

Risk Assessment and Risk Management Plan

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

DIR 035/2003

Title: Field trials of herbicide tolerant
(Roundup Ready[®] MON 88913) and
herbicide tolerant/insect resistant
(Roundup Ready[®] MON 88913/Bollgard[®] II) cotton

Applicant: Monsanto

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Office of the
Gene Technology Regulator

Abbreviations

ANZFA	Australia New Zealand Food Authority (now FSANZ)
AQIS	Australian Quarantine Inspection Service
Bt	<i>Bacillus thuringiensis</i>
<i>B.t.k</i>	<i>Bacillus thuringiensis</i> variety <i>kurstaki</i>
CaMV	cauliflower mosaic virus
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
kDa	kiloDalton
km	kilometre
m	metre
MAFF	UK Ministry of Agriculture, Fisheries and Food
MRL	maximum residue limit
mRNA	messenger ribonucleic acid
ng	nanogram
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOS	nopaline synthase
NLRD	Notifiable Low Risk Dealing
NRA	National Registration Authority for Agricultural and Veterinary Chemicals
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator
ppm	parts per million
PCR	polymerase chain reaction
T-DNA	transfer deoxyribonucleic acid
TGA	Therapeutic Goods Administrations
TGAC	Technical Grade Active Constituent
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 and the environment that can not be managed. To this end, Section 51 of the Act requires the Regulator to prepare a risk assessment and risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and stakeholders.

Under Section 52 of the Act, the Regulator is required to seek comment on the RARMP from those consulted in its preparation and to invite submissions from the public. Matters raised relating to the protection of human health and safety or the environment are taken into account in finalising the RARMP, which then forms the basis of the Regulator's decision on whether, or not, to issue a licence.

THE APPLICATION

Monsanto Australia Ltd (Monsanto) has applied for a licence (application number DIR 035/2003) for the limited and controlled release of genetically modified (GM) herbicide tolerant cotton (Roundup Ready[®] MON 88913) and herbicide tolerant/insecticidal cotton (Roundup Ready[®] MON 88913/Bollgard[®] II) into the environment. Monsanto proposes to conduct four trials (two in the southern summer growing season and two in the northern winter growing season) on 50 sites covering a total of 954 hectares, over three years (2003 – 2005), in the traditional cotton growing regions of New South Wales and southern Queensland and in northern Queensland, the Northern Territory and northern Western Australia.

Roundup Ready[®] MON 88913 cotton contains two copies of a gene (*cp4 epsps*), derived from a common soil bacterium, that confers tolerance to glyphosate (the active ingredient in the herbicides, Roundup[®] and Roundup Ready[®]). Conventional cotton is susceptible to glyphosate damage. The use of Roundup Ready[®] MON 88913 GM cotton thus allows the application of the herbicide Roundup[®] for the control of weeds that emerge in the crop, without damaging the crop itself.

Roundup Ready[®] MON 88913/Bollgard[®] II cotton was produced by conventional breeding of Roundup Ready[®] MON 88913 cotton with GM Bollgard[®] II cotton, which produces two insecticidal proteins, making it resistant to the major caterpillar pests of cotton.

Roundup Ready[®] MON 88913 cotton differs from the previous commercially released Roundup Ready[®] cotton in that tolerance to Roundup[®] herbicide is prolonged. Yield loss occurs if Roundup[®] is applied beyond the four-leaf growth stage of "first generation" Roundup Ready[®] cotton. Roundup Ready[®] MON 88913 cottons are able to tolerate application of Roundup[®] at later stages of plant growth without yield loss, allowing a wider window to apply herbicide during growth of the cotton crop. This is intended to give growers increased flexibility in the timing of herbicide application and not expected to increase the overall level of herbicide use.

The applicant's stated aims for the field trials are: to transfer the Roundup Ready[®] MON 88913 GM event into elite cotton varieties suitable for Australia and evaluate their performance under Australian

conditions; to combine the Roundup Ready[®] MON 88913 and Bollgard[®] II traits; to evaluate and gather data on levels of CP4 EPSPS protein expression, tolerance to glyphosate, seed composition, weed control effectiveness and glyphosate residue levels; and to produce seed for future large scale or commercial releases, which would require separate approvals.

None of the cotton plants from the release, or their by-products, will be used for animal feed or human food. However, the applicant proposes to sell lint from the release. Lint does not contain genetic material or protein. Transport of the GM material will be in accordance with the transport guidelines issued by the Regulator.

Details of the gene construct, including the plasmid map, some of the regulatory sequences and preliminary protein expression data have been declared as Confidential Commercial Information (CCI) under section 185 of the Act. However, the CCI was made available to the various prescribed expert groups that were consulted on the preparation of the risk assessment and risk management plan.

Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton have not previously been approved for limited and controlled release in Australia. First generation Roundup Ready[®] cotton (containing one copy of the *cp4 epsps* herbicide tolerance gene) and Roundup Ready[®]/INGARD[®] cotton (containing a single copy of both the herbicide tolerance and an insecticidal gene) have been approved for commercial release in cotton growing regions south of latitude 22° South since 2000 (refer licence number DIR023/2002).

Bollgard[®] II and Bollgard[®] II/Roundup Ready[®] cottons were approved for commercial release south of latitude 22° South on 23 September 2002 (licence number DIR 012/2002). The release of these GM cottons in northern Australia is only permitted in field trials under limited and controlled conditions due to ongoing concerns regarding their potential weediness in the prevailing moist, warm conditions.

THE EVALUATION PROCESS

Licence application DIR 035/2003 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 Australian Government stakeholders and the Gene Technology Technical Advisory Committee, and is available at www.ogtr.gov.au/pdf/public/raffinal.pdf.

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

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has a complimentary regulatory role in respect of this application due to its responsibility for agricultural chemical use, including insecticides and herbicides in Australia under the *Agricultural and Veterinary Chemicals (Code) Act 1994*. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits allowing restricted use of an insecticide or herbicide, for example for a limited period of time or for a limited area. In considering applications for registration or permits, the APVMA also considers a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as the efficacy of herbicides and insecticides, and herbicide and insect resistance management. The APVMA can impose conditions on the use of herbicides or insecticides in

registrations and permits. The APVMA and the OGTR work closely together to ensure thorough coordinated assessments are undertaken and, wherever possible, that timing of assessments and decisions by both agencies coincide. Further information about how the APVMA's assessment and approval processes can be obtained from their website <http://www.apvma.gov.au/>.

Roundup Ready[®] herbicide (a formulation of glyphosate) is not currently registered for use on cotton beyond the four-leaf stage of growth. A research permit from the APVMA for use of Roundup[®] herbicide after this stage on Roundup Ready[®] MON 88913 cotton is not required for 2003/2004 summer season, i.e. first season, of the proposed field trial as less than one hectare of cotton plants per state is proposed to be sprayed with herbicide. However, a research permit from the APVMA will be required for such use of Roundup[®] herbicide in the subsequent three seasons of the proposed trial or for any further larger scale release. Future releases of Roundup Ready[®] MON 88913 cotton will also require further applications and approvals by the Regulator.

In July 2003, the APVMA registered Bollgard[®] II genes (*cry1Ac* and *cry2Ab*) as insecticidal products in GM cotton. As part of the registration, the APVMA has imposed conditions on the use of Bollgard[®] II cotton, including an insect resistance management plan. A condition within this resistance management plan specifies that the amount of combined INGARD[®] and Bollgard[®] II cotton to be grown by one grower (as defined by the growers trading name) in a valley must not exceed 40% of the total cotton being grown by that grower in that valley. A grower may choose to plant up to 40% Bollgard[®] II and no INGARD[®]. These trials will comply with all conditions imposed by the APVMA.

