Risk Assessment and
Risk Management Plan

Application for licence for dealings involving an intentional release into the environment

DIR 022/2002

Title: Commercial release of insecticidal (INGARD® event 531) cotton

Applicant: Monsanto Australia Ltd

June 2003

Office of the
Gene Technology Regulator

Abbreviations

aad	aminoglycoside adenylyltransferase
ANZFA	Australia New Zealand Food Authority (now FSANZ)
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine Inspection Service
Bt	*Bacillus thuringiensis*
B.t.k	*Bacillus thuringiensis* variety *kurstaki*
CaMV	cauliflower mosaic virus
CSD	Cotton Seed Distributors Ltd
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DIR	dealing involving intentional release
DNA	deoxyribonucleic acid
DNIR	dealing not involving intentional release
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
IgG	immunoglobulin G
IOGTR	Interim Office of the Gene Technology Regulator
IPCS	International Program on Chemical Safety
IRM	Insecticide Resistance Management
MAFF	UK Ministry of Agriculture, Fisheries and Food
mg/g	milligrams per gram
MRL	maximum residue limit
mRNA	messenger ribonucleic acid
ng/g	nanograms per gram
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOS	nopaline synthase
nptII	neomycin phosphotransferase II
NLRD	Notifiable Low Risk Dealing
OGTR	Office of the Gene Technology Regulator
ppm	parts per million
TGA	Therapeutic Goods Administrations
TGAC	Technical Grade Active Constituent
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
w/v	weight per volume
µg/g	micrograms per gram

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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 cannot 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

A risk assessment and risk management plan has been prepared in response to a licence application (application number DIR 022) from Monsanto Australia Limited (Monsanto) for the continued commercial release of genetically modified (GM) insecticidal (INGARD®) cotton, event 531, in the cotton growing regions of New South Wales and Queensland south of latitude 22º South. Monsanto also proposes phasing-out INGARD® cotton over the next two growing seasons while GM insecticidal Bollgard II® cotton (which was approved for commercial release in September 2002, DIR 012/2002) is phased-in over the same period.

In 1996, the insecticidal gene present in INGARD® cotton was registered as an agricultural chemical product by the Australian Pesticides and Veterinary Medicines Authority (APVMA) (then the National Registration Authority for Agricultural and Veterinary Chemicals (NRA)) based on advice from the Genetic Manipulation Advisory Committee (GMAC) and other Commonwealth and State Government agencies. In the same year INGARD® cotton received an advice to proceed for a general (commercial) release (GR-3) under the previous voluntary system that was overseen by GMAC. A 'deemed' licence for the commercial release of INGARD® cotton was issued to Monsanto before the commencement of the Act on 21 June 2001. The deemed licence is effective during the transition period, i.e. 2 years from the commencement of the Act and expires in June 2003. Approval of application DIR 022 would enable the continued commercial release of genetically modified INGARD® cotton.

INGARD® cotton is resistant to lepidopteran caterpillar pests that attack cotton. The GM cotton contains an insecticidal gene derived from a common soil bacterium that produces a protein that is toxic to specific insects. It also contains bacterial genes conferring resistance to antibiotics that were used solely as selectable markers in the initial laboratory stages of developing the GM cotton.

Monsanto seeks approval for continued commercial release of INGARD® cotton in all Australian cotton growing regions south of latitude 22º South without limitations on transportation or storage. Monsanto intends that GM cotton plants and their by-products, including cotton seed, be used in general commerce in the same manner as conventional cotton, including for human food and stock feed. Cotton seed is processed for oil that is used in a variety of food products and for cotton linters (a type of short fibre that does not contain any genetic material) that are used as a cellulose base in some foods.

Food Standards Australia New Zealand, FSANZ, (formerly the Australia New Zealand Food Authority, ANZFA) approved the use of oil and linters from INGARD® cotton in human food in 1999.

The APVMA is responsible for determining the total planting area each season of INGARD® cotton. It currently allows up to 30% of the cotton crop to be planted to this GM cotton, to preserve the effectiveness of the product by guarding against the evolution and emergence of resistant insects.

Prior to commercial release of INGARD® cotton, 56 limited and controlled releases involving INGARD® cotton were conducted under the voluntary system overseen by GMAC. There have been no reports of adverse effects on human health or the environment resulting from any of these field trials. Nor, has an ongoing research and monitoring program revealed any such reports since the commercial release of INGARD® cotton.

THE EVALUATION PROCESS

Licence application DIR 022/2002 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.

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 website at www.ogtr.gov.au.

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

Through this process, potential hazards to human health and safety or the environment that may be posed by the release of INGARD® cotton identified. These have been evaluated on the basis of the likelihood of the 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 INGARD® cotton be harmful to human health if it were 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 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 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 cotton transfer to non-GM cotton crops, feral or native cottons, or to other organisms, with adverse consequences for the environment; and

- **insecticide resistance**: could target insects might develop resistance to the insecticidal protein produced by the introduced insecticidal gene in INGARD® cotton.

CONCLUSIONS OF THE RISK ASSESSMENT

Consistent with GMAC's original evaluation, and in the light of extensive experience with this GMO in the Australian environment, the Regulator considers that no risks to human health and safety, or to the Australian environment, will result from the continued commercial release of INGARD® cotton, above the very low risks posed by commercial production of non-GM cotton. The assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans

INGARD® cotton shows no indication of being more toxic or allergenic to humans than conventional cotton. There have been no reports of adverse health effects associated with INGARD® cotton since trials first began in 1993. As noted above, FSANZ has previously approved the use of oil and linters from INGARD® cotton in food, concluding that these products are as safe as 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

INGARD® cotton shows no indication of being more toxic to non-target organisms than conventional cotton. The introduced proteins have been found to be non-toxic to non-target organisms and INGARD® cotton seed and seed meal have been used as stock feeds 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 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 in relation to soil moisture, soil nutrients and frosts. Therefore it is not considered necessary to impose any conditions to manage the risk of weediness in southern Australia.

There is a low risk of INGARD® cotton becoming weedy in specific habitats in northern Australia, however this can be managed. INGARD® cotton seed has been used as stockfeed in northern Australia since its commercial release in 1996. Currently there is no indication that INGARD® cotton volunteers have become a problematic weed species, or are more prevalent than conventional cotton. Although the risk is low, licence conditions have been imposed to monitor INGARD® cotton volunteers at sites in northern Australia where the GMO is fed to stock.

Transfer of introduced genes to other organisms

Some gene transfer from INGARD® cotton to other cultivated cotton is likely, but the overall frequency of out-crossing will be 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 the geographical isolation of known feral populations in Western Australia and the Northern Territory from areas of NSW and Queensland in which 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. For this reason, a licence condition has been imposed requiring the applicant to document the location of these populations, if they exist, in Queensland and determine their distance from cotton production locations.

The likelihood of transfer of the introduced genes to other organisms is negligible, but even if such transfer occurred, it will not pose any hazard to human health and safety or the environment.

Insecticide resistance

The likelihood 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.

THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)

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

Management of toxicity or allergenicity

Based on the risk assessment, it is not considered necessary to impose any management conditions in relation to potential toxicity or allergenicity of the GM cotton.

Management of weediness

Based on the risk assessment, it is not considered necessary to impose any conditions to manage the risk of weediness in southern Australia.

Conditions have been imposed to limit the spread and persistence of GM cotton that may be used for stockfeed in northern Australia. The licence includes conditions that require:

- the use of covered vehicles for transport of GM cottonseed north of latitude 22° South;
- the licence holder to develop, in consultation with the OGTR, a strategy to communicate the importance of control of volunteer cotton plants to recipients of GM cottonseed north of latitude 22° South; and
- the licence holder to conduct an annual survey of the incidence of volunteer cotton in areas where GM cottonseed is used as stockfeed and of the effectiveness of the communication strategy required above.

Management of gene transfer

A licence condition has been imposed requiring the applicant to determine the remnants of feral cotton populations in this region, as indicated by the herbarium records, and their distance from cotton production locations.

Management of insecticide resistance

The licence does not impose any management conditions in relation to insecticide resistance because this risk is being managed effectively by the APVMA, as the relevant statutory authority. The licence indicates the applicant's obligation to comply with the APVMA's Insecticide Resistance Management Strategy and conditions of this licence do not replace or over-ride any conditions set by the APVMA.

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 or persons covered by the licence and informing the Regulator if the applicant becomes aware of any adverse effects or additional information about risks to human health and safety or to the environment.

Monitoring and enforcement of compliance by the OGTR

As well as 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 this application, including the licence conditions imposed, is available in the RARMP document, which can be obtained from the web site of the OGTR (www.ogtr.gov.au), or by calling 1800 181 030 (please quote application number DIR 022/2002).

CHAPTER 1 BACKGROUND

1. This chapter provides information about the background to the application and previous releases of relevant GMOs into the environment.

SECTION 1 THE APPLICATION

Project Title:	**Commercial release of insecticidal (INGARDâ event 531) 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):	Insecticidal action and antibiotic resistance
Identity of the gene(s) responsible for the modified trait(s):	• *cry1Ac* gene from the bacterium *Bacillus thuringiensis* (insecticidal gene) • *nptII* gene from bacterial Tn5 transposon (antibiotic resistance gene)
Proposed Release Location:	Cotton growing regions of New South Wales and Queensland south of latitude 22º South.
Proposed Release Size:	As required by the Australian cotton industry. Currently a maximum of 30% of the total cotton area can be planted to INGARD® cotton. The limit is imposed as part of the Insecticide Resistance Management Strategy for INGARD® cotton, regulated by the APVMA. The applicant proposes the phasing-out of INGARD® cotton to coincide with the phased introduction of Bollgard II® cotton over the next 2 growing seasons.
Proposed Release Date:	The proposed release is a continuation of the current commercial release, approved in 1996 under the former voluntary system.

2. The OGTR has received an application (licence application number DIR 022) from Monsanto Australia Ltd (Monsanto) for the intentional release of genetically modified (GM) insecticidal INGARD® cotton (event 531) into the environment in the cotton growing regions of New South Wales (NSW) and Queensland (Qld) south of latitude 22º South. Approval would enable the continued commercial release of the genetically modified cotton that was authorised to proceed under the previous voluntary system (see Section 2.2 of this chapter).

3. In accordance with section 190 of the Act, a ‘deemed’ licence (GR-3) for the general (commercial) release of INGARD® cotton (events 531 and 757) was issued to Monsanto, before the commencement of the Act on 21 June 2001, based on an approval issued under the previous voluntary system administered by the Genetic Manipulation Advisory Committee (GMAC). The deemed licence is effective during the transition period, i.e. 2 years from the commencement of the Act. The deemed approval for commercial cultivation of INGARD® cotton will expire in June 2003. Note that, under the application DIR 022/2002, Monsanto does not seek approval to continue releases of INGARD® cotton event 757.

Section 1.1 The proposed dealings

4. Monsanto seeks approval for commercial release of INGARD® cotton (event 531) in all Australian cotton growing regions south of latitude 22º South. No limitations on transportation or storage of the GMO or its by-products are proposed (see below for further explanation). However, the Australian Pesticides and Veterinary Medicines Authority (APVMA), formerly the National Registration Authority for Agricultural and Veterinary Chemicals (NRA), remains responsible for determining the total planting area each season of INGARD® cotton. Currently a maximum of 30% of the cotton crop can be planted to this GM cotton to guard against the emergence of resistant insects (see Section 2.2 of this chapter).

5. It is intended that INGARD® cotton (event 531) plants and their by-products, including cottonseed, be used in the same manner as conventional cotton, including for human food and stockfeed. Cottonseed is processed for oil (that does not contain any genetic material) used in a variety of food products and for cotton linters (a type of short 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 previously approved the use of oil and linters from INGARD® cotton in human food.

6. Monsanto also proposes the phasing-out of INGARD® cotton over the next two cotton growing seasons while GM insecticidal Bollgard II® cotton (which was approved for commercial release in September 2002, DIR 012/2002) is phased-in over the same period. INGARD® contains an insecticidal gene that produces a protein that is toxic to lepidopteran caterpillar pests that attack cotton. Bollgard II® cotton contains the same insecticidal gene as INGARD® plus a second insecticidal gene. This combination is expected to reduce the likelihood that resistance will emerge.

Section 1.2 Parent organism

7. 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. More detailed information on cotton can be found in a review document ‘The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia’ that was produced in order to inform this risk assessment process for licence applications involving GM cotton. It can be accessed at the OGTR website (http://www.ogtr.gov.au).

Section 1.3 Genetic modification and its effect

8. INGARD® cotton contains 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.

9. INGARD® cotton plants also contain two bacterial genes conferring resistance to the antibiotics kanamycin and neomycin (*nptII gene*), and streptomycin and spectinomycin (*aad gene*). The *nptII* gene was used as a selectable marker in the initial laboratory stages to select cotton plants that were genetically modified. The *aad* gene 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.

10. Short regulatory sequences that control expression of the genes (promoters and terminators) are also present in the genetically modified cotton. These sequences are derived from the cauliflower mosaic virus, *Agrobacterium tumefaciens* and soybean. Although the first two 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.

11. Detailed information on the *cry1Ac* and *nptII* genes, and characterisation of the inserted genetic material and the new proteins expressed by INGARD® cotton (event 531) are provided in Appendix 1.

Section 1.4 Method of gene transfer

12. INGARD® cotton is generated by inserting the *cry1Ac*, *nptII* and *aad* genes into cotton on a plasmid vector carried by *Agrobacterium tumefaciens* (a bacterium). The vector is 'disarmed' since it lacks the genes that encode the tumour-inducing functions of *A. tumefaciens* (see Appendix 1, Section 5 for details).

SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

Section 2.1 Previous Australian Releases

13. INGARD® cotton (events 531 and 757) was approved for general (commercial) release in 1996 (GR-3) by the APVMA (then the NRA), on the basis of advice from GMAC. This commercial release was restricted to the cotton-growing regions of NSW and Qld south of latitude 22° South because of concerns about the potential weediness of the cotton in tropical areas, as well as the potential for out-crossing to feral or naturalised cotton species in these areas.

14. Prior to commercial release, numerous limited and controlled releases involving INGARD® cotton were conducted under the voluntary system overseen by GMAC, as listed below:

- INGARD® cotton — 44 limited and controlled releases undertaken by:
  - Qld Department of Primary Industries (PR-141),
  - Deltapine Australia Pty Ltd (PR-17, PR-31, PR-33, PR-34, PR-47, PR-47X, PR-47X2, PR-47X3, PR-47X4, PR-50, PR-50X, PR-51, PR-51X, PR-51X2, PR-51X3, PR-51X5, PR-98, PR-98X, PR-112X and PR-112X2),

- CSIRO Plant Industry (PR-20, PR-36, PR-36X, PR-36X2, PR-36X3, PR-36X4, PR-36X6, PR-38, PR-38X, PR-44, PR-44X, PR-44X2, PR-56, PR-89, PR-89X, PR-89X2, PR-131, PR-131X and PR-131X2) and
- Western Australian Department of Agriculture (PR-87, PR-87X, PR-87X2 and PR-144).

➢ INGARD®/Roundup Ready® cotton — 12 limited and controlled releases undertaken by:

- Deltapine Australia 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 (PR94X, PR-94X2 and PR-94X3).

15. On 28 March 2002 the Regulator issued a licence to the Western Australian Department of Agriculture for a limited and controlled release of INGARD® cotton (licence number DIR 008/2002) in northern Western Australia under the new regulatory system.

16. The limited and controlled releases approved by GMAC and under licence number DIR 008/2002 ranged in size from 0.1 – 1600 hectares. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

Section 2.2 Approvals for general release of INGARD® cotton and issuing of the deemed licence

17. On 5 August 1996, the insecticidal gene present in INGARD® cotton (events 531 and 757) was registered as an agricultural chemical product by the APVMA (then the NRA), on the basis of advice provided by GMAC and other Commonwealth and State Government Agencies. Commercial planting of INGARD® cotton initially was limited to 30 000 hectares, but the area has been extended gradually. The APVMA remains responsible for determining the total planting area each season and for other conditions of registration including the implementation of insecticide resistance management plans (see Appendix 6). In June 2000, the APVMA varied the conditions of registration allowing up to 30% (165 000 hectares) of the cotton crop to be planted to INGARD® cotton for the 2000-2001 season. The APVMA's 30% cap on INGARD® cotton planting is still in place and includes any plantings of Roundup Ready®/INGARD® cotton that is subject to a separate deemed licence (GR-9).

18. Under transitional arrangements set out in Section 190 of the Act, GMAC's advice to proceed with the general release of INGARD® cotton, issued to Monsanto in 1996, 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 INGARD® cotton could only be for a maximum of two years after the legislation took effect, on 21 June 2001.

Section 2.3 Approvals by Other Australian Government Agencies

19. The OGTR is responsible for assessing the 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 GMO, including the requirements of the APVMA and FSANZ.

2.3.1 Australian Pesticides and Veterinary Medicines Authority (APVMA)

20. The insecticidal gene present in INGARD® cotton is subject to regulation by the APVMA as it falls under the *Agricultural and Veterinary Chemicals Code Act (1994)* definition of an agricultural chemical product. As INGARD® cotton is genetically modified to control key insect pests of cotton, the APVMA has imposed conditions in connection with the insecticidal activity of these cottons to manage the development of insecticide resistance in the cotton industry as a whole. These have included specifying maximum areas for release of the insecticidal cotton, as noted in Section 2.2 of this chapter.

21. Therefore the Regulator has not imposed licence conditions in relation to insecticide resistance management. The licence does however indicate the applicant's obligation to also comply with the requirements of the APVMA.

22. Further information about the management of insecticide resistance is available from the APVMA;

Australian Pesticides and Veterinary Medicines Authority
PO Box E240
KINGSTON ACT 2604
Phone: (02) 6272 5158
Fax: (02) 6272 4753
Email: apvma.contact@apvma.gov.au
http://www.affa.gov.au/apvma

2.3.2 Food Standards Australia New Zealand (FSANZ)

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

24. Only cottonseed oil and linters (short fibre removed from the seed coat) are consumed by humans in Australia (OGTR, 2002). FSANZ has determined that refined oil and linters derived from INGARD® cotton are as safe for human consumption as refined oil and linters derived from other conventional cotton varieties (see Appendix 2).

