, Boca Raton, pp 53-84.

Paget, E., Simonet, P. (1997). Development of engineered genomic DNA to monitor the natural transformation of *Pseudomonas stutzeri* in soil-like microcosms. *Canadian Journal of Microbiology* **43**: 78-84.

Paget, E., Simonet, P. (1994). On the track of natural transformation in soil. *Fems Microbiology Ecology* **15**: 109-118.

Palm, C.J., Schaller, D.L., Donegan, K.K., Seidler, R.J. (1996). Persistence in soil of transgenic plant produced *Bacillus thuringiensis* var. *kurstaki* δ-endotoxin. *Canadian Journal of Microbiology* **42**: 1258-1262.

Palmer, S.J. and Beavers, J.B. (1993a). *B.t.k.* HD-73 protein: Dietary toxicity study with green lace wing larvae (*Chrysopa carnea*). Report No. WL 93-233, Monsanto Company, St. Louis.

Palmer, S.J. and Beavers, J.B. (1993b). B.t.k. HD-73 protein: Dietary toxicity study with ladybird beetles (*Hippodamia convergens*). Report No. WL 93-232, Monsanto Company, St. Louis.

Palmer, S.J. and Beavers, J.B. (1993c). B.t.k. HD-73 protein: dietary toxicity study with parasitic Hymenoptera (*Nasonia vitripennis*). Report No. WL 93-234, Monsanto Company, St. Louis.

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

Perry, R.L.(2002). Roundup Ready® cotton as a volunteer weed. Bachelor of Rural Science Honours. University of New Egland,

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

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

Pyla, P.D., McCann, M.C., Breeze, M.L., Sorbet, R.D., Nemeth, M.A., and Astwood, J.D. (2001). Compositional analysis of seed from cotton expressing both Roundup Ready® and Bollgard® traits grown in 1999 U.S. field trials. Report No. MSL-16831, Monsanto Company, St. Louis, MO, USA.

Rayssiguier, C., Thaler, D.S., Radman, M. (1989). The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch repair mutants. *Nature* **342**: 396-401.

Ream, J.E. (1994a). Expression of *B.t.k.* HD-73 protein in nectar and pollen collected from insect resistant cotton lines 531, 747 and 1076. Report No. MSL-13738, Monsanto Company, The Agricultural Group, St. Louis, USA.

Ream, J.E. (1994b). Aerobic soil degradation of *Bacilus thuringiensis* var. *kurstaki* B.t.k. HD-73 protein bioactivity. Report No. MSL 13299, Monsanto Company, St. Louis USA.

Richins, R.D., Scholthof, H.B., Shepherd, R.J. (1987). Sequence of figwort mosaic virus DNA (caulimovirus group). *Nucleic Acids Research* **15**: 8451-8466.

Sanger, M., Daubert, S., Goodman, R.M. (1990). Characteristics of a strong promoter from figwort mosaic virus: comparison with the analogous 35S promoter from cauliflower mosaic virus and the regulated mannopine synthase promoter. *Plant Molecular Biology* **14**: 433-443.

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

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

Schoelz, J.E., Wintermantel, W.M.(1993). Expansion of viral host range through complementation and recombination in transgenic plants abstract no. 11). Plant Cell 5: 1669-1679

Schubbert, R., Renz, D., Schmitz, B., Doerfler, W. (1997). Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proceedings of the National Academy of Sciences of the United States of America* **94** : 961-966.

Schuler, M.A., Schmitt, E.S., Beachy, R.N. (1982). Closely related families of genes code for the alpha and alpha' subunits of the soybean 7S storage protein complex. *Nucleic Acid Research* **10**: 8225-8261.

Schulz, A., Kruper, A., Amrhein, N. (1985). Differential sensitivity of bacterial 5-enolpyruvyl-shikimate-3-phosphate synthases to the herbicide glyphosate. *FEMS Microbiology Letters* **28**: 297-301.