The risk assessment considered information relevant to the evaluation of potential impacts on human health and safety and the environment contained in the application (including information required by Act and the Regulations on the GMO, the parent organism, the proposed dealings and containment measures), submissions received during consultation with expert groups and authorities, and current scientific knowledge.

Through this process, potential hazards to human health and safety or the environment that may be posed by the proposed release of Roundup Ready[®] MON 88913 cotton and Roundup Ready[®] MON 88913/Bollgard[®] II cotton were identified. These have been evaluated on the basis of the likelihood of each hazard occurring and the likely impact of the hazard, were it to be realised. The identified potential hazards relate to:

- toxicity and allergenicity for humans: could Roundup Ready[®] MON 88913 cotton or Roundup Ready[®] MON 88913/Bollgard[®] II cotton be more toxic or allergenic than non-GM cotton, as a result of the novel gene products or because of unintended effects?
- toxicity for non-target organisms: could Roundup Ready[®] MON 88913 cotton or Roundup Ready[®] MON 88913/Bollgard[®] II cotton be harmful to non-target organisms as a result of the novel gene products or because of unintended effects?
- weediness: could the genetic modifications be harmful to the environment by increasing the potential for the Roundup Ready[®] MON 88913 cotton or Roundup Ready[®] MON 88913/Bollgard[®] II cotton to establish as problem weeds?

- transfer of introduced genes to other organisms: could there be adverse consequences from potential transfer of the introduced genes to non-GM cotton crops, feral or native cottons, or to other organisms? and
- herbicide and insecticide resistance: could weeds develop resistance to glyphosate if the Roundup Ready[®] crop-herbicide combination is used inappropriately and could target insects develop resistance to the insecticidal proteins produced by the introduced insecticidal genes in Roundup Ready[®] MON 88913/Bollgard[®] II cotton?

CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator considers that the limited and controlled release of Roundup Ready[®] MON 88913 cotton, or Roundup Ready[®] MON 88913/Bollgard[®] II cotton, will not pose any significant risk to public health and safety, or to the Australian environment, that cannot be managed. The assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans

Roundup Ready[®] MON 88913 cotton and Roundup Ready[®] MON 88913/Bollgard[®] II cotton are unlikely to prove more toxic or allergenic to humans via occupational exposure than conventional cotton. The introduced proteins are the same as those expressed by existing commercially released GM cottons (Roundup Ready[®], Roundup Ready[®]/Bollgard[®] II and Bollgard[®] II cottons). Even though the levels of expression may be different, none of the introduced proteins have any known toxicity or allergenicity and the proteins are naturally widespread in the environment. However, Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment, and FSANZ approval will be needed before products of these GM cottons could be used in human food. FSANZ has previously approved the food use of oil and linters derived from Roundup Ready[®] and Bollgard[®] II cotton for use in human food. Cottonseed from the releases will not be used for human food or animal feed. However, lint from the release will be sold commercially for use in fabric, upholstery and other non-food products, as lint contains no DNA or protein and is not used in food.

Toxicity to non-target organisms

Roundup Ready[®] MON 88913 cotton and Roundup Ready[®] MON 88913/Bollgard[®] II cotton are unlikely to prove more toxic to non-target organisms than conventional cotton. The introduced proteins are the same as those expressed by existing commercially released GM cottons, are naturally widespread in the environment and have no known toxicity to mammals, birds, fish, non-target invertebrates and soil microorganisms. Exposure of non-target organisms to the introduced proteins will be low and cottonseed from the release will not be used for stockfeed.

Weediness

The risk of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton establishing as problematic weeds in the southern cotton growing areas of Australia is low and not likely to be greater than that of non-GM cotton. This is because the germination and persistence of both GM and non-GM cottons in southern Australia are likely to be limited by the availability of adequate soil moisture, nutrients, plant competition and/or frosts. It is highly unlikely that the genetic modifications

will affect the response of the GM cottons to these variables and, thereby, alter the weediness of the GM cottons.

Limited experimental data suggests that insecticidal GM cotton may have the potential to be weedier than non-GM cotton in certain habitats in northern Australia. Therefore, the licence conditions imposed include the same containment measures as DIR 012/2002, in order to minimise this risk.

Roundup Ready® MON 88913 and Roundup Ready® MON 88913/Bollgard® II cottons express the same introduced proteins as commercially released Roundup Ready® cotton, although the site of insertion and the level of expression of the introduced genes may differ. There is, therefore, a possibility that potential pleiotropic effects resulting from the genetic modifications could alter some aspect of the GM cottons' biology that may affect their potential weediness. Licence conditions have been imposed to minimise the persistence and spread of introduced genes from the release sites (refer to key licence conditions below).

In greenhouse and field trials of Roundup Ready® MON 88913 cotton conducted in the United States, no unintended or secondary effects on the GM cotton plants, including on their fertility, have been observed. As part of the field trial, the applicant proposes to evaluate agronomic characteristics of the MON 88913 cottons under Australian conditions. More data on potential pleiotropic effects of the genetic modifications on the GM cottons and how these may affect potential weediness will be required before any future application for a large scale or commercial release of these GMOs could be assessed. These will require separate applications and approval processes.

Transfer of introduced genes to other organisms

Some gene transfer from the GM cottons to other cultivated cottons would be likely under uncontrolled conditions, even though the overall frequency of out-crossing would be very low. It is highly unlikely, however, that the inserted genes would alter the likelihood or frequency of such gene transfer. Transfer of introduced genes to other cultivated cotton would pose the same risks as the low risks posed by Roundup Ready® MON 88913 cotton and Roundup Ready® MON 88913/Bollgard® II cotton themselves. However, licence conditions have been imposed to minimise the transfer of introduced genes to other cotton crops (refer to key licence conditions below).

Transfer of genes to feral/naturalised cotton is unlikely due to geographic isolation and the risk of transfer of the introduced genes to native cotton is negligible, because of genetic incompatibility. Similarly, the likelihood of transfer of the introduced genes to other organisms is negligible because of even greater genetic incompatibility. Even if such transfer occurred it would be unlikely to pose any hazard to human health and safety and the environment.

As part of the OGTR's ongoing commitment to the review of data, specific research conditions have been imposed in the licence. The research is intended to confirm research on gene flow undertaken prior to the implementation of the Act and prior to the commercial release of GM cottons, and in so doing validate the containment measures imposed during the field trials of GM cottons. In order to facilitate this review and data collection, the Regulator has imposed a licence condition to establish 400 metre research zones around proposed field trial sites (locations) in excess of 1 hectare. This research program will be developed in consultation with the OGTR.

Insecticide and herbicide resistance

These risks will be managed by the APVMA through conditions on the existing registration for the use of the insecticidal genes as an insecticide in the Bollgard® II cotton (refer to page III) and any permit or registration for the use of Roundup® herbicide on Roundup Ready® MON 88913 and Roundup Ready® MON 88913/Bollgard® II cottons beyond the four leaf stage.

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 identified (refer to Conclusion of the risk assessment, above). This plan is given effect by licence conditions. The key licence conditions are outlined below.