25. Further details of the risk analysis for 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 for INGARD® cotton

26. Countries that have assessed the use of INGARD® cotton include:

- The United States: The US Department of Agriculture and the Food and Drug Administration approved the commercial release and use in food of INGARD® cotton in 1995;
- Canada: The Canadian Food Inspection Agency and Health Canada approved the commercial release and use in food of INGARD® cotton in 1996;
- Japan: The Japanese Ministries of Agriculture, Forestry and Fisheries, and Health and Welfare approved the commercial release and use in food of INGARD® cotton in 1997;
- China: Approved the commercial release and use of INGARD® cotton in food and feed in 1997;
- Mexico: Approved the commercial release and use of INGARD® cotton in food and feed in 1997;
- Argentina: Approved the commercial release and use of INGARD® cotton in food and feed in 1998;
- Indonesia: The National Biosafety Committee approved the limited commercial release and use in food of INGARD® cotton in 1999;
- South Africa: The Department of Agriculture approved the commercial release and use in food of INGARD® cotton in 2000; and
- India: Approved the commercial release and use of INGARD® cotton in food and feed in 2002.

27. Other countries where INGARD® varieties have been approved, or are pending approval, include Israel, and the European Union. No country has refused an application for the release of INGARD® cotton.

There have been no reports of adverse effects on human health or the environment resulting from any of the international or Australian releases of INGARD® cotton, including since its commercial release in Australia, in 1996.

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

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

SECTION 1 ISSUES RAISED IN CONSULTATION ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

29. 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.

30. Written submissions received from the agencies prescribed by Section 50 of the Act, in relation to DIR application number 022/2002, suggested that the following issues should be addressed in the risk assessment and the risk management plan:

- the potential toxicity and allergenicity of INGARD® cotton, particularly through occupational exposure (Appendix 1, Section 2 refers);
- the potential for gene transfer to other cotton crops (Appendix 5, Section 1 refers);
- the emergence of insects resistant to the insecticidal protein and the adequacy of the Insecticide Resistance Management plan (Appendix 6, Section 2 refers); and
- the use of Australian species in the testing of the non-target effects of INGARD® cotton (Appendix 3, Section 2 refers).

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

- secondary effects of INGARD® cotton on non-target organisms (Appendix 3 Section 2 refers);
- the weediness of INGARD® cotton in the Australian environment (Appendix 4 Section 2 refers);
- the emergence of insects resistant to the insecticidal protein and the adequacy of the Insecticide Resistance Management plan (Appendix 6, Section 2 refers).

32. 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.

33. In total, the OGTR received 6 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 is provided in Appendix 9.

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

34. 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.

35. 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.

36. The following table (Table 1) lists each of the potential hazards that were considered during the risk assessment process in the *Hazard Identification* column and summarises the assessment of each hazard under the column headed *Risk*. A comprehensive assessment of each identified hazard is provided in Appendices 2 - 6, as cross-referenced in the column headed *Summary of Risk Assessment*.

37. 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 Method*). 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.

38. 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.

39. 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

40. 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.

41. 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 insecticidal INGARD® cotton 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. Therefore the Regulator has issued licence number DIR 022/2002.

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

INGARD® cotton: the GM insecticidal cotton proposed for release.
Cry1Ac: insecticidal protein that is produced by the introduced *cry1Ac* gene and is toxic to the target lepidopteran caterpillar pests.
NPTII: this protein is produced by the introduced *nptII* gene (marker gene) and provides antibiotic resistance that allows identification of modified plants in the laboratory.
Lepidoptera: the caterpillar insect pests targeted by INGARD® cotton belong to this order of insects.
Bt toxin: the Cry1Ac protein may also be referred to as a Bt toxin, because it is one of many proteins that are produced by the bacteria *Bacillus thuringiensis* (Bt) in nature.

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 Method(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** ▪ FSANZ has approved the use of oil and linters from INGARD® cotton in human food. ▪ Cotton seed oil and cotton linters used in food do not containDNA or protein and these products from INGARD® cotton cannot be distinguished from non-GM products. ▪ The toxicity of Cry1Ac (insecticidal protein) is specific to lepidopteran caterpillar pests and the conditions required for its toxicity do not occur in the human digestive system. ▪ Compositional analyses indicate that INGARD® cotton lint does not differ from non-GM cotton and that cotton seed differs only in the expression of the introduced proteins. ▪ Feeding studies indicate that neither the introduced proteins (in their purified form) nor INGARD® cotton elicit toxic responses in test animals. ▪ The introduced proteins do not have properties characteristic ofknown allergenic proteins and evidence indicates that they are not allergenic.	No	N/A	N/A	N/A	None required
TOXICITY AND ALLERGENICITY FOR HUMANS: Occupational exposure	**Very low**	**See Appendix 2** ▪ Humans are commonly exposed to Cry1Ac and NPTII, as these proteins are naturally ubiquitous in the environment. ▪ Exposure to the introduced proteins through working with cotton plants is very low. ▪ Like non-GM cotton, INGARD® cotton pollen is not wind-dispersed and is unlikely to be aeroallergenic. ▪ Fine lint particles and dust is generated during the processing of cotton however the fibre characteristics of INGARD® cotton are the same as for non-GM cotton and the use of personal protection equipment prevents respiratory irritation. ▪ The APVMA has registered the *cry1Ac* gene in INGARD® cotton as a pesticide with no requirement for Safety Directions on the product label. ▪ There have been no toxic or allergic effects from INGARD® cotton reported since its commercial release in Australia in 1996.	No	N/A	N/A	N/A	None required
TOXICITY AND ALLERGENICITY FOR HUMANS: Wearing & using household items	**Very Low**	**See Appendix 2** ▪ The lint, linters and cotton seed oil products from INGARD® cotton, that may be used in clothing and household items, contain no proteins or DNA - these are removed or destroyed during processing. Products from INGARD® cotton cannot be distinguished from those made with non-GM cotton.	No	N/A	N/A	N/A	None required

containing 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 Method(s)]	Reason(s) for selecting RM methods	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 toxicity of the insecticidal protein is highly specific to Lepidopteran larvae and the conditions required for its toxicity do not occur in any non-target organisms. ▪ The introduced proteins are already widespread in the environment through the presence of the bacteria to which they are native. ▪ Toxicity studies with the Cry1Ac protein and/or INGARD® cotton tissue indicate that INGARD® cotton will not be more toxic to mammals, birds or fish than non-GM cotton. ▪ Levels of nutrients and anti-nutrients in cotton seed and processed cotton seed products used for stock feed the same as non-GM cotton. ▪ Studies have revealed no differences in the performance of stock fed INGARD® cotton seed compared to stock fed non-GM cotton seed.	No	N/A	N/A	N/A	None required
TOXICITY FOR OTHER ORGANISMS: Non-target invertebrates, including soil insects	**Low**	**See Appendix 3** ▪ The toxicity of Cry1Ac is highly specific to Lepidopteran insect larvae. ▪ Studies conducted under controlled conditions and in the field indicate 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 associated decreases in the use of broad-spectrum insecticides.	No	N/A	N/A	N/A	None required
TOXICITY FOR OTHER ORGANISMS: Microbial organisms	**Low**	**See Appendix 3** ▪ Laboratory studies indicate that the Cry1Ac protein has no adverse effect on the growth of various bacteria, fungi or protozoans. ▪ The presence of INGARD® cotton plant material in the soil may only produce transient changes in soil microbial communities. ▪ Natural degradation of Cry1Ac in the soil limits bioaccumulation.	No	N/A	N/A	N/A	None 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 Method(s)]	Reason(s) for selecting RM methods	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, including INGARD® cotton, does not possess characteristics commonly associated with weediness, and is not known to be a problematic weed in any environment. ▪ Cotton, including INGARD® cotton, has a low potential for dispersal by natural means. ▪ Cotton volunteers, whether INGARD® or non-GM, can establish on roadsides but do not persist or lead to population spread into the wider environment. ▪ Soil-water availability, soil nutrients and frost are predominant factors limiting the germination and/or persistence of both INGARD® and non-GM cotton in off-farm habitats.	No	N/A	N/A	N/A	None required
WEEDINESS: **North of latitude 22º South environment**	**Low**	**See Appendix 4** Monsanto seeks approval to <u>grow</u> INGARD® cotton plants in southern Australia, but the GMO may be <u>transported</u> north and <u>used as stockfeed</u>. ▪ In favourable conditions, such as where soil-moisture is not limiting and plant competition is absent, both INGARD® cotton and non-GM cotton show some ability to establish self-perpetuating populations. ▪ In some stockyards in Broome, GM insecticidal cotton showed increased boll production than non-GM cotton which may, in some seasons, represent a selective advantage.	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. 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.	*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. *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.	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) 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 Method(s)]	Reason(s) for selecting RM methods	Is risk managed?	Licence conditions (refer to Appendix 7 for detailed licence conditions)
GENE TRANSFER: **Plants** ▪ Other cotton crops ▪ Volunteer cotton	**Low**	**See Appendix 5** ▪ Gene transfer to non-GM cotton would not pose any risks additional to those posed by INGARD® cotton. Conventional farming practices, such as the use of certified (pure) seed protects varietal integrity. ▪ Cotton volunteers in the cotton growing regions of NSW and QLD south of latitude 22º South, already include INGARD® cotton however surveys indicate that these are not more weedy than non-GM cotton.	No	N/A	N/A	N/A	None required
GENE TRANSFER: **Plants** ▪ Feral (naturalised) cotton	**Low**	**See Appendix 5** ▪ Gene transfer to feral cotton populations is thought to be unlikely because of geographic isolation. ▪ However the distribution of feral cotton in Queensland, as indicated by historical herbarium records, has not recently be confirmed.	No However, research is required to update information on the existence of feral cotton populations in Queensland, as indicated by herbarium records.	N/A	N/A	Yes	*Research on the distribution of naturalised populations of cotton*: the licence holder must, in consultation with the OGTR, conduct a survey of feral cotton populations in Queensland, south of latitude 22º South, based on existing herbarium records of *G. hirsutum* and *G. barbadense.*
GENE TRANSFER: **Plants** ▪ Native cottons ▪ Other plant species	**Negligible**	**See Appendix 5** ▪ Genetic incompatibility and geographical isolation prevent the production of fertile hybrids of INGARD® cotton and native cottons. ▪ Well established genetic incompatibility prevent successful cross pollination with other plant species.	No	N/A	N/A	N/A	None required
GENE TRANSFER: **Microorganisms**	**Negligible**	**See Appendix 5** ▪ All of the introduced genes in INGARD® cotton are already widespread in the environment and are readily available for transfer from these sources via demonstrated natural mechanisms. ▪ Gene transfer from INGARD® cotton to microorganisms has not been demonstrated under natural conditions and the likelihood of such a transfer occurring is greatly exceeded by the likelihood of transfer from the native sources of these genes.	No	N/A	N/A	N/A	None required
GENE TRANSFER: **Animals, including humans**	**Negligible**	**See Appendix 5** ▪ The most likely means of such a transfer occurring is by the exposure of animal cells to free DNA during digestion. The introduced genes are not present in ▪ human food products. ▪ The fate of DNA in the digestive tract of various animals has been studied and exposure of animals to introduced DNA from GM crop material is negligible compared with normal exposure to non-GM DNA. ▪ The likelihood of gene transfer to animal cells is extremely low, and not greater than the likelihood of transfer occurring from other sources of the introduced genes in the environment.	No	N/A	N/A	N/A	None required

		▪ In the rare event of plant DNA uptake by animal cells, the further step of chromosomal integration has not been demonstrated.					

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 Method(s)]	Reason(s) for selecting RM methods	Is risk managed?	Licence conditions (refer to Appendix 7 for detailed licence conditions)
INSECTICIDE RESISTANCE	**High**	**See Appendix 6** ▪ Laboratory studies have shown that resistance to Bt toxins can be generated experimentally in insects by repeated exposure. ▪ High levels of resistance to Cry1A toxins, including Cry1Ac, already occur in field populations of the diamondback moth. ▪ A management strategy for this risk has been in place since INGARD® cotton was first commercially released in 1996.	Yes The APVMA oversees the Insecticide Resistance Management Strategy for INGARD® cotton and ensures that the cotton industry continues to refine the Strategy as required to minimise this risk.	**Continued use of insecticide resistance management strategies in accordance with APVMA requirements.**	Decreases likelihood of insecticide resistance developing while INGARD® cultivation continues.	Yes	None required. Licence will note the requirement to adhere to the APVMA's Insecticide Resistance Management Strategy.

APPENDIX 1 INFORMATION ABOUT THE GMO

42. 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 genetic modification.

43. This part of the document addresses these matters and provides detailed information about the GMO 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 GMO

44. INGARD® cotton is derived from an original genetic modification event referred to as INGARD® cotton event 531, which has subsequently been transferred into Australian cotton varieties by conventional plant breeding. INGARD® cotton is currently grown commercially in the Australian cotton growing regions south of latitude 22º South and the applicant seeks approval for the continuation of these dealings. Monsanto also proposes the phasing-out of INGARD® cotton over the next two cotton growing seasons while GM insecticidal Bollgard II® cotton (which was approved for commercial release in September 2002, DIR 012/2002) is phased-in over the same period.

45. 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 (Van Rie et al. 1989); (Widner & Whiteley 1989); (Van Rie et al. 1990); (Dankocsik 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 Sections 3 and 4, respectively, of this Appendix.

46. The modified 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 II (*nptII*) gene, confers resistance to the antibiotics kanamycin and neomycin. The aminoglycoside adenylyltransferase (*aad*) gene confers spectinomycin and streptomycin resistance. The antibiotic resistance genes are discussed in more detail in Section 3 of this Appendix. Potential hazards relating to transfer of these genes to bacteria are discussed in Appendix 5.

47. The Coker 312 cotton variety was used to produce the initial event 531 GM plants. Coker 312 was grown in the US through the 1970s but has not been and will not be commercialised in Australia. The INGARD® cotton grown in Australia is backcross progeny of conventional crosses between INGARD® transformation event 531 (in Coker 312) 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 5 of this Appendix.

SECTION 2 THE PARENT ORGANISM

48. 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 on the OGTR website.

SECTION 3 THE INTRODUCED GENES

Section 3.1 The *cry1Ac* gene

49. The *cry1Ac* gene in INGARD® cotton is a chimeric gene that combines parts of two genes isolated from the bacterium *Bacillus thuringiensis* variety *kurstak*i (Btk). Part of the Btk *cry1Ab* gene (nucleotides 1 - 1398, corresponding to amino acids 1 - 466 (Fischhoff et al. 1987) was linked to a portion of the Btk *cry1Ac* gene (nucleotides 1399-3534, corresponding to amino acids 467 - 1178 (Adang et al. 1985). The *cry1Ab* region is identical to the analogous region of the *cry1Ac* gene with the exception of 6 amino acid differences. The hypervariable region responsible for insecticidal specificity is from the Btk *cry1Ac* gene. The chimeric gene is therefore referred to in this document as the *cry1Ac* gene.

50. To ensure the bacterial gene was expressed optimally in plants, a plant-preferred version of the chimeric *cry1Ac* gene was synthesised using the strategy described by (Perlak et al. 1990; Perlak et al. 1991). The amino acid sequence encoded by the synthetic gene is identical to that of the native Btk protein with the exception that a serine is encoded at position 766, rather than leucine. This was the result of an unintentional change that occurred during the synthesis of the plant-preferred version of the gene. However, the altered amino acid is not present in the insecticidally-active trypsin-resistant core and will not change the host range, which is determined by the amino-terminal portion of the protein (Bietlot et al. 1989). The Cry1Ac protein expressed in INGARD® cotton is 99.4% identical to the native Btk Cry1Ac protein (Adang et al. 1985). The properties of the protein are discussed in Section 4 of this Appendix.

51. Expression of the *cry1Ac* gene is driven by a modified (enhanced) 35S promoter (a region of DNA that determines whether or not a gene is expressed, and to what extent) from cauliflower mosaic virus (CaMV) (Odell et al. 1985;(Odell et al. 1985; Kay et al. 1987).

52. Also required for gene expression in plants is a mRNA termination region, including a polyadenylation signal. For the cry1Ac gene this 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).

Section 3.2 The *nptII* gene and encoded protein

53. The *nptII* gene was isolated from the bacterial Tn5 transposon (Beck et al. 1982). It is widespread in the environment and in food chains, occurring naturally in kanamycin-resistant microorganisms found in soil and in mammalian digestive systems (Flavell et al 1992).

54. The *nptII* gene encodes the enzyme neomycin phosphotransferase type II (NPTII) which confers resistance to aminoglycoside antibiotics such as 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.

55. Expression of the *nptII* gene is controlled by the CaMV 35S promoter (Odell et al. 1985). The mRNA termination region of the gene is from the 3' non-translated region of the *nos* gene from *A. tumefaciens* (Rogers et al. 1985). Since these regulatory sequences are active in plants, the NPTII enzyme is produced in INGARD® cotton.

56. The *nptII* gene functions as a selectable marker in the initial laboratory stages of cotton plant cell selection following genetic modification (FIFRA Scientific Advisory Panel 2000), allowing modified cells to grow while effectively inhibiting the growth of non-GM cells. The potential toxicity of the antibiotic resistance protein and potential risk of gene transfer are discussed in Appendices 2 and 5, respectively.

Section 3.3 The *aad* gene and encoded protein

57. 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.

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

SECTION 4 BT TOXIN

59. *Bacillus thuringiensis* is a gram positive, spore-forming, soil bacterium that is ubiquitous in the environment. During sporulation, *B. thuringiensis* produces a parasporal crystal composed of proteins may be referred to as δ-endotoxins, insecticidal crystal proteins or protoxins. The formation of the parasporal crystal distinguishes *B. thuringiensis* from other *Bacillus* species. The crystal (Cry) proteins of each *B. thuringiensis* subspecies are often toxic to specific insect genera.

60. Cry proteins (Bt proteins or Bt toxins) are a diverse family of insecticidal proteins. 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).