Sims, S. and Martin, J. (1996). Effect of the *Bacillus thuringiensis* insecticidal proteins Cry1Ab, Cry1Ac,Cry2A and Cry23A on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola). Report No. 93-081E1, Monsanto Company, St. Louis.

Sims, S.R. (1994). Sensitivity of insect species to the purified Cry 1Ac insecticidal protein for *Bacillus thuringiensis* subsp. *kurstaki* (B.t.k. HD-73). Report No. MSL 13273, Monsanto Company, St. Louis, USA.

Sims, S.R. (1995). *Bacillus thuringiensis* subsp. *kurstaki* (Cry1Ac) protein expressed in transgenic cotton: effects on beneficial and other non-target insects. *Southwestern Entomologist* **20**: 493-500.

Sims, S.R., Berberich, S.A., Nida, D.L., Segalini, L.L., Leach, J.N., Ebert, C.C., Fuchs, R.L. (1996). Analysis of expressed proteins in fibre fractions from insect-protected and glyphosate-tolerant cotton varieties. *Crop Science* **36**: 1212-1216.

Smalla, K., Borin, S., Heuer, H., Gebhard, F., van Elsas, J. D., and Nielsen, K. (2000). Horizontal transfer of antibiotic resistance from transgenic plants to bacteria - are there data to fuel the

debate?Fairbairn, C., Scoles, G., and McHughen, A. eds, University Extension Press, Saskatchewan, Canada. pp. 146-154.

Smalla, K., Gebhard, F., van Elsas, J. D., Matzk, A., and Schiemann, J. (1994). Bacterial communities influenced by transgenic plants.Jones, D. D. eds, Division of Agriculture and Natural Resources, University of California, Oakland, USA. pp. 157-167.

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

Stachel, S.E., Nester, E.W. (1986). The genetic and transcriptional organisation of the *vir* region of the A6 Ti plasmid of *Agrobacterium tumefaciens*. *EMBO Journal* **5**: 1445-1454.

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

Steinrucken, H.C., Amrhein, N. (1980). The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. *Biochemical and Biophysical Research Communications* **94**: 1207-1212.

Stotzky, G. (2000). Release, persistence, and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis*.

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

Tapp, H., Stotzky, G. (1998). Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. *Soil Biology and Biochemistry* **30**: 471-476.

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

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

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

van Frankenhuyzen, K.(1993). The challenge of *Bacillus thuringiensis*. *In* PF Entwistle, JS Cory, MJ Bailey, S Higgs, eds "*Bacillus thuringiensis*, an environmental biopesticide: theory and practice". John Wiley & Sons, Chichester, England, pp 1-35.

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

Van Rie, J., Jansens, S., Hofte, H., Degheele, D., Van Mellaert, H.V. (1990). Receptors on the brush border membrane of the insect mid-gut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. *Applied and Environmental Microbiology* **56**: 1378-1385.

Vulic, M., Dionision, F., Taddei, R., Radman, M. (1997). Molecular keys to speciation: DNA polymorphism and control of genetic exchange in enterobacteria. *Proceedings of the National Academy of Sciences of the United States of America* **94**: 9763-9767.

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

Widmer, F., Seidler, R.J., Donegan, K.K., Reed, G.L. (1997). Quantification of transgenic plant marker gene persistence in the field. *Molecular Ecology* **6**: 1-7.

Widmer, F., Seidler, R.J., Watrud, L.S. (1996). Sensitive detection of transgenic marker gene persistence in soil microcosms. *Molecular Ecology* **5**: 603-613.

Widner, W.R., Whiteley, H.R. (1989). Two highly related insecticidal crystal proteins of *Bacillus thuringiensis* subsp. *kurstaki* possess different host range specificities. *Journal of Bacteriology* **171**: 965-974.

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

Xia, J.Y., Cui, L.D., Ma, L.H., Dong, S.X., Cui, X.F. (1999). The role of transgenic Bt cotton in integrated insect pest management. *Acta Gossypii Sim* **11**: 57-64.

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