Toxicity or allergenicity to humans

Licence conditions have been imposed which require the applicant to:

- prevent entry of the GMOs and products derived from the GMOs into the human food supply;
- destroy all seed not required for future trials;
- securely transport and store the GMOs; and
- limit the size of field trial sites to a maximum of 50 hectares each.

Toxicity to non-target organisms

Licence conditions have been imposed which require the applicant to:

- prevent cottonseed being used as stockfeed;
- destroy all seed not required for future trials;
- securely transport and store the GMOs; and
- limit the size of field trial sites to a maximum of 50 hectares each.

Weediness

Licence conditions have been imposed which require the applicant to:

- surround the GM cotton by a 20 m pollen trap of non-GM cotton at sites north of 22°S;
- surround the GM cotton by a 20 m pollen trap of non-GM cotton, or GM Roundup Ready® cotton approved for commercial release in Australia at sites south of 22°S;
- surround trial sites in excess of one ha with a 400 m wide research zone for the purpose of conducting research for the ongoing review of the data on spread and persistence of the GMOs and to validate previous research on containment measures;

- securely transport and store the GMOs;
- prevent cottonseed being used as stockfeed;
- clean equipment used at release sites;
- monitor field trial sites after harvest and destroy volunteers; and
- limit the scale of the release (size of individual sites equal to, or less than 50 ha).

Transfer of introduced genes to other organisms

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release (size of individual sites equal to, or less than 50 ha);
- surround the GM cotton by a 20 m pollen trap of non-GM cotton at sites north of 22°S;
- surround the GM cotton by a 20 m pollen trap of non-GM cotton, or GM cottons approved for commercial release in Australia at sites south of 22°S;
- surround trial sites in excess of one ha with a 400 m wide research zone for the purpose of conducting research for the ongoing review of the data on spread and persistence of the GMOs and to validate previous research on containment measures;
- securely transport and store the GMOs;
- clean equipment used at release sites; and
- monitor field trial sites after harvest and destroy volunteers.

Insecticide and herbicide resistance

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

General conditions

The licence issued by the Regulator also contains a number of general conditions, which are also relevant to risk management. These include, for example,

- identification of the persons or classes of person covered by the licence;
- a requirement that the applicant allow access to the release sites by the Regulator, or persons authorised by the Regulator, for the purposes of monitoring or auditing; and
- a requirement to inform the regulator if the applicant becomes aware of any additional information about risks to human health or safety or to the environment.

Monitoring and enforcement of compliance by the OGTR

As well as the legislative capacity to enforce compliance with licence conditions, the Regulator has additional options for risk management. The Regulator can direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment. The OGTR also independently monitors releases that the Regulator has authorised. At least 20% of all field trial sites will be inspected each year, in accordance with a monitoring and compliance strategy based on risk profiling, to determine whether licence holders are complying with the licence conditions, or whether there are any unintended effects.

FURTHER INFORMATION

Detailed information on the evaluation of the application, including the proposed licence conditions, is available in the risk assessment and risk management plan document for this application, which can be obtained from the website of the Office of the Gene Technology Regulator (www.ogtr.gov.au), or by calling 1800 181 030 (please quote application number DIR 035/2003).

CHAPTER 1 BACKGROUND

1. This chapter provides background information about the application and previous releases of relevant genetically modified organisms (GMOs) into the environment.
2. The OGTR has received an application (licence application number DIR 035/2002) from Monsanto Australia Limited (Monsanto) for the intentional release of genetically modified (GM) herbicide tolerant (Roundup Ready[®] MON 88913) and herbicide tolerant/insecticidal (Roundup Ready[®] MON 88913/Bollgard[®] II) cotton into the environment, on a limited scale and under controlled conditions. Key information on the application is given below:

SECTION 1 THE APPLICATION

Project Title:	Field trials of herbicide tolerant (Roundup Ready[®] MON 88913) and herbicide tolerant/insect resistant (Roundup Ready[®] MON 88913/Bollgard[®] II) cotton		
Applicant:	Monsanto Australia Ltd PO Box 6051 Melbourne, VIC 8008		
Common name of the parent organism:	Cotton		
Scientific name of the parent organism:	<i>Gossypium hirsutum</i> L.		
Modified trait(s):	Prolonged herbicide tolerance, insecticidal action, antibiotic resistance, reporter gene expression		
Identity of the gene(s) responsible for the modified trait(s):	<ul style="list-style-type: none"> • <i>cp4 epsps</i> gene from <i>Agrobacterium</i> sp. strain CP4 (herbicide tolerance) • <i>cryIAc</i> and <i>cry2Ab</i> genes from the bacterium <i>Bacillus thuringiensis</i> (insecticidal) • <i>nptII</i> gene from bacterial Tn5 transposon (antibiotic resistance) • <i>uidA</i> gene from <i>Escherichia coli</i> (reporter gene) 		
Proposed Release Location	New South Wales (NSW), Queensland (Qld), Northern Western Australia (WA), Northern Territory (NT) (see Appendix 7 for possible release shires)		
Proposed Release Sizes and Dates:	Dates	Number of sites	Maximum area (hectares)
	Summer 2003/04	6	7.8
	Winter 2004	4	30
	Summer 2004/5	32	885.8
	Winter 2005	4	30
	Total	46	953.6

3. In accordance with Section 185 of the Act, Monsanto has received approval for details of the gene construct, including the plasmid map, some of the regulatory sequences (promoters and terminators) and preliminary protein expression data as Confidential Commercial Information (CCI). However the CCI was made available to the prescribed expert groups that were consulted on the preparation of the risk assessment and risk management plan.
4. Roundup Ready[®] MON 88913 cotton contains two copies of a gene (*cp4 epsps*), derived from a common soil bacterium, that confers tolerance to glyphosate (the active ingredient in the herbicides, Roundup[®] and Roundup Ready[®]). The *cp4 epsps* gene encodes an enzyme (CP4 EPSPS) that is tolerant to glyphosate. Conventional cotton is susceptible to glyphosate damage because the equivalent plant enzyme is inhibited by the herbicide. The use of Roundup Ready[®] MON 88913 GM cotton thus allows the application of the herbicide Roundup[®] for the control of weeds that emerge in the crop, without damaging the crop itself.
5. Roundup Ready[®] MON 88913/Bollgard[®] II cotton was produced by conventional breeding of Roundup Ready[®] MON 88913 cotton with GM Bollgard[®] II cotton, which expresses two insecticidal proteins, making it resistant to the major caterpillar pests of cotton.
6. Roundup Ready[®] MON 88913 cotton differs from the previous commercially released Roundup Ready[®] cotton in that tolerance to Roundup[®] is prolonged. Yield loss occurs if glyphosate is applied beyond the four-leaf growth stage of “first generation” Roundup Ready[®] cotton. Roundup Ready[®] MON 88913 cottons are able to tolerate application of glyphosate at later stages of plant growth without yield loss, allowing a wider window to apply herbicide for weed control during growth of the cotton crop. This is intended to give growers increased flexibility in the timing of herbicide application and not expected to increase the overall level of herbicide use.