61. The specificity of the Bt protein results from a series of steps which must occur before the toxicity is realised. Firstly, the Bt protein crystal requires alkaline conditions, as in the larval insect gut, with pH values of 10 or above, to be soluble. The Bt protein, which is in the form of a protoxin, must then be partially digested by a specific protease in the insect gut to create its 'active' (toxic) form. The specific proteases cleave the carboxyl-terminal domain of the Cry1Ac protein and approximately 28 amino acids from the amino-terminal end of the protein, leaving an active protease-resistant core of approximately 600 amino acids (Bietlot et al. 1989; Chroma & Kaplan 1990). The active Bt protein must then diffuse through the midgut membrane and bind with specific receptors found on the midgut epithelium surface in order to exert its toxic activity (Hofmann et al. 1988; Van Rie et al. 1989; Karim et al. 2000). If receptor binding does not occur, the Bt protein will have no effect on that organism. Non-target insects, mammals (including humans), birds and fish do not possess these receptors and therefore are not susceptible to the toxic effects of the Bt proteins.

62. Binding of activated Bt toxin to the insect epithelial gut cell receptors leads to formation of pores in the cell membrane, allowing leakage of intracellular contents (for example potassium ions) into the gut lumen and water into the cell (Sacchi et al. 1986; English & Slatin 1992; Knowles & Dow 1993). The

larval gut epithelial cells swell due to osmotic pressure and lyse. The gut becomes paralysed because of changes in the electrolyte and pH balance and the insects stop eating and die (Goldburg et al. 1990).

63. Bt spores have been used as microbial insecticide products in agriculture over several decades, especially by organic farmers (Cannon 1993). Sims (1994a) 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 microbial Btk.

SECTION 5 METHOD OF GENE TRANSFER

64. The genes were inserted into the genome of cotton cells by *Agrobacterium*-mediated DNA transformation (Zambryski 1992) to produce INGARD® cotton event 531. *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 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) 7 region of Ti plasmids.

65. 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).

66. 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.

67. In this case the plasmid used was PV-GHBK04, a binary, single-border transformation vector. The plasmid contains well characterised DNA segments required for replication and selection in bacteria and for transfer of DNA from *Agrobacterium* and integration into the plant cell genome (Bevan 1984; Wang et al. 1984); Bevan 1984).

68. The cotton cells used were from the Coker 312 variety because of their positive response to the tissue culture process used for the regeneration of cotton plants. Coker 312 was grown commercially in the US but, as stated in Section 1, will not be commercially produced in Australia. Conventional plant breeding methods were then employed to transfer the INGARD® event 531 into elite Australian cotton varieties.

SECTION 6 CHARACTERISATION OF THE INSERTED GENETIC MATERIAL AND STABILITY OF THE GENETIC MODIFICATION

Section 6.1 Characterisation by Southern blot and PCR analysis

69. Detailed Southern blot analysis of INGARD® cotton GM event 531 has revealed three insertions of T-DNA, at two sites within the cotton genome. One insertion contains the T-DNA right border (RB) and one complete copy of each of the *cry1Ac*, *nptII* and *aad* genes. This insertion is responsible for the modified traits of INGARD® cotton. The second insertion, immediately adjacent to the first and in opposite ('head-to-head') orientation, contains only the RB and a portion of the *cry1Ac* gene (approximately 900 base pairs from the 3' of the *cry1Ac* coding sequence plus the 7S 3' terminator).

70. A third insertion identified in southern blotting was characterised by DNA sequencing to be 242 base pairs of the 7S 3' terminator sequence. This insertion is at a different location within the cotton genome to the functional insertion.

71. Southern blot and polymerase chain reaction (PCR) analysis of three generations of back-crossed progeny and segregation data indicate that all of the insertions are stably integrated into the genome and that the first two are tightly linked.

72. The non-functional 7S 3' terminator insertion was maintained along with the functional insertion through six generations of propagation of the original GM line. However, in two US commercial INGARD® cotton seed lots (produced from INGARD® cotton event 531 by repeated crossing to elite commercial varieties) this insertion was no longer detectable by Southern blotting.

73. The much more sensitive PCR technique was able to detect this insertion at a low level in one of the two commercial seed lots tested. This suggests that this insertion segregates independently of the functional insertion and is lost during conventional breeding. Thus this insertion is likely to be present in Australian commercial INGARD® cotton varieties at a very low frequency.

74. To determine if RNA transcripts are produced from the 7S 3' terminator insertion, reverse transcription (RT)–PCR analysis was conducted. No RNA transcript corresponding to this insertion was found.

75. Similarly, RT-PCR analysis was used to assess transcription of the second partial insertion of *cry1Ac*. A mRNA transcript corresponding to the 3' portion of the *cry1Ac* coding sequence and flanking cotton genomic DNA was detected in INGARD® cotton leaf tissue. From DNA sequence analysis it was concluded that, in the unlikely event that this RNA was translated, the resulting protein would be 94% identical to the corresponding portion of the C-terminus of the Cry1Ac protein.

Section 6.2 Characterisation of Bt toxin by Western blot analysis

76. (Berberich & Fuchs 1992) compared the Cry1Ac protein expressed in INGARD® cotton with commercially available microbial pesticides containing numerous Bt spores and protoxins. Western Blot analysis showed that the Bt protein expressed by INGARD® cotton is of similar molecular weight and immunological reactivity to one or more proteins contained in the commercial Bt product Dipel® (Valent BioSciences), that is currently used commercially in Australia. (Refer to Section 6.2.).

Section 6.3 Characterisation and stability of inserted genetic material as indicated by classical trait inheritance

77. The tight linkage of the first two insertions, as shown by the Southern blot analysis, was confirmed by the analysis of elite commercial varieties after crossing with INGARD® cotton event 531. Comparison of eight different progenies from two commercial lines, with three generations of backcrossing, demonstrated that the smaller T-DNA insertion was maintained in all progenies.

78. The stability of the *cry1Ac* gene has been demonstrated over four generations of backcrossed derivatives of INGARD® cotton event 531 in several elite varieties. Mendelian inheritance of the INGARD® trait is observed after self pollination or backcrossing with other cotton varieties. The insecticidal efficacy has been maintained during the development of commercial varieties, as confirmed by ELISA tests showing similar levels of the Cry1Ac protein (and NPTII protein) over eight years of testing (details in Section 7).

Section 6.4 Stability of the *aad* gene in the INGARD^â^ cotton genome

79. The stability of the *aad* gene in INGARD® cotton was demonstrated in a study conducted on cotton grown in Greece during 1998 (Reed & Hontis 1999). The study aimed to determine whether genetic rearrangement had occurred at the site of the *aad* gene insertion that would expose the functional *aad* coding region to an active plant promoter. ELISA testing was used to determine whether the AAD protein was present in seed of INGARD® cotton. The AAD protein was not detected in seed lots of either INGARD® cotton or non-GM control cotton. The result confirms that the *aad* gene is stably integrated into the genome of INGARD® cotton.

SECTION 7 EXPRESSION OF THE INTRODUCED PROTEINS

80. Expression levels of Cry1Ac and NPTII proteins in INGARD® cotton plants grown under field conditions were estimated in order to gauge whether expression may change from season to season, within a season and between different parts of the same plant. Samples of leaves, pollen, nectar, seed and seed fractions were analysed by ELISA. Some representative data from these analyses are presented in this section.

Section 7.1 Young leaf samples

81. Samples of young leaves from six sites across the US cotton growing region in 1992 (first leaf, (Fuchs 1994) and in 1993 (third leaf, (Berberich et al. 1993a)) and from four sites in 1999 (first leaf, (Kolwyck et al. 2000)) were analysed to determine the concentrations of Cry1Ac and NPTII proteins.

82. On average across the trial sites, Cry1Ac protein was found at 1.56 µg/g, 2.59µg/g, and 5.00 µg/g of leaf (fresh weight) in 1992, 1993 and 1999 respectively (see Table 2). Variation between sites was around two and seven fold for 1992 and 1993 respectively. Information for individual sites was not available for 1999 trials.

83. The NPTII protein was also expressed at low levels. Average concentrations of 3.14 µg/g, 2.05 µg/g and 4.94 µg/g were detected in 1992, 1993 and 1999 respectively (Table 2). Variation between sites was around two and four fold for 1992 and 1993 respectively. Information for individual sites was not available for 1999 trials.

Section 7.2 Leaves collected throughout the season

84. In 1992 and 1993 sampling continued at one of the six sites on a five and four weekly basis, respectively, until the end of the season (giving a total of 4 sampling dates in each season, see Table 2). In 1992 similar concentrations of Cry1Ac were observed on the first, second and fourth sampling dates while a two fold peak occurred on the third sampling date. The peak was thought to be due to resurgence in vegetative growth. In 1993 Cry1Ac protein levels declined as the plant aged. Prior to harvest, the level of Cry1Ac present in young leaves was approximately twenty fold lower than that present in the young leaves at the start of the season (or five fold when adjusted for total protein content, data not shown).

85. In another study, (Greenplate 1999) also found a fall in Cry1Ac protein levels throughout the growing season in INGARD® cotton. Cry1Ac protein was determined by bioassay on *Heliothis virescens* larvae. Results showed that, in general, the level of Cry1Ac protein in terminal foliage and in maturing fruit tissue declined over time.

86. Australian studies have also found a decline in Cry1Ac level in leaves of INGARD® cotton over the growing season, either by bioassay (Fitt et al. 1998) or by ELISA (Holt 1998), consistent with the drop in efficacy of INGARD® cotton towards the end of the growing season (see Section 7.7).

Section 7.3 Cottonseed

87. The Cry1Ac and NPTII proteins were expressed in harvested INGARD® cottonseed at low levels in the 1992, 1993 and 1999 field trials (see Table 2). Variation between sites was in the order of two to three fold in both 1992 and 1993 field trials (individual site data not available for 1999). As for expression in leaf tissue, expression in seeds seems to be subject to environmental factors. Generally, expression levels of the novel proteins in harvested seed were similar to those for young leaves sampled shortly after crop emergence.

Section 7.4 Pollen and nectar

88. Pollen and nectar samples were collected from cotton plants grown in a greenhouse as part of a separate study in 1992, 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 11.5 ng/g fresh weight. In nectar Cry1Ac protein was below the limit of detection (1.6 ng/g fresh weight).

89. Pollen extracts contained large amounts of total protein, 7.0 mg/g fresh weight in the parent variety and 12.6 mg/g in the INGARD® cotton variety. This difference may have been due to differences in the moisture content of the pollen preparations. No protein was detected in nectar, consistent with the absence of Cry1Ac protein.

Table 1 Cry1Ac and NPTII expression levels in INGARD® cotton

Season	1992[1]		1993[2]		1999[3]	
Variety	Coker 312 with INGARD® event 531		COKER 312 WITH INGARD® event 531		DP 5415 with INGARD® event 531	
Protein	Cry1Ac* AVERAGE (range)	NPTII* AVERAGE (range)	Cry1Ac* average (range)	NPTII* average (range)	Cry1Ac* average ± SD	NPTII* average ± SD
Young Leaf (all sites)	1.56 (1.10 – 2.04)	3.14 (2.30 – 3.98)	2.59 (0.41 – 5.91)	2.05 (0.41 – 4.97)	5.00 ± 1.84	4.94 ± 1.70
Leaf over season (one site)	Jun: 1.40 Jul: 1.49 Aug: 3.55 Sep: 1.30	Not reported	Jun: 5.12 Jul: 3.21 Aug: 0.13 Sep: 0.23	Not reported	Not reported	Not reported
Cotton Seed (all sites)	0.86 (0.49 – 1.62)	2.45 (1.70 – 3.16)	2.18 (1.13 – 3.41)	3.18 (1.13 – 6.56)	4.30 ± 0.86	5.48 ± 0.83

[1] (Fuchs 1994); [2] (Berberich et al. 1993a); [3] (Kolwyck et al 2000)
* µg/g fresh weight

Section 7.5 Fruiting Structures and Terminal Foliage

90. A field study was conducted at four sites in the US in the 1994 growing season to map changes in the expression of the Cry1Ac protein as fruit of INGARD® cotton matured and the crop aged (Greenplate, 1995). Terminal foliage and primary (first position) fruit on branches arising from nodes 7, 11 and 13 were sampled on a two weekly basis from 46 to 116 days after planting (DAP).

91. Mean comparisons within individual field sites for specific sampling times showed significantly higher Cry1Ac activity in terminal foliage over levels in fruiting structures. In most cases the primary fruit from higher nodes exhibited greater Cry1Ac activity than fruit from lower nodes.

92. When results from all four field sites were combined, primary fruiting structures showed an average Cry1Ac concentration of 259 µg/g dry weight at 46 DAP. This level declined in an apparent exponential manner to 43 µg/g at 116 DAP. On several sampling dates, significant differences in Cry1Ac activity was seen between fruit from different fruiting branches. In all cases these differences were seen as lower expression in more mature fruit. Expression in terminal foliage declined from 370 µg/g dry weight at 46 DAP to 144 µg/g at 116 DAP.

Section 7.6 Roots

93. Roots of INGARD® cotton also express the Cry1Ac and NPTII proteins (Fuchs 1994). Fuchs (1994) reported expression of both proteins to be lower in roots than in other tissues (leaves, stems, bolls) of mature plants at the end of the growing season. (Gupta et al. 2002) measured Cry1Ac in

INGARD® cotton plants four to nine weeks after germination: in roots 1 – 43 µg/g dry weight; in stems 0.2 – 3 µg/g; and in leaves 2 – 24 µg/g. Roots were also found to release Cry1Ac protein into soil during growth, although this was not quantified in soil, nor is the mechanism known. Root breakage causes a significant increase in Cry1Ac release.

Section 7.7 Field Efficacy of INGARD® cotton

94. 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 (data provided by the applicant) (Fitt et al. 1998). This has been measured in terms of larvae survival in feeding studies, field counts of surviving larvae and surveying damage to fruiting structures.

95. No differences in egg lays on conventional cotton compared to INGARD® cotton varieties have been observed in Australian field evaluations. This supports the conclusion that *Helicoverpa* moths are unable to distinguish INGARD® cotton plants and hence continue to lay their eggs in a normal random pattern.

96. Bioassays using Australian field grown INGARD® cotton showed that early and mid season, first instar (0-1 day old) *H. armigera* larvae suffered high mortality when fed on leaves or squares from INGARD® cotton plants. After three days of feeding on GM squares 86% mortality occurred and no larvae survived beyond 7 days. There was also a marked effect on larval growth. Those few larvae which survived to 5 days were still first instars, whereas larvae fed on the non-GM cotton had reached second or third instar. Towards the end of the season the rate of larval development was still suppressed however there was an increasing proportion surviving to second and third instar. The pattern of increasing survival as the plants matured follows the decline in Cry1Ac expression levels (see Section 7.2).

97. In a study during the Australian 1996/1997 cotton growing season (Fitt et al. 1998), *H. armigera* larvae suffered high mortality (70 – 100 %) when fed on INGARD® cotton leaves from plants up to 90 days after planting. Mortality was lower when larvae were fed leaves from older plants (0 – 30 %), however larval growth was still markedly reduced compared to non-GM fed controls.

98. The first stage of fruit development in cotton is the formation of squares. This delicate new growth is susceptible to *Helicoverpa* damage. Protection of fruiting positions is critical to potential cotton yield. In a study during the Australian 1994/1995 cotton growing season consistently fewer damaged squares were counted on untreated INGARD® cotton compared to conventional cotton, treated or untreated, at several locations, indicating that a high level of *Helicoverpa* control was achieved (data provided by the applicant).

SECTION 8 COMPARISON OF GM AND NON-GM COTTON CHARACTERISTICS

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

100. All varieties of INGARD® cotton intended for commercial release have undergone laboratory analysis and field trials to establish their equivalence with their conventional parent variety. Incidental,

adverse effects on the performance of the cotton plant that may occur during the crossing of the Coker 312 event 531 with the Australian elite varieties are identified during pre-release studies. Material not providing equivalent agronomic performance to non-GM standards is discarded. Backcrossing with the conventional parent is repeated until virtual equivalence is achieved. Through this process the INGARD® varieties proposed for commercial production are proven to be essentially similar morphologically, structurally and physiologically to the non-GM parental varieties. The only phenotypic difference between INGARD® cotton for commercial release and conventional cotton is the intended expression of the Cry1Ac and NPTII proteins.

Section 8.1 Agronomic performance of INGARD® cotton

101. Agronomic performance of INGARD® cotton in Australia has been researched in great detail in numerous studies in a collaborative effort by Monsanto Australia Limited, CSIRO, Cotton Seed Distributors Ltd and Deltapine Australia Pty Ltd (refer to Chapter 1 Section 2 for details of previous releases).

102. The cotton breeding programs in Australia have transferred the INGARD® gene from the Coker 312 line into elite Australian varieties. After repeated backcrossing to the parent variety, the INGARD® cotton varieties for commercial release have been found to be no different agronomically from the conventional variety as measured by germination, establishment, rate of vegetative growth, duration of flowering, fruiting potential, fibre yield and fibre quality (data provided by the applicant). It has been established through 10 years of field evaluation, including six years of commercial production, that INGARD® cotton is phenotypically the same as the conventional parent variety and has no genetic potential to produce a greater (or lesser) fibre yield.

103. All agronomic performance and varietal selection trials conducted during development of INGARD® cotton were monitored on a regular basis for differences in disease susceptibility between GM and non-GM plants. Both above and below ground plant parts were examined for the presence of disease development. Because the cotton plants were transformed using a disarmed *Agrobacterium tumefaciens* vector, plants were specifically examined for the development of crown gall throughout the growing season. Field trials indicated that INGARD® cotton possesses no disease or pest susceptibilities different from the parental non-GM cotton, other than resistance to the target Lepidopteran pest (information provided by the applicant).

Section 8.2 Compositional analysis of INGARD® cotton seed

104. The results of extensive compositional analyses of whole cottonseed and processed cottonseed fractions demonstrates that the levels of the important nutritional and anti-nutritional components in INGARD® cotton are comparable to the parental variety and are within established ranges for commercial cotton varieties.

105. Levels of the important nutritional cottonseed components (protein, oil, carbohydrate, moisture, ash and calories) in INGARD® cottonseed are comparable to the Coker 312 parental control variety or are within established and reported ranges for commercial cotton varieties (Berberich et al. 1996); (Reed & Olson 1997). There were no significant differences observed in the total lipid contents or levels of individual fatty acids of INGARD® and Coker 312 cotton. Amino acid composition of INGARD® cottonseed was also very similar to that of Coker 312. In only one of these studies, several

amino acids showed statistically different values but these were close to those previously reported for commercial cotton (Berberich et al. 1996).