Section 1.1 The proposed dealings

7. Monsanto seeks approval for the limited and controlled release of herbicide tolerant Roundup Ready[®] MON 88913 and herbicide tolerant/insect resistant Roundup Ready[®] MON 88913/Bollgard[®] II cotton into the environment.
8. Monsanto proposes to conduct four trials (two in the southern summer growing season and two in the northern winter growing season) on 50 sites covering a total of 954 hectares, over three years (2003 - 2005) in the cotton growing regions of New South Wales and Queensland and in northern Western Australia and the Northern Territory.
9. The aims of the proposed field trials are: to transfer the MON 88913 GM event into elite cotton varieties suitable for use under Australian conditions; to combine the Roundup Ready[®] MON 88913 and Bollgard[®] II traits; to evaluate and gather data on levels of CP4 EPSPS protein expression, tolerance to glyphosate, seed composition, weed control effectiveness and glyphosate residue levels; and to produce seed for future large scale or commercial releases, which will require separate approvals.
10. None of the cotton plants from the release, or their by-products, will be used for animal feed or human food. However, the applicant proposes to sell lint from the release. Lint does not contain genetic material or protein.

Section 1.2 Parent organism

11. 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 WA and the NT. 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 the risk assessment processes for licence applications involving GM cottons. This document is available from the OGTR or at www.ogtr.gov.au.

Section 1.3 Genetic modification and its effect

12. Roundup Ready[®] MON 88913 cotton contains two copies of the *epsps* gene from *Agrobacterium* species strain CP4. The *cp4 epsps* gene encodes an enzyme (CP4 EPSPS) that is tolerant to inhibition by glyphosate (Padgett et al. 1993), the active constituent of Roundup[®]/Roundup Ready[®] herbicide, and has been introduced into the cotton plants to confer tolerance to the foliar application of glyphosate.

13. In plants the native *epsps* (5-enolpyruvylshikimate-3-phosphate synthase) gene encodes an enzyme (EPSPS) critical for the biosynthesis of aromatic amino acids (tryptophan, tyrosine and phenylalanine), essential building blocks for cellular proteins. The herbicide Roundup[®]/Roundup Ready[®] functions by inhibiting the activity of the naturally occurring EPSPS enzyme in plants, thus blocking the biosynthesis of aromatic amino acids and eventually leading to cell death (Steinrucken & Amrhein 1980). The *cp4 epsps* gene from *Agrobacterium* is insensitive to the effects of glyphosate (Padgett et al. 1993) and the CP4 EPSPS enzyme is still able to function normally in the presence of glyphosate to synthesize aromatic amino acids. Consequently, in GM plants containing the *Agrobacterium cp4 epsps* gene, biosynthesis of aromatic amino acids is not blocked by the presence of glyphosate and the plants are not killed by Roundup[®]/Roundup Ready[®] when applied as a herbicide.

14. Roundup Ready[®] MON 88913 cotton differs from the currently commercially released Roundup Ready[®] cotton, which contains only one copy of the *cp4 epsps* gene, in that tolerance to glyphosate is prolonged. The applicant has indicated that Roundup[®] can be applied to control weeds during plant growth of up to 14 nodes (note that the earliest development of first fruit can occur at about 16 nodes or 165 days after planting). By contrast, in a Roundup Ready[®] cotton crop if Roundup[®] was not applied for weed control, for instance due to rainfall, by 4 nodes of plant growth or 35 days after planting, it cannot be applied at later stages of plant growth. Hence Roundup Ready[®] MON 88913 cotton is intended to give growers increased flexibility in timing of herbicide application for controlling weeds.

15. Roundup Ready[®] MON 88913/Bollgard[®] II cotton plants will be produced during the field trials through the conventional breeding of Roundup Ready[®] MON 88913 cotton and Bollgard[®] II cotton. Roundup Ready[®] MON 88913/ Bollgard[®] II cotton plants will contain all of the genes introduced into each of the parental GM varieties.

16. Bollgard[®] II cotton contains two insecticidal genes, *cryIAc* and *cry2Ab*, both derived from a common soil bacterium, *Bacillus thuringiensis* (Bt). These genes encode proteins that are toxic to lepidopteran caterpillars, including the two key *Helicoverpa* pests of cotton.

17. Bollgard[®] II cotton plants also contain bacterial antibiotic resistance genes (*nptII* gene, conferring resistance to kanamycin and neomycin; and *aad* gene, conferring resistance to streptomycin and spectinomycin) and a reporter gene (*uidA* gene, which enables visualisation of plant tissues in which this gene is being expressed). The *aad* gene is not expressed in the plants because the promoter that is required for its expression is not present in plants. This gene was used as a marker in the laboratory prior to the production of the genetically modified plants to select for bacteria containing the modified DNA.

18. Short regulatory sequences that control expression of the genes are also present in the genetically modified cottons. These sequences are derived from the cauliflower mosaic virus, figwort mosaic virus, *Agrobacterium tumefaciens* and from soybean and two other plant species. Although three of these organisms are plant pathogens, the regulatory sequences comprise only a small part of their total genome and are not in themselves capable of causing disease.

19. Further details on the introduced genes and their encoded proteins are provided in Appendix 1, Section 3.

Section 1.4 Method of gene transfer

20. Roundup Ready MON 88913[®] cotton was generated by introducing the two copies of the *cp4 epsps* gene into cotton on a plasmid vector carried by *Agrobacterium tumefaciens*. The vector is ‘disarmed’ since it lacks the genes that encode the tumour-inducing functions of *A. tumefaciens* (See Appendix 1, Section 4 for details).

21. Bollgard[®] II cotton was produced by introducing the *cry2Ab* and *uidA* genes into GM INGARD[®] cotton (containing the *cryIAc*, *nptII* and *aad* genes) using microprojectile bombardment. This technique involves coating the DNA containing the genes onto very small tungsten or gold particles which are ‘shot’ into the cotton tissue. INGARD[®] cotton was produced using a disarmed plasmid vector carried by *A. tumefaciens*.

22. Roundup Ready[®] MON 88913/ Bollgard[®] II cotton will be produced by conventional crossing of Roundup Ready MON 88913[®] cotton and Bollgard[®] II cotton.

SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

Section 2.1 Previous Australian Releases of similar GM cottons

23. Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/ Bollgard[®] II cotton have not been previously approved for release in Australia. However, first generation Roundup Ready[®] cotton, which contains one copy of the same *cp4 epsps* gene present in the MON 88913 cottons, has been approved for commercial release since 2000 (refer licence DIR 023/2002). Bollgard[®] II cotton and Bollgard[®] II/Roundup Ready[®] cotton were approved for commercial release in September 2002 (refer to licence number DIR 012/2002).

24. These commercial releases were 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 cotton in these areas. Therefore ongoing field trials, of Bollgard[®] II cotton and Bollgard[®] II/Roundup Ready[®] cotton, north of 22° South are required to be conducted under limited and controlled conditions.

25. Prior to commercial release, numerous limited and controlled releases of Roundup Ready[®] cotton were conducted under the voluntary system overseen by GMAC, and three licences for limited and controlled releases of Bollgard[®] II and/or Roundup Ready[®]/Bollgard[®] II cotton were issued under the current regulatory system:

- Roundup Ready[®] cotton - 23 limited and controlled releases undertaken by:
 - Deltapine Australia Pty Ltd (PR-32, PR-52, PR-52X, PR-52X2, PR-52X3, PR-71, PR-71X, PR-71X2, PR-83, PR-83X, PR-83X3, PR-140, PR-140X and PR-143);
 - CSIRO Division of Plant Industry (PR-55, PR-55X, PR-55X2, PR-55X3 and PR-55X5 and PR-55X6);
 - Cotton Seed Distributors Pty Ltd (PR 55-X4); and
 - Monsanto (PR-83X2 and PR-83X4).