106. Compositional analyses (Fuchs 1994); (Berberich et al. 1996); (Reed & Olson 1997)(Reed & Olson 1997) of cottonseed samples were also conducted to measure the levels of key anti-nutritional components native to cotton: gossypol and cyclopropenoid fatty acids, as well as aflatoxin, a toxicant produced by fungal infection. No statistically significant differences were observed in gossypol levels between the insecticidal cotton line 531 and the Coker 312 control. Levels of cyclopropenoid fatty acids (dihydrosterculic, sterculic and malvalic) also showed no statistical differences between INGARD® and the conventional parent. Levels of the four primary aflatoxins in INGARD® cottonseed were shown to be undetectable at a sensitivity of 1 ppb. All toxicant levels determined for INGARD® cotton were within the range determined for the conventional commercial cotton varieties.

107. Cotton contains allelochemicals that may be involved in pest control. Three of the most important are flavonoids, tannins and anthocyanin. The levels of these classes of compounds in squares and in terminal leaves were analysed (data supplied by applicant). No meaningful differences in the levels of these factors were detected between INGARD® cotton and the Coker 312 control.

108. Yields of the processed cottonseed fractions (linters, linter motes, delinted seed, hulls, kernels, toasted meal, crude oil and refined oil) were statistically similar for INGARD® cotton and the parental cotton line, and comparable to the means and ranges previously reported for processed fractions from other cotton varieties (Fuchs 1994).

109. The demonstration of agronomic and compositional similarity between INGARD® cotton and conventional cotton indicates that no significant pleiotropic effects have occurred.

110. The following appendices, 2 through 6, focus on potential hazards to human health and safety and to the environment associated with the introduction of new genes and expression of new proteins in INGARD® as compared to non-GM cotton.

APPENDIX 2 HUMAN HEALTH AND SAFETY

111. 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. This Appendix considers potential hazards that may be posed to human health and safety by the continued release of the GMO. In this context, the potential toxicity and allergenicity of the GMO and its novel proteins were considered.

112. It should be noted that since the commercial release of INGARD® cotton in 1996, there have been no reported adverse toxic or allergenic effects on human health resulting from the release of INGARD® cotton.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

113. 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 2000). 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 (FAO/WHO 2000) (Taylor & Lehrer 1996). Allergy has a well defined etiology (ie. Biochemical cause) that is quite different from toxicity.

114. INGARD® cotton differs from conventional cotton in the expression of two additional proteins, the Cry1Ac and NPTII proteins. The potential for cotton expressing these proteins to be toxic or allergenic to humans has been considered in detail in this Appendix. This could occur if the genetically modified cotton were toxic or allergenic because of the novel gene products expressed in the plants or because of unforeseen, unintended effects of the genetic modification.

SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

115. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of INGARD® cotton on human health and safety, a number of factors were considered, including:

- the potential exposure of people to cotton, cotton products, and the new proteins which are expressed in INGARD® cotton;
- the potential exposure of people to the CP4 EPSPS, Cry1Ac and NPTII proteins from other sources in the environment;
- the inherent toxicity and allergenicity of conventionally bred cotton;
- the toxicity and allergenicity of the purified form of the introduced proteins;
- the toxicity and allergenicity of Cry proteins applied to crops as microbial insecticides.
- the toxicity and allergenicity of INGARD® cotton (the GMO);

Section 2.1 Exposure of people to INGARD® cotton

116. Humans are in contact with cotton plants and cotton products through a variety of circumstances. Potentially harm could occur if the GM cotton became toxic or allergenic for people;

- eating foods containing cottonseed oil or cotton linters; or
- working with cotton or living in and near the areas where cotton is grown; or
- wearing cotton clothing or using household items made from cotton lint or cotton linters or cottonseed oil.

117. Segregation of cotton lint and cotton seed from GM cotton crops does not occur. All stages of cotton processing, transport and storage are co-mingled. Thus where ever people are exposed to cotton products, the cotton plants or origin may have been GM, non-GM or a combination of both.

118. Since INGARD®'s commercial release in 1996/97, there have been no reported adverse toxic or allergic effects on health through occupational exposure, ingestion of foods derived from INGARD® cotton, or use of products containing oil, lint or linters from INGARD® cotton.

2.1.1 Exposure of people to cotton through food products

119. Cotton seed 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 of 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). Whole cottonseed, seed meal and seed hulls may also be used for animal feed, particularly cattle, which are less affected by gossypol (OGTR 2002). For information regarding the safety of animal feed, refer to Appendix 3, Section 2.1.3.

120. In Australia exposure to cotton products through food is in the form of cottonseed oil and cotton linters (short fibres removed from the seed coat) (OGTR 2002). Many food products eaten on a daily basis contain these ingredients. Examples of common food products that may contain cotton seed 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.

121. There is no detectable nitrogen and hence no DNA or protein in the highly refined and processed cotton linters or cotton seed oil that people are exposed to through food products (Leffler & Tubertini 1976) (Sims et al. 1996). Processing and refining both chemically and thermally destroys or removes proteins and nucleic acids to below detectable levels (Sims 1994b; (Sims 1994c; Sims & Berberich 1996). Oil and linters derived from INGARD® cotton cannot be distinguished from oil and linters derived from non-GM cotton.

122. It is possible that limited amounts of GM cotton pollen might be incorporated in honey, since honey may contain small amounts of pollen. However, the expression level of the Cry1Ac protein is low in pollen (see Section 7.4) and hives are generally not placed near cotton fields because of the potential for exposure and loss of foraging bees or contamination of honey, due to the range of pesticides normally applied to cotton.

123. 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 human food products (oil and linters) derived from INGARD® cotton are as safe as those

derived from conventional cotton varieties (ANZFA 1999). The Regulator sought advice from FSANZ on the application and on the risk assessment and the risk management plan (see Appendix 8).

Exposure of people to cotton through their occupation or living in cotton growing regions

124. People working with cotton plants would be primarily exposed to the outer waxy cuticle layer at the plant surface, or to the cotton fibres, which are essentially free of protein. Exposure to proteins (including the new proteins expressed in INGARD® cotton) or to other cellular components of the cotton plants only occurs when plant cells are ruptured. Even if the cells rupture, exposure to the new proteins expressed in INGARD® cotton will be very low, due to their very low levels of expression in the cotton tissues.

125. The Cry1Ac protein is also foliarly-applied to cotton crops in Btk biological insecticides. The exposure of people to the protein through residues of biological insecticides on the crop is potentially greater than exposure to the plant-incorporated Cry1Ac protein produced in INGARD® cotton.

126. The primary processing of cotton at cotton gins, and the bulk handling of cotton seed creates and stirs up fine dust and lint particles. The use of personal protective equipment by exposed workers is commonplace in such facilities to prevent respiratory irritations. Fibre characteristics (length, strength, fineness) of INGARD® cotton are the same as for non-GM cotton varieties and all cotton is processed using exactly the same methods. Thus exposure to INGARD® cotton lint is not more likely to induce adverse responses in workers than is conventional cotton.

127. Cotton pollen, be it from INGARD® cotton or non-GM cotton is large, sticky and not easily transported by wind (OGTR 2002). Thus the potential for cotton pollen to act as an airborne allergen is very limited. People living in cotton growing areas are unlikely to be exposed to cotton pollen.

2.1.3 Exposure to cotton through wearing cotton clothing and using household products made from cotton lint, cotton linters and cotton seed oil.

128. Cotton fabrics, used in clothing, upholstery, towels and other household products, are made from the cotton lint (log 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 a variety of plastics (Gregory et al. 1999). Cotton fibre is widely used in pharmaceutical and medical applications because of its very low allergenicity. Cotton seed oil may also be used in soaps and cosmetics.

129. Processed cotton lint, linters and oil contain no detectable DNA or protein (Leffler & Tubertini 1976) (Sims et al. 1996). Cotton lint consists almost entirely (99.8%) of cellulose (AgraFood Biotech 2000). Fibre characteristics (length, strength, fineness) of INGARD® cotton are the same as for non-GM cotton varieties (Cotton Seed Distributors 2002). Therefore when people are exposed to cotton lint products such as clothing, or products containing cotton seed oil such as soap, it cannot be distinguished, by any method, whether the cotton fibre or cotton seed oil originated from GM or non-GM cotton plants.

Section 2.2 Exposure of people to other sources of Cry1Ac and NPTII in the environment

130. The Cry1Ac and NPTII proteins expressed in INGARD® cotton are widespread in the environment, through their expression in the bacteria to which they are native. Both proteins can be found in or on fresh foods.

131. 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 are their crystal toxins are found widely in soils, on plant leaves and in grain stores (Meadows 1993).

132. The exposure of people to the Cry1Ac protein in fresh food has in 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 a cabbage, lettuce and tomato with no reported toxic or allergic responses (ANZFA 1999). The Cry1Ac protein produced by INGARD® cotton is almost identical (greater than 99.4% homology) to the protein found in nature and in the Btk microbial insecticide formulations (Adang et al. 1985).

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

Section 2.3 Toxicity and allergenicity of conventionally bred cotton

134. Summary information on the toxicity and allergenicity of non-GM cotton is included here to establish a baseline for comparison with INGARD® cotton. 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 hirstum*) in Australia' (OGTR 2002). This document was produced in order to inform the risk assessment processes for licence applications involving GM cotton and can be accessed at *www.ogtr.gov.au*.

135. Cotton is a well established field crop with a long history of safe use. It is not known to be capable of causing disease or other ill health in people, plants or animals, except from excessive consumption of cotton tissues.

136. Cotton tissue, particularly the seeds, can be toxic if ingested in excessive quantities because of the presence of anti-nutritional and toxic factors including gossypol (a sesquiterpene) and cyclopropenoid fatty acids (including dihydrosterculic, sterculic and malvalic acids). However oil extracted from the cotton seed has been in common use since the middle of the nineteenth century and has 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.

137. Cotton and cotton products are not recognised allergens. Cotton pollen has a very low potential to act as an aeroallergen as it is large and sticky and hence not easily dispersed by wind. Processed cotton fibre contains 99.8% cellulose and is widely used in pharmaceutical and medical applications

because of its very low allergenicity. However inhalation of fine particles of cotton lint and dust generated during cotton processing can cause byssinosis, an asthma-like condition, in sensitive individuals. The use of facemasks has been successful in lowering the incidence of this condition among exposed workers.

Section 2.4 Toxicity and allergenicity assessment of the introduced proteins

138. INGARD® cotton varies from conventional cotton in the expression of two additional proteins, Cry1Ac and NPTII. The NPTII protein has no intrinsic toxicity, while the Cry1Ac protein is specifically toxic to lepidopteran insects. For details of the mode of action of the Cry1Ac toxin, refer to Appendix 1, Section 4.

139. The toxic effects of Cry1Ac are highly specific for Lepidopteran insects (see Appendix 1, Section 4). Once ingested, the Cry1Ac toxin requires an alkaline pH of 10 for effective solubility. The highly acidic pH of the human gastrointestinal tract, pH 1.2, severely limits Cry1Ac solubility (English & Slatin 1992). Hence the toxicity of Cry1Ac observed in lepidopteran insects cannot be manifested in humans.

140. Unlike Cry1Ac, NPTII has no inherently toxic properties. In nature, NPTII works to prevent the death of the bacteria targeted by neomycin and kanamycin. The enzymatic activity of NPTII requires the co-factor ATP. ATP is unstable at the low pH of the digestive system (Flavell et al 1992). In the absence of ATP, NPTII cannot confer resistance to the Aminoglycoside antibiotics.

141. Studies using the purified forms of the introduced proteins, have been conducted, as the very low expression of the introduced proteins in INGARD® cotton means it is generally not possible to feed test animals the quantity of the plant material that would ensure a broad margin of safety for humans. Through the use of the purified proteins it is possible to test mammalian toxicity at much higher concentrations than the concentrations produced in the GM plants (FIFRA Scientific Advisory Panel 2000).

2.4.1 Acute toxicity studies

142. An acute oral toxicity study in mice, with purified *B.t.k.* Cry1Ac protein at doses of up to 4300 mg/kg (2150 mg/kg administered twice, three hours apart), did not shown any adverse effects (Naylor 1996). There were no treatment related differences in mortality, weight gain, food consumption, behaviour, clinical signs or gross pathology.

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

2.4.2 Comparison of Cry1Ac and NPTII with known properties of allergens

144. 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 (Taylor & Lehrer 1996) (Metcalfe et al. 1996).

145. GM crops may be able to trigger food allergies in one of two ways: either they introduce proteins from foods known to trigger allergic reactions; or they introduce proteins completely new to the food supply that cause reactions (2002). In the case of INGARD® cotton, neither of these scenarios arise. The introduced genes have not been sourced from other foods, both occur naturally in bacteria (explained in Appendix 1, Section 3). Neither is the introduction of either of Cry1Ac or NPTII completely new to the food supply, as both are ubiquitous in nature and are commonly present on human food and animal feeds (Flavell et al. 1992; ANZFA 1999).

146. Taylor (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 allergenic response. Taylor (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 to the protein-free oil or linters derived from INGARD® cotton.

2.4.2.1 Sequence Homology

147. Searches of allergen sequence databases have shown no significant matches of any of the Cry1 proteins, including Cry1Ac, to known allergens (Astwood & Fuchs 1996; Metcalfe et al. 1996). Cry1Ac does not display characteristics common to known food allergen proteins.

148. The NPTII protein does not display characteristics common to known food allergen proteins (FDA 1998; Fuchs et al. 1993b; ANZFA 1999); Fuchs 1993b). NPTII is not derived from a known allergen and shows no significant DNA or protein sequence homology to known allergens in the Genbank, EMBL, Pir and Swiss-Prot databases. It is present in very low levels in INGARD® cotton, as discussed in Appendix 1, Section 7.

2.4.2.2 Stability of Cry1Ac and NPTII in the human digestive tract

149. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases (Astwood et al. 1996). This is because it is necessary that a protein is sufficiently stable to reach and cross the mucosal membrane for it to stimulate an allergenic response following oral ingestion (Kimber et al. 1999).

150. The Cry1Ac protein is heat labile and rapidly degraded, in under 30 seconds, under simulated gastrointestinal conditions of the mammalian system (Fuchs et al. 1993a). These cleavage products in essence become nutrients, and there is no evidence that they bind to or accumulate in mammalian tissues FIFRA Scientific Advisory Panel 2000).

151. The NPTII protein is also heat labile and degrades rapidly in simulated human gastric fluid. (Fuchs et al. 1993b) reported that no NPTII was detected 10 seconds after addition simulated gastric fluid as measured by both Western blot and enzymatic activity.

2.4.3 Assessment by other agencies

152. 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' (IPCS 2000).

153. 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. Food products for human consumption that are derived from INGARD® cotton were approved for the inclusion into Standard 1.5.2 – Food derived from Gene Technology – of the Australia New Zealand Food Standards Code – on 28 July 2000 (A341 Full Assessement Report – Oil and Linters derived from Insect Resistant Cotton).

154. The use of NPTII enzyme in tomatoes, canola and cotton has been previously evaluated by the US FDA. The FDA concluded that this enzyme does not have any of the recognised characteristics of food allergens or any attributes that would distinguish it toxicologically from other phosphorylating enzymes in the food supply (FDA 1994), cited in (ANZFA 1999).

155. The APVMA (then the NRA) has issued a Technical Grade Active Constituent (TGAC) exemption for the Cry1Ac protein from the requirement to establish a maximum residue limit (MRL) when present in INGARD® cotton or when used as a topical application on food crops (TGAC Exemption 48404, NRA toxicology evaluation and approval 48296, 5 June 2000).

156. The US EPA considers Cry1Ac protein to be non-toxic to mammals and has also established an exemption from tolerance requirements (EPA 2001b). The US EPA has also established an exemption from the requirement of a tolerance for residues of NPTII when used as a plant pesticide inert ingredient (EPA 1994).

157. The UK Ministry of Agriculture, Fisheries and Food (MAFF, now the Department for Environment, Food and Rural Affairs) has carried out a study using honey containing pollen from genetically modified canola that expressed the nptII gene. They estimated that a 500 g pot of honey would contain up to 0.005µg of NPTII protein (MAFF 1997). This is equivalent to 0.00001 ppm. To put this in perspective, it has been estimated that although amounts as low as 100 µg of peanut protein may cause mild reactions in a limited number of individuals, amounts of 2000 to 5000 µg are required to produce significant reactions in the same patients (MAFF 1997).

Section 2.5 Toxicity and allergenicity assessment of Cry (Bt) proteins in microbial insecticides

158. There have been no confirmed adverse effects on health either through occupational exposure or ingestion of fresh produce sprayed with Bt insecticides, despite significant oral, dermal and inhalation exposure to the product (Entwistle et al., 1993;US EPA 2000).

2.5.1 Acute and repeat-dose toxicity studies

159. The Cry1Ac protein, as a component of various Bt microbial products has shown no observable effects when tested in acute oral feeding studies with rats and rabbits at doses up to thousands of milligrams of per kilogram of body weight (EPA 1986; EPA 1996; David 1989; Carter & Ligget 1994; McClintock et al. 1995; Barbera 1995). These studies all reported no treatment-related effects on survival, body weight, food consumption, clinical observations, and gross pathology findings at necropsy.

160. In two separate studies, human volunteers have been fed 1000 mg of Bt microbial preparations per day for up to 5 days and exhibited no symptoms of toxicity or other ill (McClintock et al. 1995).

2.5.2 Sub-chronic and chronic toxicity studies

161. Subchronic feeding studies with rats (up to 8400 mg Dipel®/kg/day for 90 days) and with sheep (up to 10^{12} spores Dipel®/day for 153 days) showed no observable effects (Hadley et al. 1987; McClintock et al. 1995).

162. 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 at the highest dose, but in the absence of any other adverse findings this was not considered to be related to Cry protein toxicity (McClintock et al. 1995).

2.5.3 Allergenicity studies

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

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

Section 2.6 Toxicity and allergenicity assessment of INGARD® cotton

165. The nutrient composition of INGARD® cottonseed is within the normal range for cottonseed in terms of the concentration of protein, oil, carbohydrate and ash, and amino acid and fatty acid profiles. The levels of known anti-nutritional or toxic factors in INGARD® cottonseed or cottonseed oil, including gossypol, and cyclopropenoid fatty acids, are also within the range of non-GM cotton controls (Keck et al. 1994). Additional information on the composition of INGARD® cottonseed is presented in detail in Appendix 1, Section 8.

2.6.1 Toxicity studies

166. Four-week rat feeding studies using raw, ground cottonseed were carried out to compare INGARD® cotton with the parental line (Naylor 1993; Naylor & Folk 1994). There were no significant difference 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%, however, there was some evidence of decreased consumption and weight gain in some groups of animals. This may have been due to reduced palatability as a result of slightly higher levels of sterculic acid in the INGARD® cottonseed compared to the parental line. There was no other evidence for toxicity or other adverse clinical signs during the study or in post mortem analysis of the organs.

2.6.1 Assessment by other Agencies

167. FSANZ concluded that oil and linters derived from INGARD® cotton are suitable for human consumption (ANZFA 1999).

168. The APVMA assesses evidence from various types of toxicological studies in the registration process for agricultural chemicals in Australia. The INGARD® gene (*cry1Ac*) in INGARD® cotton is registered for use as a plant-incorporated pesticide in Australia (NRA product no. 48296, registered since 05/08/95). From a toxicological perspective, the APVMA has found the *cry1Ac* gene present in INGARD® cotton is suitable for use as an insecticide. Safety directions for handling and using INGARD® cotton are not provided on the product label, indicating that the APVMA does not consider precautions need to be taken to protect the health and safety of humans when using this product.

SECTION 3 CONCLUSIONS REGARDING TOXICITY AND ALLERGENICITY

169. It is considered that the risk of INGARD® cotton being toxic or allergenic to humans exposed to cotton products is, in general, very low because:

- the toxicity of Cry1Ac is specific to Lepidopteran larvae and the conditions required for its toxicity do not occur in mammalian digestive systems;
- compositional analyses indicate that INGARD® cotton lint does not differ from non-GM cotton lint and seed from INGARD® cotton differs only in the expression of the introduced proteins;
- feeding studies indicate that neither the introduced proteins (in their purified form) nor INGARD® cotton elicit toxic responses in test animals;
- evidence indicates that the introduced proteins are not allergenic, nor do they have properties common with known allergenic proteins;
- humans are commonly exposed to Cry1Ac and NPTII, as these proteins are naturally ubiquitous in the environment;
- there have been no reported toxic or allergic effects from INGARD® cotton since its commercial release in Australia in 1996.

170. For people exposed to cotton products in foods that are derived from INGARD® cotton, the risk of toxicity or allergenicity is very low because;

- FSANZ has approved the use of oil and linters from INGARD® cotton in human food; and
- Cotton seed oil and linters used in food do not contain DNA or protein and those from the GM cottons cannot be distinguished from the non-GM products.

171. For people exposed to INGARD® cotton plants and cotton products in their work, or who are living in cotton growing areas, the risk of toxicity or allergenicity is very low because;

- exposure to the introduced proteins through working with INGARD® cotton plants is very low;
- like non-GM cotton, INGARD® cotton pollen is not wind-dispersed and is unlikely to be aeroallergenic;

- fine lint particles and dust is generated during the processing of cotton, however the fibre characteristics of INGARD® cotton are the same as for non-GM cotton and the use of protective equipment prevents respiratory irritation;
- the APVMA has registered the *cry1Ac* gene in INGARD® cotton as a pesticide with no requirement for Safety Directions on the product label.

172. For people exposed to cotton clothing and household items that are derived from INGARD® cotton, the risk of toxicity or allergenicity is very low because;

- The lint, linters and cotton seed oil products from INGARD® cotton, that may be used in clothing and household items, contain no DNA or proteins – these are removed or destroyed during processing. Products from INGARD® cotton cannot be distinguished from those made with non-GM cotton.

173. The licence holder will be 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

174. 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. This Appendix considers potential hazards that may be posed to the environment through any potential toxicity and allergenicity of the GMO or its novel proteins to non-target organisms.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY HAZARD

175. INGARD® cotton differs from conventional cotton in the expression of two additional proteins, the Cry1Ac protein and the NPTII protein. The possibility was considered that INGARD® cotton may be harmful to organisms other the target lepidopteran insect pests, either due to the expression off the novel gene products or because or unforeseen, unintended effects of the genetic modification.

176. If INGARD® cotton is toxic for other 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); and
- microbial organisms, particularly soil microorganisms, with direct impact on growth of crops on farms.

177. Toxicity for the lepidopteran target organisms 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.

SECTION 2 LIKELIHOOD OF THE TOXICITY HAZARD OCCURRING

178. In assessing the likelihood of adverse impacts on non-target organisms due to toxicity of INGARD® cotton, a number of factors were considered including:

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

179. It should be noted that since its commercial release in 1996/97, there have been no reported adverse toxic effects on non-target organisms through exposure to INGARD® cotton plants, lint or seed.

Section 2.1 Toxicity of conventionally bred cotton

180. Summary information on the toxicity and allergenicity of non-GM cotton is included here to establish a baseline for comparison with INGARD® cotton. 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 hirstum*) in Australia' (OGTR 2002). This document was produced in order to inform the risk assessment processes for licence applications involving GM cotton and can be accessed at *www.ogtr.gov.au*.

181. Cotton is a well established field crop with a long history of safe use. It is not known to be capable of causing disease or other ill health in people, plants or animals, except from excessive consumption of cotton tissues. Cotton tissue, particularly the seeds, can be toxic if ingested in excessive quantities because of the presence of anti-nutritional and toxic factors including gossypol (a sesquiterpene) and cyclopropenoid fatty acids (including dihydrosterculic, sterculic and malvalic acids). Despite this, cotton seed and cotton seed products are frequently used in a controlled manner to supplement livestock feed. According to the US National Cottonseed Products Association, more than half of the cotton seed products, meal and hulls, go into animal feed (data provided by Monsanto).

182. Cotton seed and cotton seed products are the only forms of cotton tissue used for livestock feed. Most mammals avoid feeding on cotton plants due to both the gossypol content and the morphology of the plant. 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 might be present in the cotton trash and stubble.

183. The presence of gossypol and cyclopropenoid fatty acids in cotton seed limits the use of whole cotton seed as a protein supplement in animal feed, except for ruminants (such as cattle and sheep) which can tolerate higher levels of free gossypol because it binds to proteins in their rumen (Morgan 2003). Whole cotton seed can usually be fed at about 0.5% body weight for mature cows and about 0.33% for weaned calves.

184. Inactivation or removal of these components during processing enables the use of cotton seed meal and hulls for catfish, poultry and swine as well as for cattle and sheep. Cotton seed meal provides an excellent protein supplement. Ruminant animals do not show ill effects even when they consume large quantities of cotton seed meal (FAO 2003). However the use of meal for feeding other stock remains limited to a smaller portion of the diet and must be introduced gradually to avoid potential toxic effects. Australian Pork estimates that cotton seed meal comprises up to 5% of pigs diets in Australia, but could be safely raised to 20% in times of feed shortages.

185. The herbivory of cotton plants by arthropods may also be affected by plant morphology, gossypol content and levels of allelochemicals. Conventional breeding of cotton varieties has used various techniques to optimise host plant resistance to arthropod attack and consequently improve lint yields. The development of early maturing varieties enables host evasion of late-season generations of pests. Levels of allelochemicals and gossypol are manipulated to deter arthropod attack and various

morphological characters can also confer host plant resistance. However with most of these traits increased resistance to one pest is often associated with increased susceptibility to another pest.

Section 2.2 Modified characteristics of INGARD® cotton

186. As discussed in detail in Appendix 1, Section 7, the Cry1Ac and NPTII proteins are present at low levels in INGARD® cotton. The concentrations within different tissues vary significantly (Greenplate, 1995) and may also vary seasonally, depending on climatic conditions (specifically see Appendix 1, Section 7, Table 1). Monsanto's data indicate that highest levels of expression occur in young leaves and in seed. Mature whole plants contain less of the introduced proteins and expression in pollen is even lower. Expression in nectar is below the limit of detection. Given these low concentrations, the level of exposure to the novel proteins in the GM crop is not likely to be significant and may be limited further, depending on the possible routes of exposure.

187. Also as detailed in Appendix 1, the nutrient composition of seed from INGARD® cotton is within the normal range for cotton seed in terms of the concentration of protein, oil, carbohydrate and ash and amino acid and fatty acid profiles. This along with INGARD®'s agronomic equivalence with conventional cotton, (see Appendix 1, section 8) suggests that no unintended effects have occurred as a result of the genetic modification of this cotton.

Section 2.3 Other sources of Cry1Ac and NPTII in the environment

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

189. The native Cry1Ac protein is naturally produced by the bacterium *Bacillus thuringiensis* variety *kurstaki* (Btk). Related Cry toxins are also produced by this and other varieties of *Bacillus thuringiensis* (see Appendix 1). Btk 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).

190. The presence of the Cry1Ac protein in agricultural situations has increased due to the commercial use of Bt microbial sprays to protect crops from insect attack. Btk protein (and spore) insecticides, produced by mass fermentation of Bt, including Btk, 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 INGARD® cotton is almost identical (greater than 99.4% indentity) to that found in nature and in the Btk microbial formulations (Adang et al. 1985).

191. 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 INGARD® cotton

192. As discussed in Section 2.1, most mammals avoid feeding on cotton due to the presence gossypol and other components in cotton tissues. This, coupled with the high fat content of whole cotton seed, limits its use as stock feed to only a portion of the diet. For example approximately 0.5% of the body weight can be fed to mature cows and 0.33% of body weight for weaned calves. However cotton seed meal (oil removed) provides an excellent protein supplement for ruminant and monogastric stock. For example Australian Pork estimates that cotton seed meal comprises up to 5% of pigs' diets in Australia, but could be safely raised to 20% in times of feed shortages.

193. 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 insecticidal proteins expressed in the seeds of INGARD® plants. Some exposure could occur following planting or through the use of delinted seed as cattle feed in stockyards. Cotton seed meal can also be used in rations for growing chickens.

194. Cottonseed or pollen is not expected to enter aquatic habitats in any significant quantity, and therefore aquatic species will not be exposed. Because of irrigation practices used by cotton growers in eastern Australia, water runoff following irrigation remains on farm and the first 15mm of storm water runoff is also retained on farm to minimise the entrance of soil residues into natural waterways. In New South Wales this is a legislative requirement, while in Queensland this is part of Best Management Practice for the cotton industry.

2.4.2 Toxicity of INGARD® cotton to stock and wild life

195. The toxic effects of Cry1Ac are highly specific for lepidopteran insects. The naturally occurring Btk proteins have been shown to have no deleterious effects to fish, avian species, mammals and other non-target organisms, as the digestive tract tissues of these organisms do not contain the receptors that bind the Cry1Ac toxin (EPA 2001a; Betz et al. 2000). For details of the specificity of the toxin's mode of action refer to Appendix 1, Section 4.

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

197. The performance of cows that were fed controlled diets including cottonseed from conventional cotton and INGARD® cotton has been compared (Hartnell et al. 2001);(Castillo et al. 2001). There were no significant differences between cows fed the alternative forms of cottonseed, as reflected by their body condition, milk yield and milk composition. Moreover, Western blot assays of the milk tested negative for the proteins inserted into INGARD® cotton.

198. A dietary toxicity study was conducted to compare the growth and survival of commercial catfish fed a diet comprising 20% processed cottonseed meal derived from either Bollgard II® or conventional cotton (Li & Robinson 2000). The Bollgard II® cotton expresses the Cry1Ac and NPTII proteins as well as the Cry2Ab2 and GUS proteins. At the end of the study (8 weeks), there were no differences

in survival, weight gain, feed conversion ratio or fillet composition in catfish fed with GM cotton compared to control treatments fed cottonseed meal from the parental cotton line (Li & Robinson 2000).

199. Dietary toxicity studies with raw INGARD® cottonseed meal were conducted on the Northern Bobwhite Quail (Campbell 1985; Gallagher et al. 2000). In each study there was no mortality in birds fed up to 100,000 ppm (10%w/w, equivalent to 100 seeds/bird/day) for five days. There were no significant differences in feed consumption or body weight between birds fed INGARD® cottonseed meal compared to birds fed cottonseed meal from the parental cotton line (Campbell 1985; Gallagher et al. 2000).

200. In the United States, there have been anecdotal reports of increases in the populations of hummingbirds in fields of the INGARD® cotton, thought to be associated with reductions in the use of various insecticides in these crops (Betz et al. 2000).

201. Information provided by Monsanto on the compositional analysis of seed from INGARD® cotton demonstrates that the levels of gossypol and cyclopropenoid fatty acids (naturally occurring toxicants, see (OGTR 2002) are similar to those of other commercially available cotton varieties. The levels of nutrients (protein, fat, ash, crude fibre, carbohydrate, calories, amino acids and fatty acid) and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc) for INGARD® cotton were also comparable to those of the non-GM parental cotton varieties (Hamilton 2000).

202. INGARD® cotton tissues, the purified form of the introduced proteins and Cry1Ac as a component of *B.t.k* microbial insecticides, have been tested for their acute, subchronic and chronic toxicity to mammals, including rats, rabbits and sheep with no adverse effects on health detected. Refer to Appendix 2, Section 2 for details.

Section 2.5 Potential toxicity hazard for invertebrates, including beneficial insects

2.5.1 Exposure of invertebrates to INGARD® cotton

203. Non-target invertebrates may be exposed directly, through feeding on the INGARD® plants and potentially, via the soil, when INGARD® cotton tissues break down and are incorporated into the soil. Exposure may also occur indirectly, through eating other organisms that have been exposed to the toxin including target and non-target Lepidoptera and other arthopods that may feed on the plants.

204. Relative exposure will be greatest for other herbivorous species feeding on the cotton plants. Sap feeders such as aphids, will have minimal exposure to the introduced proteins because sap is primarily composed of sugars and mineral salts dissolved in water. However, species feeding on lepidopteran larvae may be exposed to both the full length Cry1Ac protein and the activated core toxin, since their lepidopteran prey may have ingested INGARD® cotton tissues and metabolised the full-length protein, leaving the core toxic element 'free' in the insect's gut. The feeding behaviour of potential lepidopteran predators and parasitoids is, therefore, likely to affect their potential exposure to the toxin.

205. Pollinator species and various adult insects that feed on pollen and/or nectar will have minimal exposure to the proteins because of the much lower expression in pollen, relative to that in other plant tissues, and undetectable expression in nectar (see Appendix 1, Section 7 for details).

206. Non-target lepidopteran species will be exposed to INGARD® cotton and may be affected by the Cry1Ac protein. However, cotton is not the preferred food source for any of these species and their populations would be sustained on other types of plants found around the location.

2.5.2 Toxicity of INGARD® cotton for invertebrates

2.5.2.1 Studies conducted under controlled conditions

207. A series of studies has been undertaken to demonstrate the effect of Cry1Ac on non-target insects (Macintosh et al. 1990). These 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. Seven insects, all lepidopterans, were susceptible to the toxin. None of the remaining 11 non-lepidopteran species were susceptible. Other studies have compared the core Btk toxin with recombinant protein equivalent to the full-length Cry1Ac protein expressed in INGARD® cotton (Sims 1994b);(Sims 1995). Of 14 species tested (representing seven orders), only lepidopteran species were susceptible to either form of Cry1Ac. The biological activities of the full length and core toxins were very similar.

208. More extensive studies have also been carried out on beneficial non-target insects 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*) (Palmers & Beavers 1993c); (Sims 1994b);
- 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 (Palmers & Beavers 1993b); (Sims 1994b), and
- green lacewing larvae (*Chrysopa carnea*), a beneficial predatory insect commonly found on cotton and other cultivated crops (Palmers & Beavers 1993a); (Sims 1994b).

209. There were no adverse effects seen for any of the species tested at the highest dose of full-length recombinant Cry1Ac tested (20 μg/g), which is well above the level of Cry1Ac protein found in INGARD® cotton (see Appendix 1, Section 7, Table 2).

210. The effects of feeding purified Cry1Ac toxin to collembolans (springtails, species *Folsomia candida* or *Xenylla grisea*) has also been investigated (Sims & Martin 1996). No adverse effects on the survival or reproduction of the collembolans were observed, at doses of up to 200 μg/g.

211. Other studies have demonstrated no adverse effects from feeding leaves of transgenic cotton containing Cry1Ac to two non-target soil arthropods, a collembolan *(Folsomia candida*) and an orbatid mite (*Oppia nitens*), organisms that play key roles as primary feeders or detritivores in soil ecosystems (Yu C.G. et al. 1997).

212. A number of laboratory studies have analysed prey-mediated effects of Bt toxins on beneficial predators using restricted-diet feeding studies. Experiments were conducted in which larvae of the predator *Chrysoperla carnea* (green lacewing) were fed an artificial diet containing high levels of purified Cry1Ab protein or fed on lepidopteran larvae that had either fed on purified Cry1Ab protein or

fed on corn expressing the Cry1Ab protein (Hilbeck et al., 1998; Hilbeck et al. 1998; Hilbeck et al., 1999). In all cases, mortality of *C. carnea* larvae was increased relative to non-Bt controls, suggesting that the Cry1Ab protein may have a toxic effect on *C. carnea* larvae.

213. It is difficult to extrapolate the results of these studies with Cry1Ab expressed in corn to potential impacts of Cry1Ac expressed in cotton, because corn expresses the core Cry1Ab toxin, whereas INGARD® cotton expresses the full-length protein that requires activation in the insect gut before becoming toxic. One study suggests that the longevity of adult heteropteran predators (*Orius tristicolour* and *Geocoris punctipes*) may be reduced when fed on lepidopteran prey that have ingested cotton expressing the Cry1Ac protein (Ponsard et. al. 2002). However, the inconsistent experimental approach among other technical considerations reported in this paper highlight the need to interpret the study's results with caution.

214. The significance of the results reported by Hilbeck et al. (1998a; 1998b; 1999) and Ponsard et al. (2002) for non-target populations of predators in an agricultural field environment is unclear, particularly as these studies employed continuous force feeding of both predator and prey with high toxin doses and do not allow for potential avoidance behaviours that may limit exposure to the toxins in the field. *C. carnea* larvae have shown a preference for feeding on lepidopteran larvae that have not consumed Bt-expressing corn over those that have, and a preference for feeding on aphids over lepidopteran larvae, which are unlikely to be exposed to Bt toxins due to feeding on phloem (see Section 2.5.1) (Meier and Hilbeck, 2001). Studies of Bt toxin-expressing plants conducted in field situations are significantly more informative in relation to impacts on beneficial and other non-target insect populations.