- Bollgard[®] II and Roundup Ready[®]/Bollgard[®] II cotton - three limited and controlled release:
 - Cotton Seed Distributors, in Queensland (Licence No. DIR 005/2001);
 - CSIRO, in Western Australia and Northern Territory (Licence No. DIR 006/2001);
 - Department of Agriculture (WA) in Western Australia (Licence No. DIR 009/2002, Bollgard[®] II cotton only).

26. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

Section 2.2 Approvals by other Australian government agencies

27. The OGTR is responsible for assessing the biosafety risks to human health and the environment associated with development and use of GMOs. Other government regulatory requirements must also be met in respect of the release of the GMOs, and the use of products of the GMOs, including the requirements of the Australian Pesticides and Veterinary Medicines Authority (APVMA) and Food Standard Australia New Zealand (FSANZ).

2.2.1 Australian Pesticides and Veterinary Medicines Authority

28. The APVMA has a complimentary regulatory role in respect of this application due to its responsibility for agricultural chemical use, including insecticides and herbicides in Australia under the *Agricultural and Veterinary Chemicals (Code) Act 1994*. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits allowing restricted use of an insecticide or herbicide, for example for a limited period of time or for a limited area. In considering applications for registration or permits, the APVMA also considers a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as the efficacy of herbicides and insecticides, and herbicide and insect resistance management. The APVMA can impose conditions on the use of herbicides or insecticides in registrations and permits.

29. Roundup Ready[®] herbicide (a formulation of glyphosate) is not currently registered for use on cotton beyond the four-leaf stage of growth. A research permit from the APVMA for use of Roundup[®] herbicide after this stage on Roundup Ready[®] MON 88913 cotton is not required for 2003/2004 summer season, i.e. first season, of the proposed field trial as less than one hectare of cotton plants per state is proposed to be sprayed with herbicide. However, a research permit from the APVMA will be required for such use of Roundup[®] herbicide in the subsequent three seasons of the

proposed trial or for any further larger scale release. Future releases of Roundup Ready[®] MON 88913 cotton will also require further applications and approvals by the Regulator.

30. In July 2003, the APVMA registered Bollgard[®] II genes (*cryIAc* and *cry2Ab*) as insecticidal products in GM cotton. As part of the registration, the APVMA has imposed conditions on the use of Bollgard[®] II cotton, including an insect resistance management plan. A condition within this resistance management plan specifies that the amount of combined INGARD[®] and Bollgard[®] II cotton to be grown by one grower (as defined by the growers trading name) in a valley must not exceed 40% of the total cotton being grown by that grower in that valley. A grower may choose to plant up to 40% Bollgard[®] II and no INGARD[®]. These trials will comply with all conditions imposed by the APVMA.

31. Further information about the use of glyphosate on glyphosate-tolerant crops and about the management of herbicide and 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: contact@apvma.gov.au
<http://www.apvma.gov.au>

2.2.2 Food Standard Australia New Zealand

32. FSANZ is responsible for human food safety assessment. Currently the applicant has not applied to FSANZ for evaluation of material from the GM cottons proposed for release for use in human food. FSANZ approval will need to be obtained before such material could be used for this purpose. However, oil and linters from one of the parental GMOs, Bollgard[®] II, has already been approved by FSANZ for use in human food.

33. Further information about food safety and 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 2.3 International approvals

34. Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/ Bollgard[®] II cottons have not been released commercially in other countries, as these GM cottons are still in development. However, field trials of Roundup Ready[®] MON88913 cotton are currently in progress in the United States of America. First generation Roundup Ready[®] and Bollgard[®] II cotton have been approved for commercial release in other countries:

- The United States - the US Department of Agriculture and the Food and Drug Administration approved the commercial release and use in food of Roundup Ready[®] cotton in 1995 and Bollgard[®] II cotton in 2002;
- Canada - the Canadian Food Inspection Agency and Health Canada approved the commercial release and use in food of Roundup Ready[®] cotton in 1996, and in June 2003 it authorised the use of Bollgard[®] II cotton event 15985 for livestock feed;
- Japan - the Japanese Ministries of Agriculture, Forestry and Fisheries and Health and Welfare approved the commercial release of Roundup Ready[®] cotton in 1997 and use in animal feed in 1998. Bollgard[®] II cotton was approved for use in food and animal feed in 2002 and 2003 respectively;
- Argentina - approved the commercial release of Roundup Ready[®] cotton in 1999 and its use in human food and stock feed in 2000 and 2001 respectively.

35. Other countries where Roundup Ready[®] cotton varieties have been approved, or are pending approval, include India, Israel, Mexico and the European Union. Limited and controlled releases of Bollgard[®] II cotton have been approved and carried out in Argentina, Costa Rica, India, Mexico and South Africa.

36. No country is known to have refused an application for the release of Roundup Ready[®], Roundup Ready[®] MON 88913 or Bollgard[®] II cottons.

37. There have been no reports of adverse effects on human health or the environment resulting from any of the Australian or international releases of Roundup Ready[®] or Bollgard[®] II cotton or from field trials of Roundup Ready[®] MON 88913 cottons in other countries.

CHAPTER 2 SUMMARY OF RISK ASSESSMENT AND RISK MANAGEMENT PLAN

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

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

39. Comments received in response to consultation with expert groups and authorities on the preparation of the risk assessment and risk management plan (RARMP) under Section 50 of the Act, and with the same stakeholders and the public on the RARMP, under Section 52 of the Act (see Appendix 8), were very important in finalising the plan, which formed the basis of the Regulator's final decision on the application.

40. Written submissions in relation to DIR 035/2003 raised the following issues relating to risks to human health and safety or the environment that have been addressed in the RARMP:

- the potential toxicity and allergenicity of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton (Appendices 2 and 3 refer);
- the potential for increased weediness of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton (Appendix 4 refers);
- the potential for, and management of, gene transfer to other cotton crops, naturalised cotton populations and native cottons (Appendices 4 and 7 refer);
- the potential for adverse impacts arising from gene transfer to other organisms (Appendix 5 refers);
- the potential for, and management of, development of herbicide resistance in weed species (Appendix 6 refers); and
- the potential for, and management of, development of insect resistance (Appendix 6 refers).

41. Prescribed agency submissions also raised issues such as impacts on existing and future export markets, ethical consequences of biotechnology, adverse effects on local cropping systems, economic viability of GM crops, which are outside the scope of evaluations conducted under the Act and therefore have not been considered as a part of the assessment process.

42. In total the Regulator received four submissions from the public on this application. A summary of these written submissions is provided in Appendix 9. The key issues raised by the public that related to human health and safety or the environment were:

- potential for adverse impacts on human health (Appendix 2 refers);
- potential for adverse impacts on the environment (Appendix 3, 4, 5 and 6 refers);

- disclosure of site locations (Appendix 7 refers);
- management of potential weediness of the GM cottons in northern Australia (Appendix 4 and 7 refers);
- Potential for weeds to develop herbicide resistance (Appendix 5 and 6 refers);
- conclusions regarding gene transfer (Appendix 5 refer); and
- the beneficial impacts of the current insecticidal and herbicide tolerant cottons to the environment.

43. Public submissions also raised issues such as impacts on domestic markets and export expansion, labelling of GM products, maintaining product integrity, food safety, and effective tracking and tracing of GM material, which are outside the scope of evaluations conducted under the Act and therefore have not been considered as a part of the assessment process.

44. In accordance with Section 56 of the Act, the Regulator has taken into account all issues raised in written submissions that related to the protection of human health and safety and to the environment in preparing 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.

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

45. The Regulator has conducted a risk assessment in relation to the proposed dealings and prepared a risk management plan. The risk assessment process, detailed in Appendix 8, identified a number of 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 identified risks.

46. 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*.

47. Where it is considered that risk management may be required to protect the health and safety of humans and/or the environment, the *Risk Management* column identifies the selected methods and the reasons they were chosen. A risk management plan for the proposed dealing has been given effect by specific conditions within the licence. These conditions are summarised in the final column, headed *Licence Conditions*, and detailed in Appendix 7.

48. Further information on the additional research requirements that have been imposed is contained in Section 4. The research is intended to confirm previous work on gene flow and to validate the

containment measures applied to limited and controlled GM cotton trials. It will also generate data required for the assessment of future applications

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

GM cotton: the genetically modified cotton proposed for release.
 Lepidoptera: the caterpillar insect pests targeted by the GM cotton belong to this order of insects.
 Bt toxins: the Cry1Ac and Cry2Ab proteins may also be referred to as a Bt toxins, because they are some of the many proteins that are produced by the bacteria *Bacillus thuringiensis* (Bt) in nature.
 CP4 EPSPS: the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, the gene of which was derived from the bacterium *Agrobacterium* species strain CP4, and is tolerant to the herbicide glyphosate.
 GUS the enzyme β -glucuronidase, encoded by a reporter gene (*uidA*), which enables visualisation of plant tissues in which this gene is being expressed.
 NPT II neomycin phosphotransferase type II, encoded by an antibiotic resistance gene (*nptII*) which enables selection of GM cotton plant cells when grown in the presence of antibiotics (kanamycin or neomycin) in the laboratory.
 N/A: not applicable.
 NA: not assessed.

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management ?	Risk Management Method(s) and Reason(s) for selection	Is risk managed?	Licence conditions (See Appendix 7 for detailed licence conditions)
TOXICITY AND ALLERGENICITY FOR HUMANS: Food	Very Low	See Appendix 2 <ul style="list-style-type: none"> none of the GM material from the release will be used in human food or animal feed; and FSANZ approval will be required before the GM materials could be used for human food. 	Yes	<ul style="list-style-type: none"> <u>Prevent seed from entering human food supply</u>: prevents exposure through food. <u>Destroy all seed not required for further trials</u>: prevents unintended exposure. <u>Ensure secure transport and storage of retained seed</u>: prevents unintended exposure. 	Yes	<ol style="list-style-type: none"> <u>Prevent entry into human food supply</u>: no cottonseed to enter human food supply. <u>Destroy seed</u>: destroy all seed not required for future trials. <u>Secure transport and storage</u>: GMOs must not be transported unless contained within a primary, sealed container packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.
TOXICITY AND ALLERGENICITY FOR HUMANS: Occupational exposure	Very Low	See Appendix 2 <ul style="list-style-type: none"> pleiotropic effects of the genetic modification could alter aspects of plant metabolism; cotton pollen is not wind-dispersed and therefore unlikely to be an air-borne allergen; exposure to the introduced proteins through working with cotton plants is very low; humans are commonly exposed to the CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII proteins, as these proteins are naturally widespread in the environment; feeding studies indicate that the introduced proteins are not 	Yes	<ul style="list-style-type: none"> <u>Limit scale of release</u>: decreases likelihood of exposure. <u>Destroy all seed not required for further trials</u>: prevents unintended exposure. <u>Ensure secure transport and storage of retained seed</u>: prevents unintended exposure. <u>Report any adverse impacts on human health and safety</u>: ensures identification of unexpected adverse impacts. 	Yes	<ol style="list-style-type: none"> <u>Limit scale</u>: restrict area to 1000 hectares over four growing seasons or up to 46 individual sites of not more than 50 ha. <u>Destroy seed</u>: destroy all seed not required for future trials. <u>Secure transport and storage</u>: GMOs must not be transported unless contained within a primary, sealed container packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management ?	Risk Management Method(s) and Reason(s) for selection	Is risk managed?	Licence conditions (See Appendix 7 for detailed licence conditions)
		<p>toxic;</p> <ul style="list-style-type: none"> evidence indicates that the introduced proteins are not allergenic, nor do they have properties of known allergenic proteins; there have been no reported toxic or allergic effects from similar GM cottons expressing the same proteins that have been extensively field trialed and are commercially released in Australia; and although dust and lint from cotton can be created at processing facilities, the use of protective equipment prevents respiratory irritation, and fibre characteristics of the GM cottons are likely to be the same as for non-GM cotton. 				<p>indicate GM cotton is stored within.</p> <p>4. <u>Report adverse impacts</u>: any adverse impacts on human health and safety must be reported to the Regulator.</p>
<p>TOXICITY AND ALLERGENICITY FOR HUMANS: Wearing & using household items containing cotton products</p>	Very Low	<p>See Appendix 2</p> <ul style="list-style-type: none"> cotton lint used in clothing and household items contains no proteins or DNA and cotton products from GM cottons can not be distinguished from non-GM products. 	No	N/A	N/A	None required
<p>TOXICITY FOR OTHER ORGANISMS: Mammals and wildlife including birds and fish</p>	Low	<p>See Appendix 3</p> <ul style="list-style-type: none"> exposure of stock and wild life to these GM cottons is low, cotton seed will not be used as stock feed; the introduced proteins are native to bacteria that are already widespread in the environment; the toxicity of Cry1Ac and Cry2Ab is highly specific to lepidopteran insect larvae; the CP4 EPSPS, GUS and NPTII enzymes are not known to be toxic to any organism; toxicity studies with the purified proteins or commercial Bt microbial products indicate that these proteins are not toxic to mammals, birds or fish. however, molecular characteristics of the inserted genetic material and expression levels of the introduced proteins in herbicide tolerant/insecticidal cottons under Australian field conditions is not known. 	Yes	<ul style="list-style-type: none"> <u>Limit scale of release</u>: decreases likelihood of exposure. <u>Prevent seed from being used as stockfeed</u>: prevents exposure of other animals. <u>Destroy all seed not required for further trials</u>: prevents unintended exposure. <u>Ensure secure transport and storage of retained seed</u>: prevents unintended exposure <u>Further research</u>: provides information on the molecular characteristics of the inserted genetic material and the expression of the introduced proteins. 	Yes	<ol style="list-style-type: none"> <u>Limit scale</u>: restrict area to 1000 hectares over four growing seasons or up to 46 individual sites of not more than 50 ha. <u>Prevent seed from being used as stockfeed</u>: no cottonseed to be used as stockfeed. <u>Destroy seed</u>: destroy all seed not required for future trials. <u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container that is packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within. <u>Require further research</u>: on characteristics of insert and expression of introduced proteins under Australian field conditions.