2.5.2.2 Studies conducted in the field

215. The effects of INGARD® cotton on non-target arthropod populations were studied over two seasons in cotton fields near Dalby, QLD (Addison 2001a; Addison 2001b). These studies used two sampling methods, pitfall trapping and suction sampling, to compare total arthropod abundance in unsprayed INGARD® fields with their abundance in unsprayed fields of conventional cotton on several occasions during the growing season. After classifying the samples to the level of Order, the results for both seasons suggested that, with the exception of Lepidoptera, the total (cumulative) abundance of arthropods collected from INGARD® cotton fields was comparable to their abundance in control (unsprayed conventional cotton) plots.

216. A series of large scale field experiments over three growing seasons in Australia showed no significant difference in invertebrate faunal diversity or abundance between unsprayed INGARD® cotton and unsprayed conventional cotton, except for a reduction in parasitoids of *Helicoverpa* in one season (Fitt & Wilson 2002). A reduction in specialist parasitoids is expected due to reductions in the abundance of their host species (the target pests of INGARD® cotton). This is unlikely to threaten their persistence in the cropping system, however, since a significant proportion of the *Helicoverpa* population is always present on other crops and in uncultivated areas. Studies of invertebrate abundance were also conducted in commercial cotton crops in the 1996/1997 growing season. Total abundance of predators and several other non-target invertebrate groups in INGARD® commercial cotton crops that had received synthetic insecticide sprays (to control non-lepidopteran pests) was not significantly different to total abundance in unsprayed conventional cotton and was higher than that in sprayed conventional cotton (Fitt & Wilson 2002).

217. Field studies were also conducted on commercial cotton crops in NSW in the 1996/1997 and 1997/1998 growing seasons used suction sampling, to compare the abundance of predatory beetles, bugs, lacewings and spiders in INGARD® and conventional cotton fields (Mensah, 2002). In these studies both crops were sprayed with synthetic insecticides as necessary to produce a commercial crop. INGARD® cotton crops received between 25 % and 60 % fewer synthetic insecticide sprays than the non-GM cotton crops and generally had higher levels of all classes of predators studied.

218. In addition to pitfall trapping, an American study used sweep nets, beat-buckets and plant inspections to compare the impact on non-target insects of conventional cotton and cotton varieties expressing Cry1Ac, relative to the impact of insecticides that may be used in cotton plantations (Naranjo & Ellsworth 2002). As well as measuring arthropod abundance, this study measured species diversity and investigated the possibility that the relative importance of predators and parasitoids may be affected by the insecticidal gene. The results indicated that the diversity of arthropods and the abundance of natural enemies was unaffected by the Cry1Ac toxin present in the cotton, but affected by the use of insecticides (Naranjo & Ellsworth 2002). The relative level of parasitism and predation of two key cotton pests was also unaffected by the insecticidal toxin.

219. The commercial release of INGARD® cotton has reduced the use of broad-spectrum insecticides on Australian cotton crops and several studies have found that overall numbers of non-target invertebrates in INGARD® cotton fields are the same or higher than in conventionally sprayed fields of non-GM cotton. Increased numbers of non-target invertebrates are likely to relate directly to reductions in chemical insecticide usage. Similarly, the use of INGARD® cotton in China, with the concomitant reduction in insecticide use, resulted in an average increase of 24% in the number of insect predators over conventional cotton fields (Xia et al. 1999).

2.5.2.3 Multi-trophic studies of Cry toxins

220. The potential for insecticidal Cry toxins expressed by GM plants to impact on ecological communities in the natural environment in unexpected indirect ways, by affecting interactions between organisms elsewhere in the 'food web', has been considered. Indirect impacts have been demonstrated in several field studies (see review paper by Groot and Dicke 2002) and, where detected, relate to altered arthropod community structure in the agricultural field in which the GM insecticidal plant is cultivated. Groot and Dicke (2002) suggest that, in instances where such observations of changed community structure have been made, they probably reflect the dispersal of predator species from GM-crops to nearby non-GM habitats, possibly in response to decreased prey abundance, rather than implying a direct toxic effect mediated via prey species.

221. Potential impacts of such dispersal on ecological communities in the habitat to which predator species disperse does not appear to have been investigated. Nonetheless, data from Australian (Addison 2001a; Addison 2001b) and American (Naranjo & Ellsworth 2002) studies demonstrate that the abundance of potential predator and parasitoid species is not adversely affected by cultivation of GM insecticidal cotton. These studies imply, therefore, that any dispersal to nearby non-GM habitats, as may have occurred in the studies referenced by Groote and Dicke (2002), is unlikely to be ecologically significant. It appears that, even if potential 'ripple effects' through the food web can be detected, their significance is likely to be minimal in terms of ecosystem structure and function.

Section 2.6 Potential toxicity hazard for microorganisms, particularly soil microorganisms

2.6.1 Exposure of microorganisms to INGARD® cotton

222. Microorganisms may be exposed to cotton plants during growth or during decomposition of plant material. While cotton plants are living, exposure of soil microorganisms to Bt residues may occur as a result of root exudations, as has been observed in Bt corn expressing Cry1Ab (Saxena et al. 1999); (Stotzky 2000b). 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 in soil (see Appendix, Section 7.6 for details) and the mechanism is not clear. Root breakage could also increase the release of Cry1Ac protein into the soil.

223. After harvest of lint and seed, the remaining cotton plant residues are typically tilled into the soil. Soil microorganisms are likely to be exposed to the introduced proteins as the GM cotton residues are broken down. (Other soil biota are discussed in Section 2.2.2 of this Appendix.) The level of exposure is likely to decrease with time, as a result of soil biodegradation. (Ream 1994b) compared the rate of soil biodegradation of Cry1Ac protein in INGARD® cotton plants to that of the purified toxin. The plant-encoded Cry1Ac degraded with a half-life of 41 days, compared to 9.3 to 20.2 days for the purified toxin. In another study, the results were variable but indicated half-lives for Cry1Ac in the order of 2.2 to 46 days (Palm et al. 1996). 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. However, low levels of Cry1Ac were still detectable several months after treatment in some soils.

224. 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 season's 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. The typically rapid breakdown of Bt proteins in the soil, including that of Bt potatoes (Cry3Aa) and Bt corn (Cry1Ab), indicates that these proteins are not likely to accumulate at biologically significant levels (Palm et al. 1994; Sims & Holden 1996; Head et al. 2002). In addition, generally in Australia cotton is grown in alkaline soil, with a pH ranging from 7.5 – 8.5 (Cotton CRC NUTRIpak) (Tapp & Stotzky 1998), in which Bt endotoxins would desorb from clay soils and be degraded by soil microorganisms. Thus the Cry1Ac protein is not likely to accumulate in agricultural soils as a result of successive seasons of cultivation of INGARD® cotton.

2.6.2 Toxicity of INGARD® cotton for microorganisms

225. The effect of Cry 1Ac toxin on soil microorganisms was examined by incubating various soils with purified Cry1Ac toxin or cotton leaves containing Cry1Ac (0.05 µg/g Cry1Ac protein in each treatment). The numbers and types of protozoans, bacteria and fungi were determined at various time points. Substrate utilisation tests and DNA fingerprinting of eubacterial ribosomal sequences were also used to analyse the composition of bacterial soil community (Donegan et al. 1995).

226. In these experiments, addition of purified Cry1Ac toxin to the soil did not cause any detectable effects on populations of culturable aerobic soil bacteria, fungi or protozoa after exposure for up to 56 days. The transgenic cotton lines expressing Cry1Ac protein caused a transient increase in total bacterial and fungal population levels, that was significantly higher on several sample dates, compared to levels in other treatments. (Donegan et al. 1995) noted that the stimulatory effects observed were short term and suggested that the transgenic plants may have decomposed faster than the parental plants and thus more rapidly provide nutrients for microbial growth.

227. In a second study, (Donegan & Seidler 1998) again examined the impact on soil organisms of cotton containing the Cry1Ac toxin or purified Cry1Ac toxin, when added to soil at equivalent levels. The numbers and types of protozoans, bacteria and fungi were determined at various time points. The addition of purified Btk endotoxin to natural soil had no measurable effects on the indigenous soil microorganisms. In contrast, the increase in culturable, aerobic bacteria and fungal populations that occurred when leaves of cotton were added to soil was significantly higher in the transgenic treatments relative to the parental cotton treatment (Donegan & Seidler 1998). Transient changes in the composition of the soil microbial community for soil containing GM cotton expressing Cry1Ac, relative to soil containing leaves from the parent line, was also reported. (Donegan & Seidler 1998) suggested that while the temporary stimulation of microbial growth would not generally be considered to be detrimental, the apparent changes in microbial species composition may have the potential to impact on soil processes and may be of ecological significance.

228. 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.

229. Many of the experiments examining persistence of Bt proteins reported in the published literature have been conducted in bulk soils or soil components (e.g. (Palm et al. 1996; Koskella & Stotzky 1997; Stotzky 2000a). Bulk soil generally does not support populations of microorganisms as high as those in the rhizosphere or as high as in cropping situations where plant residues are incorporated into the soil (Griffiths et al. 1999) — conditions that are more likely to favour the rapid degradation of Bt toxin. CSIRO is currently studying the impact of INGARD® cotton on rhizosphere microflora and microfauna.

230. The direct effects of the NPTII enzyme on microorganisms have not been tested. NPTII is a phosphorylating enzyme which does not possess any properties that distinguish it toxicogically from other phosphorylating enzymes present in microoganisms, 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 scientific literature. 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 (canola, corn, cotton, tomato), over several years of agronomic performance testing and commercial cultivation, has not been linked to any increased occurrence of disease.

SECTION 3 CONCLUSIONS REGARDING TOXICITY TO NON-TARGET ORGANISMS

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

- the toxicity of Cry1Ac is specific to Lepidoptera and the conditions required for its toxicity do not occur in any non-target organisms;
- the introduced proteins are already widespread in the environment through the presence of the bacteria to which they are native;
- toxicity studies with the Cry1Ac protein and/or INGARD® cotton tissue indicate that INGARD® cotton will not be more toxic to mammals, birds or fish than non-GM cotton;
- levels of nutrients and anti-nutrients in cotton seed and processed cotton seed products used for stockfeed are the same for INGARD® and non-GM cotton;
- there are no differences in the performance of stock fed INGARD® cotton seed compared to stock fed non-GM cotton seed;
- studies conducted under controlled conditions and in the field indicate 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 associated decreases in the use of broad-spectrum insecticides;
- laboratory studies indicate that the Cry1Ac protein has no adverse effect on the growth of various bacteria, fungi and protozoans;
- the presence of INGARD® cotton plant material in the soil only produce transient changes in soil microbial communities; and
- natural degradation of Cry1Ac in the soil limits bioaccumulation.

232. The licence holder will be required to report and adverse effects on human health and safety or the environment (for example, any indication of toxicity of INGARD® cotton for non-target organisms).

APPENDIX 4 ENVIRONMENTAL SAFETY - WEEDINESS

233. 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. This part of the document considers potential hazards that may be posed to the environment by the proposed dealing. In this context, the potential for the GMO to become a problematic weed was considered.

234. 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

235. INGARD® cotton differs from conventional cotton in the expression of two additional proteins, Cry1Ac and NPTII (see Appendix 1 for details of protein expression in the GM cotton). The possibility was considered that INGARD® cotton might have the potential to be harmful to the environment, because of inherent weediness or increased potential for weediness either as a direct result of genetic modification or as a result of pleiotropic effects. This could occur if INGARD® cotton displayed altered characteristics such as increased fitness due to higher levels of insect resistance or increased fecundity. If the GM cotton were to spread in the environment as a weed, this could result in impacts such as loss of native biodiversity or adverse effects on agricultural systems.

SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

Section 2.1 Inherent Weediness of Conventional Cotton

236. Attributes of conventional 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 process 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, temperature sensitivity and roadside management practices limit the establishment and/or persistence of cotton seedlings. Further discussion of the weediness of conventional cotton is included here to establish a base-line for comparison with INGARD® cotton.

237. 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).

238. Other aspects of plant's character used in predicting its potential for weediness are; taxonomic relationships, weediness of the taxon's relatives, and its history of weediness in the rest 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 listed as a weed in

Australia (Tothill et al. 1982). There are about 50 species of *Gossypium* (Fryxell et al. 1992; Craven et al. 1994) of which only one (*G. tomentosum*) is listed as a weed in the USA (Holm et al. 1997).

239. Observations and/or experiments on the dispersal, seed germination, survival, reproduction and invasiveness of cotton indicate that expression of these proteins in INGARD® cotton does not alter any of the characteristic attributes of cotton that may affect weediness in the natural environment (Eastick 2002; OGTR 2002; and see below for details).

Section 2.2 Dispersal of cotton seed in the environment

240. Natural dispersal of cotton seed in the environment is very limited. The lint covered seed that forms on the cotton plant (seed cotton) is too heavy to be dispersed by wind. While the seed cotton could float for some distance along a waterway, cotton farms generally offer very poor access to waterways beyond the on-farm irrigation systems. Thus there is only very limited potential for movement of seed cotton without the assistance of human intervention.

241. The proposed dealings include cultivation of INGARD® cotton in the cotton growing regions of NSW and Qld south of latitude 22º South and entry of cotton products into general commerce after harvest. Through these dealings seed prepared for planting and the harvested seed cotton will be transported within and between cotton growing regions. The seed product from ginning (fuzzy seed) will be transported throughout the cotton growing regions and may also be moved beyond these regions, including north of latitude 22º South for use as stockfeed.

242. Surveys by Eastick (2002) and Farrell & Roberts (2002) have found that seed cotton is the most likely form of seed to be dispersed in the environment. Seed cotton readily falls from cotton modules during transport to cotton gins after harvest, greatly increasing seed dispersal off-farm. Planting seed however is rarely spread into the off-farm environment. It is a value added product that is packaged to prevent losses during transport and storage. Similarly fuzzy seed is transported in a manner which minimises seed loss on-route.

243. The activities associated with using cotton seed for stock feed, also represents opportunity for cotton seed to disperse into the environment. Seed can be spilt around feeding-out areas and a very small percentage of cotton seed consumed by stock can pass through their digestive system intact, retaining capacity for germination ((Eastick 2002).

Section 2.3 The occurrence of cotton volunteers in the environment

244. Eastick (2002) found that seed type was a key factor influencing germination of cotton volunteers. Seed prepared for planting had the highest germination percentage followed by fuzzy seed and then seed cotton when planted in various off-farm environments (56.5%, 49.6% and 29.7% respectively). Eastick's results indicate that the likelihood of each of the seed types germinating is the converse of the likelihood of their dispersal into the environment. Even though seed cotton is the most readily dispersed seed type, it is the least likely to germinate in habitats such as bushland, waterways, cattle yards and roadsides.

245. The trend observed in Eastick's experiments was confirmed by a roadside survey in the Lower Namoi cotton growing region of NSW (Farrell & Roberts 2002). Conducted five years after the commercial release of INGARD® cotton the survey found cotton volunteers to establish infrequently,

despite the large quantity of seed (up to tens of thousands of seeds per kilometre) dispersed into this environment through cotton module transportation. The population of volunteer plants observed averaged 4.2 volunteers/km, indicating that very rarely do seeds successfully germinate and establish. All of the volunteers observed were less than one year old. There was no indication of perennial growth in these volunteers, most probably due to high levels of disturbance, low winter temperatures and high incidence of frosts. Nor was there any indication that the volunteers established self-perpetuating weedy populations. Finally, INGARD® cotton volunteers did not occur more frequently, nor did they produce more fruiting structures than the non-GM cotton volunteers that were observed.

246. This roadside survey was repeated in March 2003 following the sixth year of commercial production of INGARD® cotton and additionally roadsides in the Darling Downs cotton region were also surveyed. The 2003 survey was conducted after substantial rains, creating ideal conditions for new germinations of cotton volunteers. In the Lower Namoi Valley, cotton volunteers were found at 33 of the 77 sites, although at only two of these sites were the volunteers established (past the fourth leaf stage). None of the established volunteers were found at sites where volunteers had been observed in the previous season's survey, indicating that the volunteers from the previous season had not survived. In the Darling Downs, cotton volunteers were found at five of the 40 surveyed sites. At only one site were these established beyond the fourth leaf stage.

247. A survey of the transport routes between Emerald (in the Qld cotton growing region) and the Atherton Tablelands (north of latitude 22º South) indicated that cotton plants had established in the 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). Factors thought to be preventing populations of such volunteers from increasing in size, density or area in this environment were; a lack of suitable conditions for germination, competition from established species for new germinants, and low levels of soil nutrients. It was also noted that the adoption of 'roll-over tarp' systems for trailers transporting cotton seed to the Atherton Tablelands since 2001 has substantially reduced the opportunity for fuzzy seed to escape on-route.

248. In the same survey, the ability for cotton plants to establish and spread on Atherton Tableland dairy farms, the destination of the fuzzy seed, was also assessed. Populations of both conventional and INGARD® cotton were observed to germinate readily and establish. INGARD® and conventional cotton plants were observed at similar frequencies. None of the cotton plants produced mature seeds and there was no indication that INGARD® cotton had a higher reproductive potential than non-GM cotton. Most commonly, cotton seedlings died during early stages of growth because of disease, competition or physical damage. During the 12 years in which cottonseed has been transported to, and used within, these northern Queensland environments, cotton plants have not spread into the natural environment as weeds or exhibited weedy potential.

249. Cattleyards were one of the non-cropping habitats investigated by (Eastick 2002). GM cotton plants growing in a cattleyard at Broome demonstrated substantial reproductive capacity, while cattleyards at Katherine and Kununurra produced no, or only very few, mature fruit. At Broome, second generation seedlings were recruited from the seed produced by the plants that had established. Seedlings were recruited from all genotypes. Eastick (2002) considered soil nutrients, moisture availability and interspecific competition to be the key factors associated higher fruit production at Broome. Rhodes grass (*Chloris spp*) and buffel grass (*Cenchurus spp*) established in some areas of

the trial. These areas had significantly less cotton plants than areas where little interspecific competition occurred.