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management ?	Risk Management Method(s) and Reason(s) for selection	Is risk managed?	Licence conditions (See Appendix 7 for detailed licence conditions)
TOXICITY FOR OTHER ORGANISMS: Non-target invertebrates, including soil insects	Low	See Appendix 3 <ul style="list-style-type: none"> the introduced proteins are native to bacteria that are already widespread in the environment; the CP4 EPSPS, GUS and NPTII enzymes are not known to be toxic to any organism; the toxicity of Cry1Ac and Cry2Ab is highly specific to lepidopteran insect larvae; laboratory and field studies suggest that populations of key non-target invertebrates are unlikely to be affected by the Bt toxins. Rather, it is likely that their populations would be favoured by decreases in the use of broad-spectrum insecticides. expression levels of the introduced proteins in herbicide tolerant/insecticidal cottons under Australian field conditions is not known 	Yes	<ul style="list-style-type: none"> <u>Further research</u>: provides information on the molecular characteristics of the inserted genetic material and the expression of the introduced proteins. 	Yes	<ol style="list-style-type: none"> <u>Limit scale</u>: restrict area to 1000 hectares over four growing seasons or up to 46 individual sites of not more than 50 ha. <u>Destroy seed</u>: destroy all seed not required for future trials. <u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container that is packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within. <u>Require further research</u>: on characteristics of insert and the expression of introduced proteins under Australian field conditions.
TOXICITY FOR OTHER ORGANISMS: Microbial organisms	Low	See Appendix 3 <ul style="list-style-type: none"> the introduced proteins are native to bacteria that are already widespread in the environment; the Cry1Ac and Cry2Ab toxins are unlikely to have adverse effects on soil microorganisms. however, expression levels of the introduced proteins in herbicide tolerant/insecticidal cottons and potential for accumulation in soil under Australian field conditions is not known 	Yes	<ul style="list-style-type: none"> <u>Further research</u>: informs the ongoing review of the data on toxicity and potential for accumulation in the soil; 	Yes	<ol style="list-style-type: none"> <u>Limit scale</u>: restrict area to 1000 hectares over four growing seasons or up to 46 individual sites of not more than 50 ha. <u>Destroy seed</u>: destroy all seed not required for future trials. <u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container that is packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within. <u>Require further research</u>: on the potential toxicity for soil organisms; and potential for accumulation of proteins in soil.
WEEDINESS:	Low	See Appendix 4 <ul style="list-style-type: none"> cotton does not possess characteristics commonly associated with weediness, and is not known to be a problematic weed in any environment; cotton has a low potential for dispersal by natural means; the introduced genes in the GM cottons are unlikely to affect these characteristics; there is a low possibility for the genetic modification to 	Yes	<ul style="list-style-type: none"> <u>Limit scale of the release</u>: decreases likelihood of escape <u>Surround the GM cotton with a pollen trap</u>: minimises spread of the introduced genes beyond the release sites via pollen flow. <u>Ensure secure transport and storage of retained seed</u>: prevents escape of GM 	Yes	<ol style="list-style-type: none"> <u>Limit scale</u>: restrict area to 1000 hectares over four growing seasons or up to 46 individual sites of not more than 50 ha. <u>Surround the GM cotton with a pollen trap</u> of at least 20 m in all directions from the outside of the release site. <u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management ?	Risk Management Method(s) and Reason(s) for selection	Is risk managed?	Licence conditions (See Appendix 7 for detailed licence conditions)
		<p>have potential pleiotropic effects that could alter traits affecting weediness, therefore, the applicant proposes to collect data on agronomic characteristics associated with weediness during the field trials;</p> <ul style="list-style-type: none"> major constraints on weediness of GM and non-GM cotton are water availability, nutrient availability, plant competition, herbivory by non-lepidopteran species, frost and fire; the combination of herbicide tolerance and insect resistance in Roundup Ready® MON 88913/Bollgard® II cotton is unlikely to increase the weedy potential of this GMO above the low potential for either Roundup Ready® MON 88913 or Bollgard® II cotton individually, to become problematic weeds; surveys carried out since the commercial release of Roundup Ready® and Roundup Ready®/INGARD® cotton in 2000 indicate that these GM cottons have not become problematic weeds; preliminary experimental data suggest that insecticidal cotton may be weedier than non-GM cotton in certain nutrient-rich habitats such as artificial waterways and stock feeding areas; INGARD® cottonseed has been used as stockfeed in northern Australia since 1996, and Roundup Ready®/INGARD® cotton since 2000, with no indication that they have become more weedy than non-GM cotton; and applicant proposes to surround the trial sites by pollen traps and isolate the trials from feral cotton and all other cotton crops to minimise gene flow and persistence. 		<p>plant material outside the release sites.</p> <ul style="list-style-type: none"> <u>Cleaning of equipment used at the release sites</u>: prevents escape of GM plant material into the environment outside the release sites. <u>Destroy any volunteers</u>: prevents persistence. <u>Further research</u>: informs the ongoing review of the data on spread/persistence of the GMO in the environment and validates the efficacy of containment measures. 		<p>primary, sealed container packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</p> <ol style="list-style-type: none"> <u>Cleaning of equipment used at the release sites</u>: must be cleaned before used for any other purpose. If GM cotton is ginned, gin must be cleaned immediately following use, before any other cotton is ginned. <u>Destroy volunteers</u>: release sites must be monitored after harvest at least once every 2 months for at least 12 months and any volunteers destroyed before flowering. <u>Research conditions</u>: <ul style="list-style-type: none"> surround the trial site by a 400 m research zone for the purpose of conducting research on spread and persistence of the GMOs. collect data on agronomic characteristics indicative of the potential weediness of the GM cotton under Australian field conditions.
<p>GENE TRANSFER: Plants - Other cotton crops</p>	<p>Low</p>	<p>See Appendix 5</p> <ul style="list-style-type: none"> gene transfer poses the same risks as the low risks posed by Roundup Ready® MON 88913 or Roundup Ready® MON 88913/ Bollgard® II cotton. applicant proposes to surround the trial sites by pollen traps and isolate the trials from feral cotton and all other cotton crops to minimise gene flow and persistence. 	<p>Yes</p>	<ul style="list-style-type: none"> <u>Limit scale of the release</u>: decreases potential for transfer. <u>Surround the GM cotton with a pollen trap</u>: minimises spread of the introduced genes beyond the release sites via pollen flow. <u>Ensure secure transport and storage of retained seed</u>: prevents escape of GM 	<p>Yes</p>	<ol style="list-style-type: none"> <u>Limit scale</u>: restrict area to 1000 hectares over four growing seasons or up to 46 individual sites of not more than 50 ha. <u>Surround the GM cotton with a pollen trap</u> of at least 20 m in all directions from the outside of release sites. <u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management ?	Risk Management Method(s) and Reason(s) for selection	Is risk managed?	Licence conditions (See Appendix 7 for detailed licence conditions)
				<ul style="list-style-type: none"> • plant material outside the release sites. • <u>Cleaning of equipment used at the release sites</u>: prevents escape of GM plant material into the environment outside the release sites. • <u>Destroy any volunteers</u>: prevents persistence. • <u>Further research</u>: validates efficacy of containment measures. 		<p>primary, sealed container packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container in locked facility signed to indicate GM cotton stored within.</p> <ol style="list-style-type: none"> 4. <u>Cleaning of equipment used at the release sites</u>: must be cleaned before used for any other purpose. Gin for GM cotton must be cleaned immediately after use, before any other cotton is ginned. 5. <u>Destroy volunteers before flowering</u>: release sites must be monitored after harvest at least once every 2 months for at least 12 months. 6. <u>Research conditions</u>: surround the trial site by a 400 m wide research zone for the purpose of conducting research on pollen dispersal and gene transfer.
<p>GENE TRANSFER: Plants - Feral (naturalised) cotton</p>	<p>Low</p>	<p>See Appendix 5</p> <ul style="list-style-type: none"> • gene transfer would pose the same risks as the low risks posed by Roundup Ready® MON 88913 or Roundup Ready® MON 88913 Bollgard® II cotton; • applicant proposes to surround the trial sites by pollen traps and isolate the trials from feral cotton and all other cotton crops to minimise gene flow and persistence. 	<p>Yes</p>	<ul style="list-style-type: none"> • <u>Limit scale of the release</u>: decreases potential transfer. • <u>Surround the GM cotton with a pollen trap</u>: minimises spread of the introduced genes beyond the release sites via pollen flow. • <u>Ensure secure transport and storage of retained seed</u>: prevents escape of GM plant material outside the release sites. • <u>Cleaning of equipment used at the release sites</u>: prevents escape of GM plant material into the environment outside the release sites. • <u>Destroy any volunteers</u>: prevents persistence. • <u>Further research</u>: validates efficacy of containment measures. 	<p>Yes</p>	<ol style="list-style-type: none"> 1. <u>Limit scale</u>: restrict area to 1000 hectares over four growing seasons or up to 46 individual sites of not more than 50 ha. 2. <u>Surround the GM cotton with a pollen trap</u> of at least 20 m in all directions from the outside of the release site. 3. <u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container in locked facility signed to indicate GM cotton is stored within. 4. <u>Cleaning of equipment used at the release sites</u>: must be cleaned before used for any other purpose. Gin for GM cotton must be cleaned immediately after use, before any other cotton is ginned. 5. <u>Destroy volunteers before flowering</u>: release sites must be monitored after harvest at least once every 2 months for at least 12 months and