250. There was no evidence to suggest that the addition of the *cry1Ac* gene in INGARD® cotton consistently lead to increased fruit (boll) production. Fruit production from INGARD® and Bollgard II® cottons were greater after than first season than production from conventional cotton, however this trend was reversed after the second season of growth. Eastick (2002) proposed that determining the time corresponding to maximum boll numbers may be different between genotypes and may also differ between seasons. The transgenic plants may be more determinant due to more rapid boll setting and hence obtain their reproductive potential earlier in the season. However this may or may not result in greater maximum fruit production from the transgenic plants, depending in part on whether the wet season commences before the bolls on the conventional plants have time to fully develop.

Section 2.4 Potential weediness of INGARD® cotton

251. Many of the characteristics associated with weediness are also important agronomic characteristics and are consequently assessed as part of the agronomic evaluations of the new GM cotton varieties (refer Appendix 1, section 7). Seed survival, germination, vigour, yield and disease susceptibility of INGARD® cotton varieties currently being grown have been evaluated in both controlled environment and field releases and are within the range of current conventional cotton varieties (Monsanto 1995). These data suggest that the genetic modification has not affected the characteristics of cotton that may affect weediness.

252. As mentioned previously, INGARD® cotton has been in commercial release since 1996. Since then, cotton seed from INGARD® cotton has been used as stock feed in northern Australia. Over this period there has been no evidence that GM cotton has become more weedy than non-GM cotton (see Section 2.5). 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.

253. The results of this research strongly indicate that INGARD® cotton is not more likely to establish or persist as a weed in southern Australia than non-GM cotton, because it is environmental variables and not the genetic modification, that are the factors limiting cotton establishment and population growth. 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.

Section 2.5 The potential selective advantage of INGARD® cotton

254. The expression of the Cry1Ac and NPTII proteins are the only known differences between INGARD® and conventional cotton. In order for INGARD® cotton to spread and persist in the environment more so than non-GM cotton, the expression of one or both of these proteins would need to confer a selective advantage.

255. The NPTII protein could only confer a selective advantage to INGARD® cotton plants in areas where cotton plants had established but antibiotics limited their growth. Neomycin and kanomycin have no herbicidal activity, are not applied to plants 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 advantage on INGARD® cotton in relation to weediness.

256. The Cry1Ac protein could only confer a selective advantage in areas where lepidopteran insect predation limits one or more of cotton's key life stages or regulates weedy cotton populations. The production of Cry1Ac is known to decline as the plant matures. As seen in field trials and in the commercial production of INGARD® cotton, the expression of Cry1Ac is insufficient to control the target pests late in the season (see Appendix 1, Section 7). Hence, the selective advantage conferred by Cry1Ac may be relatively short lived, particularly as the protein may not protect fruit through to seed maturity.

257. With respect to cotton's key life stages, Eastick (2002) compared the germination, survival, reproduction and invasiveness of non-GM cotton with different types of GM insecticidal cotton, including INGARD® cotton, to determine whether insecticidal genes affected potential weediness in various non-cropping environments. Although Eastick's investigations were conducted in northern Australia, most of the results are also relevant to the potential weediness of INGARD® cotton in southern Australia, as summarised below:

- Seed dispersal: As cotton does not generally reproduce vegetatively (Serdy et al. 1995), spread within the environment occurs by seed dispersal (OGTR 2002). Dispersal of cottonseed is a physical process largely dependent on human intervention. Seed may be dispersed in its natural form as 'seed cotton', with the lint attached to the seed coat or following activities involved in cotton production, either as 'fuzzy seed', with the majority of lint removed, or as 'black seed', with all of the lint removed. The genes inserted into INGARD® cotton do not affect these properties of the seed and, therefore, do not affect the natural dispersal of INGARD® cotton.
- Germination: Eastick (2002) concluded that typically, the insecticidal genes in GM cotton did not affect seed germination. Although she reported statistically significant differences between the germination of conventional and GM cotton at a few sites, germination was highly variable, with seeds germinating very poorly at some sites and relatively well at others. The prevalence of site-specific impacts suggests that environmental effects are likely to affect the germination of INGARD® and conventional cotton more strongly than genotype *per se*. In combination with the *in vitro* comparisons of germination in INGARD® and non-GM cotton (Wiltse et al. 2000; Wiltse et al. 2001), Eastick's data strongly suggest that the genes inserted into INGARD® cotton are unlikely to increase INGARD®'s potential weediness by enhancing its potential for germination.
- Survival: The survival of seedlings that germinated in Eastick's field germination experiments were monitored for two years. Typically, the total number of surviving seedlings was low and highly variable. At sites where the number of surviving seedlings was sufficiently high to warrant statistical analysis, there was no indication that the genetic modifications significantly enhanced survival. Eastick (2002) concluded that factors such as water availability, soil nutrients, grazing by vertebrates, fire and plant competition are likely to affect seedling survival more so than insect pressure.

- Reproduction: Eastick (2002) also provided data from the initial germination experiments on the number of surviving seedlings that reached reproductive maturity. Surviving plants produced floral buds at the majority of sites, but frequently they were aborted before maturation of the fruit, a phenomenon that is common in cultivated cotton (OGTR 2002). Maturation of fruit occurred at 8 of the 20 study sites, although the total number of fruit was highly variable ranging from 1 to > 300. Statistical analysis was not possible for all sites because of low numbers and high variability. At individual sites where fruit production warranted analysis, there was some indication that the insecticidal genes were associated with increased fruit production, however this trend was not consistent for all sites in both seasons (refer to discussion in paragraph 246). These sites were characterised by artificially high nutrient and/or soil moisture levels, as might occur in drainage channels or where livestock are feed cottonseed in stockyards.
- Invasiveness: Eastick (2002) estimated invasiveness by an index that considers any deaths that may occur, as well as any new recruits into the population. The term therefore integrates elements of germination, survival and reproduction to provide a means of comparing the net population growth rate of GM insecticidal cottons, including INGARD® cotton, with conventional cotton. Generally the data suggested that cotton is rarely invasive, irrespective of the inserted insecticidal genes, because at most study sites invasiveness values were considerably less than one, indicating the populations were in decline. However at one waterway site, where moisture was not a limiting factor, and fruit production was relatively high, the index of invasiveness exceeded one. That this index exceeded one for both INGARD® and non-GM cotton strongly suggests that this was a site-specific response rather than implying that a selective advantage was conferred on INGARD® cotton.

258. Eastick (2002) demonstrated that for each of cotton's life stages, the response of INGARD® cotton is not significantly different to that of conventional cotton in most circumstances. While Eastick found no conclusive evidence that the *cry1Ac* gene conferred additional fitness on cotton plants in non-cropping habitats in northern Australia, in the stockyards in Broome where moisture was plentiful and nutrition high, increased fruit production was associated with insecticidal cotton in the first season of growth. Generally there were constraints limiting the weedy potential of all the cotton genotypes including herbivory by non-lepidopteran species (vertebrates and some invertebrates), plant competition, soil moisture, fire and poor soil fertility. These constraints limit the weediness of conventional cotton and non-GM cotton equally. As non-GM cotton is not weedy, it is highly unlikely that INGARD® will become a problematic weed of the environment. However the potential for more rapid development of fruit for INGARD® cotton may, in some seasons, represent a selective advantage.

SECTION 3 CONCLUSIONS REGARDING WEEDINESS

259. It is concluded that there is a very low risk of INGARD® cotton establishing as an environmental weed in cotton growing regions of NSW and Queensland south of latitude 22º South - the region in which Monsanto proposes to grow the GM cotton - because:

- cotton, including INGARD® cotton, does not possess characteristics commonly associated with weediness, and is not known to be a problematic weed in any Australian environment;
- cotton, including INGARD® cotton, has a low potential for dispersal by natural means;

- cotton volunteers, whether INGARD® or non-GM cotton, can establish on roadsides but do not persist or lead to population spread into the wider environment;
- soil-water availability, soil nutrients and frost are predominant factors limiting the germination and/or persistence of both INGARD® and non-GM cotton in off-farm habitats.

260. It is concluded that the risk of INGARD® cotton establishing as a weed north of latitude 22º South, associated with the use of cotton seed as stock feed, is also low for the same reasons. However this is yet to be determined conclusively because:

- in favourable conditions, such as where soil moisture levels are not limiting and interspecific plant competition is absent, both INGARD® cotton and conventional cotton show some ability to establish self-perpetuating populations; and
- in some stockyards in Broome GM cotton showed increased boll production, which may, in some seasons, represent a selective advantage.

261. INGARD® cottonseed has been used as stockfeed in northern Australia since 1996, with no indication that INGARD® has become more weedy than non-GM cotton, including feral cotton. The low risk of spread or persistence in the environment associated with the continuation of transporting INGARD® cotton north of latitude 22º South for stock feed can be managed. The licence requires the licence holder to provide information about cotton volunteers to endusers, including a means for endusers to report volunteers that become established, thus allowing further investigation. The licence holder will also be required to conduct surveys of areas where seed is fed to stock and report these findings to the OGTR as part of their annual report.

APPENDIX 5 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

262. 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 INGARD® cotton to other organisms.

263. 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.

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

SECTION 1 GENE TRANSFER FROM INGARD® COTTON TO OTHER PLANTS

Section 1.1 Nature of the gene transfer hazard

265. 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.

266. 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.

267. 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 INGARD® cotton to other plants

268. 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.

1.2.1 The introduced genes

1.2.1.1 *cry1Ac* (insecticidal) gene

269. 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.

1.2.1.2 *nptII* and *aad* (antibiotic resistance) genes

270. 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.

1.2.1.3 CaMV promoter and other regulatory sequences

271. 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.

272. 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.

273. 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

274. 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.

275. *Gossypium barbedense* (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. barbedense* 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. barbedense* 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 INGARD® cotton to *G. barbedense* crop plants would not present any hazards additional to those posed by the INGARD® cotton (see Appendices 2 - 6).

276. 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.

277. 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 INGARD® cotton (see Appendices 2 - 6).

1.2.3 Transfer to volunteer and naturalised (feral) cotton

278. 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 cotton plants volunteering from previous cultivations.

279. 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 INGARD® cotton is unrestricted. Surveys indicate that INGARD® cotton volunteers are not more weedy than non-GM cotton in these regions (Appendix 4, Section 2).

280. 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 geographical isolation of known feral populations in Western Australia and the Northern Territory from areas of NSW and Queensland in which 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. For this reason, a licence condition has been imposed requiring the

applicant to document the location of self-perpetuating populations of feral cotton in Queensland and determine their distance from cotton production locations.

281. 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 INGARD® cotton (see Appendices 2 - 6).

1.2.4 Transfer to native cottons and other plant species

282. 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.

283. 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 INGARD® cotton to native cottons is prevented not only by genetic incompatibility but also by geographic constraints to cross-pollination (OGTR 2002).

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

SECTION 2 GENE TRANSFER FROM INGARD® COTTON TO MICROORGANISMS

Section 2.1 Nature of the gene transfer hazard

285. 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).

286. The potential hazards associated with the introduced genes of 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 INGARD® cotton to microorganisms

287. 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.

2.2.1 The introduced genes

2.2.1.1 *cry1Ac* (insecticidal) gene

288. 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.

2.2.1.2 *nptII* and *aad* (antibiotic resistance) genes

289. 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.

290. 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.

2.2.1.3 CaMV 35S promoter and other regulatory sequences

291. 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.

292. 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.

293. 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

294. 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.

295. All of the introduced genes in 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.

2.2.2.1 *cry1Ac* (insecticidal) gene

296. The *cry1Ac* insecticidal gene expressed in 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).

297. 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).

2.2.2.2 *nptII* and *aad* (antibiotic resistance) genes

298. 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).

2.2.3 Likelihood of gene transfer from INGARD® cotton to microorganisms occurring

299. Most gene transfers have been identified through analyses of gene sequences (Ochman et al 2000;(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).

300. In contrast, transfers of plant genes to other organisms such as bacteria, fungi or viruses are exceedingly rare (Nielsen et al 1998; (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); Nielsen et al. 2000). 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); Nielsen et al 2000). 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

301. Natural transformation is a mechanism by which transfer of DNA from plants to microorganisms could have occurred during evolution (Bertolla & Simonet 1996) 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).

302. 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.

303. 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) ; De Vries 2001) and even then only, at a very low frequency.

304. 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;(Nielsen et al. 2000; De Vries et al. 2001) or in *Pseudomonas stutzeri* (De Vries 2001).

2.2.3.2 Release and persistence

305. 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).

2.2.3.3 Bacterial competence and DNA uptake

306. 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).

307. 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.

2.2.3.4 DNA integration

308. 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.

2.2.3.5 Expression and selection

309. 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* gene was modified to enhance expression in plants); and
- processing of an encoded 'pro-protein' may be required for production of a functional product (eg. cleavage of the Chloroplast Transit Peptide from plant EPSPS pro-proteins, see Appendix 1).

310. 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).

2.2.3.6 Viruses

311. 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.

312. 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).

2.2.3.7 Fungi

313. 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).

314. 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 INGARD® COTTON TO ANIMALS

Section 3.1 Nature of the gene transfer hazard

315. The potential hazards associated with the genes introduced in 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® cotton to animals (including humans)

316. 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.

3.2.1 The introduced genes

3.2.1.1 *cry1Ac* (insecticidal) gene

317. 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.

3.2.1.2 *nptII* and *aad* (antibiotic resistance) genes:

318. Animal 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.

3.2.1.3 CaMV 35S promoter and other regulatory sequences

319. 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 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.

320. 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.

321. 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

322. 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.

323. 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

324. 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.

325. 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).

326. 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 2000). 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.

327. Einspanier et al. (2001) investigated the fate of DNA from GM maize fed to cattle and chickens, using PCR to detect the introduced *cryIAb* 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 *cryIAb* gene.

328. For cattle fed GM maize silage, both the *cryIAb* 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 *cryIAb* gene was not detected in any of these samples (Einspanier et al. 2001).

329. 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 *cryIAb* gene was not detected in any tissue sample or eggs (Einspanier et al. 2001).

330. 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.

331. 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

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

- gene transfer would not pose any risks additional to those posed by INGARD® cotton.

333. Although transfer of the introduced genes from INGARD® cotton to naturalised (feral) cotton (both *G. hirsutum* and *G. barbedense*) 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 those posed by INGARD® cotton;
- cotton volunteers in the cotton growing regions south of latitude 22º South already include INGARD® cotton, however 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. However herbarium records have not recently be confirmed. The licence requires the licence holder to conduct a survey of the sites in Queensland where herbarium records indicate cotton has been observed.

334. It is considered that the risk of gene transfer from INGARD® cotton to native cotton species is negligible, because:

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

335. It is considered that the risk of gene transfer from INGARD® cotton to other plant genera is negligible, because:

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

Section 4.2 Conclusions regarding gene transfer to microorganisms

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

- all of the introduced genes in 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.

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 INGARD® cotton to animals, including humans, is negligible because:

- the introduced genes of INGARD® cotton are not present in human food products;
- the fate of DNA in the digestive tract of various animals has been studied and exposure of animals to introduced DNA from GM crop material is negligible compared with normal exposure to non-GM DNA;
- the likelihood of gene transfer into animal cells is extremely low, and not greater than the likelihood of transfer from other sources of the introduced genes in the environment; and
- in the rare even of plant DNA uptake by animal cells, the further step of chromosomal integration has not been demonstrated.

APPENDIX 6 INSECTICIDE RESISTANCE

338. 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. This part of the document considers potential hazards that may be posed to the environment. In this context, the potential for target insects to develop resistance to the insecticidal protein was considered.

SECTION 1 NATURE OF THE INSECTICIDE RESISTANCE HAZARD

339. Extensive cultivation of 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 INGARD® cotton for the control of insect pests, and could also have impacts on the use of Bt microbial sprays to control these insects in other agricultural systems. Potential adverse effects include attenuation of the benefits of growing INGARD® cotton to the environment and human health.

340. It is noteworthy that Monsanto intends to phase-out releases of INGARD® cotton over the next two growing seasons, while GM insecticidal Bollgard II® cotton is phased-in over the same period. The Regulator issued a licence (licence number DIR 012/2002) for the commercial release of Bollgard II® cotton in Australia south of latitude 22º South on 23 September 2002. Bollgard II® cotton expresses two insecticidal genes and accordingly, is likely to significantly delay the emergence of resistance in target pests (Roush 1996; Roush & Shelton 1997; Roush 1998).

SECTION 2 LIKELIHOOD OF THE INSECTICIDE RESISTANCE HAZARD OCCURRING

341. The emergence of insects resistant to the Cry1Ac protein would almost certainly occur if INGARD® cotton was grown widely without taking any steps to prevent or delay the problem. INGARD® cotton expressing a single-toxin is at particular risk of resistance developing when the targeted insect pests, such as *Helicoverpa*, are not highly sensitive to the Bt toxin (Roush 1998) and when the efficacy of the insecticidal protein declines during the later stages of the cotton-growing season, as occurs in INGARD® cotton (see Appendix 1, Section 7 for details).

342. The cotton industry in Australia has been proactive in addressing insect resistance concerns. Prior to the first commercial release of INGARD® cotton in 1996/97, the industry established the Transgenic and Insecticide Management Strategy (TIMS) committee to develop a strategy to be adopted by all farms growing Bt cotton. The strategy outlined the need for effective refuges, mandatory cultivation after harvest to destroy pupae, defined spray thresholds for applications to control survivors and a program for monitoring Bt resistance levels. A condition of registration of INGARD® cotton by the APVMA is that growers are required to implement insecticide resistance management plans that are developed by the TIMS Committee.

343. Cotton plants growing as volunteers can reduce the effectiveness of the resistance management strategy for INGARD® cotton, reduce seed purity and may act as early hosts for pests such as spider mites and aphids (Roberts & Charles 2002). Thus, it is common practice for cotton growers to vigilantly control GM cotton volunteers as part of the industry's resistance management plan for INGARD® cotton, which forms part of the grower's Technology Users Agreement with Monsanto.

Cultivation and herbicides are the two most common methods of volunteer cotton control. The extent to which either of these methods can be employed for the control of INGARD® cotton is the same as for conventional cotton.

344. INGARD® cotton has now been grown commercially for six seasons with an average associated reduction in insecticide use of around 50% (Fitt & Wilson 2002). As noted above, Monsanto intends that INGARD® cotton will be phased-out over the next two growing seasons as Bollgard II® cotton is introduced. Bollgard II® cotton was developed with the specific intention of reducing the risk of resistance developing in the target species (Gould 1998; Roush 1998; Benedect & Altman 2001).

Section 2.1 Occurrence of resistance in insects

345. Several studies have shown that resistance to Bt toxins can be selected in the laboratory by repeated exposure of insects to the toxins (Peferoen 1997); (Tabashnik et al. 1998; Tabashnik et al. 2000b). For example, two laboratory strains of *Heliothis virescens* were selected to become resistant to Cry1Ac and other Bt derived toxins (Gould et al. 1995), and (Akhurst et al. 2000) have isolated a laboratory strain of the Australian *Helicoverpa armigera* that is resistant to Cry1Ac. (Moar et al. 1995) have selected strains of *Spodoptera exigua* resistant to Cry1C and these insects were cross-resistant to Cry1Ab, Cry9C and Cry2A as well as to a recombinant Cry1E-Cry1C fusion protein. Recent studies have found evidence of cross-resistance between Cry1Ac and Cry2A proteins in Cry1Ac-resistant strains of *Pectinophora gossypiella*, *Helicoverpa zea* and *H. armigera* ((Burd et al. 2000); (Tabashnik et al. 2000a); (Akhurst 2001).

346. Bt resistance has also been studied in field populations of the diamondback moth, *Plutella xylostella* (Tabashnik et al. 1990). The diamondback moth, a major pest of cruciferous vegetables around the world, receives frequent exposure to insecticides and shows extensive resistance to most chemical insecticides in many growing areas. High levels of resistance to Cry1A toxins have been found in populations of the diamondback moth from the Philippines, Hawaii, Florida and Asia (Tabashnik et al. 1990); (Tabashnik et al. 1994b).

347. Resistance to Bt insecticide appears to be due to one (Tang et al. 1997a), or at most a few, genes (Tabashnik et al. 1992; Ferre et al. 1995; Gould et al. 1995a; Tabashnik et al. 1998). The mechanisms of insecticidal activity include reduced binding of the toxin to the midgut (Tabashnik et al. 1994a; Gould et al. 1995; Tang et al. 1997), slower interaction of gut proteinases with the protoxin, or the absence of a major gut protein (Oppert et al. 1997).

348. Genetic crosses of the laboratory-selected insecticide-resistant strains of *Heliothis virescens* demonstrated that a major portion of the resistance in this case was encoded by a single gene (or a set of linked genes) with mostly recessive inheritance (Gould et al. 1995; Gould et al. 1997); (Tabashnik et al. 1997a). Studies of resistance in insects from field populations suggest that the common mode of resistance is characterised by a high level of resistance (over 500-fold), reduced toxin binding and a recessive mutation. However, there appear to be other modes of resistance which are not recessive and which are not associated with reduced toxin binding (Moar et al. 1995); (Tabashnik et al. 1997b).

349. (Gould et al. 1997) estimated the frequency of alleles for resistance in field populations of *H. virescens* as 1.5×10^{-3}. Genetic models indicate that a recessive allele present at this frequency could lead to rapid evolution of resistant populations if Bt toxin-producing cotton is grown without

adequate refuges for toxin-susceptible larvae (Roush 1994; Gould et al. 1997). A recessive allele that confers resistance to the Cry1Ac toxin in pink bollworm in Arizona cotton fields has been reported at frequencies roughly 100-fold higher than those reported by (Gould et al. 1997) for resistance to *H. virescens*. However, the frequency of this allele did not increase significantly between 1997 and 1999, even though the Bt cotton was grown in over half the 100 000 hectares planted to cotton. Moreover, the efficacy of the Bt cotton against the target pests remained extremely high (Tabashnik et al. 1997b).

350. Reversal of resistance in laboratory strains of diamondback moth derived from resistant field populations has been observed when exposure to Bt insecticide was discontinued over many generations. Reversal of resistance was associated with restoration of binding of Cry1Ac to brush-border membrane vesicles (Tabashnik et al. 1994c).

Section 2.2 Results from monitoring for resistant insects in Australia

351. Monitoring for resistance to Bt microbial sprays, containing the Cry1Ac toxin, in Australian field populations of *Helicoverpa armigera* and *H. punctigera* has been carried out by the NSW Department of Agriculture at the Australian Cotton Research Institute since 1993. Eggs are collected from the field and the larvae fed a diet containing the Cry1Ac at a level (the discriminatory dose) that is calibrated to kill most of a susceptible laboratory-reared population. Increased survival of field collected populations at the discriminatory dose would indicate increased resistance in the insect population.

352. No changes in susceptibility to discriminating doses of commercial microbial Bt sprays have been recorded in any of the Australian field populations of *H. armigera* and *H. punctigera* collected from cotton between 1993 and 2000 (Holloway & Dang 2000). These results indicate that there has been no shift towards insect resistance in INGARD® crops since the commercial release of INGARD® in 1996 and provides evidence of the efficacy of the resistance management plan currently in place for INGARD® cotton (see Section 2.3).

353. Since the commercial release of INGARD® cotton monitoring of field populations of *H. armigera* and *H. punctigera* for resistance to the Cry1Ac protein has also been conducted. Bioassays are used to measure the growth rate of larvae fed a diet containing a discriminating dose of the protein.

354. During the 2000/2001 season the NSW Department of Agriculture recorded an increase in resistance (from 7.1 to 9.2 %) of *H. armigera* larvae to Cry1Ac. However, this apparent increase coincided with the introduction of an additional bioassay method. An investigation into the significance of the results from the new method revealed the increase in resistance to be an artefact of the new testing method. In the new method the test larvae were at the third instar stage of development when exposed to the Cry1Ac protein, while in the standard testing method test larvae were exposed from the time of hatching. In the field, larvae are exposed from hatching, making the initial protocol more representative of the field situation. The data obtained using the standard bioassay protocols for 2000/2001 were almost a repeat of the results obtained in 1997/98, demonstrating that there has been no change in the resistance of field populations to the Cry1Ac protein.

355. No evidence of the existence of the single gene resistance mechanism (present in the recognised resistant colony at Canberra, CSIRO) was found upon observation of DNA from the field colony

thought to show increased tolerance, confirming that resistance has not developed (data provided by Monsanto).

Section 2.3 Insecticide resistance management plan

356. A condition of registration of the *cry1Ac* gene in INGARD® cotton by the APVMA is that growers are required to implement an Insecticide Resistance Management (IRM) plan that is developed by the TIMS Committee of the Australian Cotton Growers' Research Association and approved by the APVMA. The plan is designed to significantly slow the rate of development of insecticide resistance in *Helicoverpa spp*. to the Cry1Ac protein in INGARD® cotton. The central Queensland region follows a slight variation of the plan prepared for New South Wales and southern Queensland growing areas to reflect the difference in climate for this region. The plans require growers to employ a number of measures as part of their Technology Users Agreement with Monsanto. These include requirements to:

- limit the total area planted to the GM cotton;
- plant refuges of non-GM unsprayed cotton or other plants where the insect pests can breed freely (this provides a population of susceptible insects to dilute out resistance genes if these develop in the insect pests);
- plant by a certain date;
- control volunteer cotton;
- after harvest, to cultivate soil to prevent *Helicoverpa* pupae in the soil surviving to adulthood ('pupae-busting', see OGTR 2002).

357. Through the adoption of these measures, the IRM plan aims to slow the development of resistance by modifying the level of selection pressure being imposed on populations of the target species. The planting of refuges is a key feature of the IRM plan. Refuge crops, which are not sprayed with foliar Bt, ensure *Helicoverpa* that have not been exposed to Bt are present in populations to dilute any occurrence of resistance alleles. The capping of the area planted to INGARD®, at a maximum of 30% of the total planted area, also contributes to the dilution of resistance potential. The requirement to destroy pupae at the end of the season reduces carryover into the next season of *Helicoverpa* that have previously been exposed to insecticide. Synchronising of planting dates limits the number of generations of *Helicoverpa spp*. exposed to the Cry1Ac toxin in a season and thus limits the opportunity for resistance alleles to accumulate. Additionally the insecticide dose in INGARD® cotton is 'topped up' by the use of other insecticides that may be applied later in the season if the expression of the *cry1Ac* gene is insufficient to prevent threshold populations occurring. The reduced need for broad spectrum insecticides has led to increased occurrence of beneficial insects which also aid *Helicoverpa spp*. control, irrespective of whether the *Helicoverpa spp*. has survived exposure to the Cry1Ac toxin.

SECTION 3 CONCLUSIONS REGARDING INSECTICIDE RESISTANCE

358. Given the large-scale of the INGARD® cotton release, it is considered that the risk of insects developing resistance to the insecticidal protein is high. This is because:

- studies have shown that resistance to Bt toxins can be selected experimentally in insects by repeated exposure; and

- high levels of resistance to Cry1A toxins, including Cry1Ac, already occurs in field populations of the diamondback moth.

359. It should be noted that INGARD® cotton is being phased-out, associated with the introduction of Bollgard II® cotton, which is expected to significantly delay the development of insecticide resistance in target pests. Nonetheless, the licence for INGARD® cotton indicates the applicant's obligation to comply with the insecticide resistance management requirements of the APVMA.

APPENDIX 7 LICENCE CONDITIONS

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

Note in relation to Insecticide Resistance Management

The genetically modified organism 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.

PART 1

This instrument, including its attachments, is a licence authorising dealings involving the intentional release of GMOs into the environment. It is issued by the Gene Technology Regulator (the Regulator) pursuant to the *Gene Technology Act 2000* (Cth).

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 GMO 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 GMO subject to the limitations on dealing with the GMO 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 GMO are authorised during any period of suspension.

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

Material changes in circumstances

4. The licence holder must immediately notify the Regulator in writing of:

(d) any relevant conviction of the licence holder occurring after the commencement of this licence;

(e) 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;

(f) 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 GMO

7. The licence holder must not enter into a Technology User's Agreement or any other agreement which would permit the GMO to be grown *outside* of the shires of New South Wales and Queensland specified in the 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 bills 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 feral cotton populations in Queensland and determine their distance from cotton production locations. The survey locations must be based on, but need not be limited to, existing herbarium records of *Gossypium hirsutum* and *Gossypium barbadense*. The results of the survey must be reported to the Regulator in the first annual report to the Regulator prepared in relation to this licence.

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 11 and 12, 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 GMO, 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 GMO 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 GMO.

ATTACHMENT

to the Licence for dealings involving an intentional release of a GMO (INGARDÒ cotton event 531) into the environment

DIR 022/2002

<u>Shires of New South Wales and Queensland in which the licence holder may permit the GMO to be grown:</u>

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 INTENTIONAL RELEASES

SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

360. 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).

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

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

363. 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).

364. 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.

365. 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

366. Applications for DIR licence 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.

367. The application must also contain:

- additional information required for a GMO that is:
  a) a plant;
  b) a micro-organism (not living in or on animals and not a live vaccine);
  c) a micro-organism that lives in or on animals;
  d) a live vaccine for use in animals;
  e) a vertebrate animal;
  f) an aquatic organism;
  g) an invertebrate animal;
  h) to be used for biological control;
  i) to be used for bioremediation; and
  j) intended to be used as food for human or vertebrate animal consumption;

- supporting information from the Institutional Biosafety Committee.

SECTION 3 THE INITIAL CONSULTATION PROCESSES

368. 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.

369. 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 respect of the application inviting written submissions on whether the licence should be issued.

370. 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

371. 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.

372. 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.

373. 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.

374. 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:

  a) be harmful to other organisms;
  b) adversely affect any ecosystems;
  c) transfer genetic material to another organism;
  d) spread, or persist, in the environment;
  e) have, in comparison to related organisms, a selective advantage in the environment; and
  f) be toxic, allergenic or pathogenic to other organisms.

- the short and long term when taking these factors into account.

SECTION 5 FURTHER CONSULTATION

375. Having prepared a risk assessment and 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.

376. All issues relating to the protection of human health and safety and the environment raised in written submissions on an application or risk assessment and 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.

377. 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 final 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 risk assessment and risk management plan.

378. 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

379. 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.

380. If a licence is issued, the Regulator may impose licence conditions (Section 62 of the Act). 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;
- limiting the dissemination or persistence of the GMO or its genetic material in the environment; and
- require contingency planning in respect of unintended effects of the dealings.

381. 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.

APPENDIX 9 SUMMARY OF PUBLIC SUBMISSIONS

ON THE APPLICATION AND
ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

Submission from:

A: agricultural organisation; **I**: individual; **E**: environmental organisation; **C**: consumer/public interest organisation .

Issues raised: **APVMA**: issues dealt with by APVMA; **C**: contamination; **D**: insufficient data/evidence; **FC**: food chain; **FSANZ**: food safety and labelling; **G**: gene transfer; **IR**: insecticide resistance; **L**: labeling; **LC**: licence conditions; **MA**: markets; **MO**: monitoring; **RA**: risk assessment; **RM**: risk management; **SEG**: segregation; **T**: toxicity; **W**: weediness.

OSA: outside scope of the assessment.

Sub. No:	Type	Summary of issues raised	Issue	Consideration of issue
1&2	A	• …the uptake of genetically modified organism (GMOs) and their use in the food chain must be accountable, with appropriate labeling of all GMO product and accurate identification to ensure product integrity and consumer confidence.	L, FC	OSA
		• …the long-term introduction of genetically modified cotton into the food chain holds strong implications for the pork industry. The Australian pork industry currently has a competitive advantage over other countries through avoidance of GM feeds in the industry. There are risks through partial or complete rejection of animals fed GM products…	MA	OSA
		• It is … imperative that tracking, tracing and 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.	C, MA	OSA
2	I	• I object to release of G.E. organisms because I do not believe they can be “managed”.	RM	Noted
		• I do not believe that they can be segregated indefinitely from the naturally evolved environment.	SEG	OSA
		• I do not believe that all risks can be identified.	RA	Noted
4	I	• ..I realise that the [Insecticide] Resistance Management Plans are … under the authority of the NRA, I am concerned that the success of these plans is heavily reliant on a consistent, high dose expression of the toxin.	IR, APVMA	Appendix 6, Section 2.3
		• I realise that Bollgard has been introduced as an attempt to address resistance problems but I see no reason to believe that environmental conditions will not also similarly affect the expression of toxins in Bollgard cotton.	IR, APVMA	Noted

		• It seems as though the specificity of the toxin as expressed by the plant is largely assumed rather than experimentally proven. Bt toxins in spore formulations and in genetically modified plants differ significantly….The organisms that are exposed to this toxin , as well as the way that they are exposed, also therefore changes. These differences prohibit a simple deduction of safety of these plants for non-target organisms from the past record of Bt-insecticide use or from studies using either spore formulations or purified forms of the toxin.	T, D	Appendix 1, Section 4 Appendix 2 Section 2.4 Appendix 3 Sections 2.4, 2.5, 2.6
		• …RARMP… did not consider the question through the context of existing food chains….Bt cotton plants should be carefully tested as a new agent in contexts that reflect ecological realities….	FC, T	Noted
		• …collection of additional data on the impacts on non-target organisms in ecologically realistic, multitrophic contexts should be a condition of licence approval.	FC, T	Noted
		• I find studies cited in answer to the question of secondary ecological in part 20.9 equally inadequate…Additional data collection on secondary effects and the impact of these plants on non-target organisms should be included as a monitoring requirement.	FC, T	Noted
		• I am concerned about the heavy reliance on a single study (Eastick 2002) to provide important information relating to the potential weediness of INGARD® cotton in non-cropping environments. The existence of site-specific impacts on cotton's key life stages indicates to me that the heavy reliance on this single study, which was performed in very different conditions to those where the majority of INGARD® would be grown, is a cause for concern.	W	Appendix 4
5	A	• It seems surprising then that clause 249 states: "Note however, that the distribution of feral cotton in Queensland is not well understood…." This implies that while INGARD plants do not pose a weediness risk, that seeds produced by feral *G. hirsutum* plants which have received the INGARD gene and at least 50% of their genes from an INGARD pollen donor, do pose a weediness risk. This is a difficult proposal to fathom.	W, G	Appendix 5
		• While the proposal to survey all herbarium documented feral populations in the release area of Queensland is laudable, the requirement to "investigate whether these populations are spreading" is highly impractical. In order to establish whether a feral population was or was not spreading would require detailed demographic studies over a minimum of two years. It is highly likely that the size of feral populations fluctuates widely from year to year in any case. Overall I find the suggestion for such studies is excessive given that INGARD varieties will be grown commercially for only one, or possibly two more seasons.	W, G	Appendix 5

		• Clause 234 says that :preliminary experimental data suggest that INGARD cotton may be more weedy than non-GM cotton in certain nutrient-rich habitats such as stock feeding areas." Eastick's data showed that cotton was more invasive when in a niche such as an irrigation channel or cattleyard – there was no difference in invasiveness between conventional and Bt cotton. Management of volunteers in these niches could be a condition easy to impose.	W	Appendix 4, 7
		• Given the conclusions in Eastick (2002), the specific conditions imposed for transport of seed and use as stockfeed seems excessive….the requirement for 25 days of monitoring seems impractical….monitoring the cattle themselves or the other environments they enter after feeding would not seem necessary given the slight risk of seed survival through livestock…A more workable condition may be to require seed transported north for stockfeed to be crushed at the gin (preferable) or at the point of feeding.	W, MO, LC	Appendix 7
6	AO	• …draw attention to the apparent inconsistencies between statements made in the RARMP. INGARD® cotton seed has been used as a stock feed in northern Australia since the commercial release of INGARD® cotton in 1996, with no indication that [it] has become a problematic weed… Despite this, in a table summary…it is stated that the spread and persistence in northern Australia requires management, as risk of weediness is relatively high. The RARMP goes on to say the risk of INGARD® cotton establishing as a weed…is yet to be determined conclusively…	W	Appendix 4
		• After …discussions with scientists and organisations with expertise in these issues… the conclusion appears to be that the risk of weediness of INGARD® …in northern Australia is low. …Eastick determines that 'there is no indication that the risk of establishment and growth of Bt cotton is greater than that of conventional cotton."	W	Appendix 4
		• It is critical that any licence conditions imposed are commensurate with the level of risk identified and that they can be complied with.	LC	Appendix 7

APPENDIX 10 REFERENCES

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