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management ?	Risk Management Method(s) and Reason(s) for selection	Is risk managed?	Licence conditions (See Appendix 7 for detailed licence conditions)
						volunteers destroyed. 6. <u>Research conditions</u> : surround the trial site by a 400 m wide research zone for the purpose of conducting research on pollen dispersal and gene transfer.
GENE TRANSFER: Plants - Native cottons	Negligible	See Appendix 5 <ul style="list-style-type: none"> genetic incompatibility and geographical isolation from native populations prevent the production of fertile hybrids. 	No	N/A	N/A	None required
GENE TRANSFER: Plants - Other genera	Negligible	See Appendix 5 <ul style="list-style-type: none"> Well-established genetic incompatibility prevents successful cross-pollination with other plant species. 	No	N/A	N/A	None required
GENE TRANSFER: Humans & other animals	Negligible	See Appendix 5 <ul style="list-style-type: none"> The most significant route of entry of foreign DNA into animals and humans is through food. However, products from the GM cottons are not intended for stockfeed or human food. Thus the likelihood of gene transfer to animals or humans is negligible. FSANZ approval will be required before the GM materials could be used for human food. 	No	N/A	N/A	None required
GENE TRANSFER: microorganisms (bacteria)	Negligible	See Appendix 5 <ul style="list-style-type: none"> all of the introduced genes in Roundup Ready® MON 88913 or Roundup Ready® MON 88913/ Bollgard® II cotton are already widespread in the environment, and are readily available for transfer from these sources via demonstrated natural mechanisms; and gene transfer from plants to bacteria has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other sources of these genes. 	No	N/A	N/A	None required
RESISTANCE Herbicide	NA	See Appendix 6 <ul style="list-style-type: none"> APVMA is responsible for assessing the risk. 	This risk is managed by the APVMA	Not required for first season (2003/04) as less than one hectare per State will be sprayed with herbicide. APVMA would impose conditions for subsequent three seasons of the trial.		Not required. Licence notes the requirement to adhere to the APVMA conditions, including any herbicide resistance management strategy.
RESISTANCE	NA	See Appendix 6 <ul style="list-style-type: none"> APVMA is responsible for assessing the risk. 	This risk is managed by	APVMA has imposed conditions		Not required. Licence notes the requirement to adhere to the APVMA conditions, including any

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management ?	Risk Management Method(s) and <i>Reason(s) for selection</i>	Is risk managed?	Licence conditions (See Appendix 7 for detailed licence conditions)
Insecticide			the APVMA			insecticide resistance management strategy.

SECTION 3 DECISION ON THE APPLICATION

49. 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 are able to be managed so as to protect human health and safety and the environment.

50. It is concluded that there are no significant risks to human health and safety or to the Australian environment arising from the proposed release of GM herbicide tolerant and herbicide tolerant/insecticidal cottons that cannot be adequately managed. Detailed risk analyses based on the available scientific information are provided in Appendices 2 - 6 in support of this conclusion.

51. Therefore the Regulator has issued licence number DIR 035/2003 in respect of this application.

SECTION 4 IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES

52. Before any application for a larger scale or commercial release of Roundup Ready MON 88913[®] or Roundup Ready MON 88913[®]/ Bollgard[®] II cotton could be evaluated, more detailed information will be required on:

- genetic segregation and molecular characterisation of the introduced genetic material;
- the levels of expression of proteins by the introduced genes under Australian field conditions;
- the stability and persistence of introduced proteins in the soil from GM cotton under Australian field conditions;
- the ecological impacts of the insecticidal protein on soil biota under Australian field conditions;
- the expression of the glyphosate tolerant phenotype during development of the GM plant;
- agronomic characteristics of GM cottons indicative of potential weediness; and
- gene transfer to non-GM cotton or feral cotton and efficacy of pollen trap under Australian field conditions.

53. As part of the OGTR's ongoing commitment to review of data, research conditions have been imposed for a number of cotton DIR licences. This research is intended to confirm previous research on pollen and gene flow undertaken prior to the implementation of the *Gene Technology Act 2000*. For the proposed release, the following research is required:

- detailed study on efficacy of pollen traps in controlling gene transfer through pollen to non-GM cotton and feral cotton.
- the potential toxicity of the GM insecticidal cotton, including more information on potential toxicity to non-target organisms and effect on soil micro-organisms; and
- the potential weediness of the GM cotton under Australian field conditions.

54. In addition, the results of research required as a condition of licences for other releases of this GMO (DIR 017/2002 and DIR 025/2002) would also be available to the Regulator.

55. It should be noted that provision of the above data during the proposed release are not required to ensure the management of risks to human health and safety and the environment from the proposed release. The risk management measures summarised in the Table above and given effect by the licence conditions, will achieve this purpose.

APPENDIX 1 INFORMATION ABOUT THE GMO

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

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

58. It should be noted that some details of the gene construct, including the plasmid map and some of the regulatory sequences, and preliminary protein expression data have been declared as Confidential Commercial Information (CCI) under Section 185 of the Act. However the CCI was made available to the prescribed expert groups which were consulted in the preparation of the risk assessment and risk management plan.

SECTION 1 SUMMARY INFORMATION ABOUT THE GMO

59. Monsanto Australia Limited (Monsanto) proposes to release two GMOs under application DIR 035/2003: herbicide tolerant Roundup Ready[®] MON 88913 cotton and herbicide tolerant/insecticidal Roundup Ready[®] MON 88913/Bollgard[®] II cotton.

60. Roundup Ready[®] MON 88913 cotton is derived from an original genetic modification event referred to as MON 88913 and will be introduced to Australian cotton varieties by conventional breeding. Roundup Ready[®] MON 88913/Bollgard[®] II cotton will be produced by conventional breeding of Roundup Ready[®] MON 88913 cotton and GM Bollgard[®] II cotton. Bollgard[®] II cotton has been approved for commercial release south of latitude 22° South in Australia and has been considered previously in detail in the risk assessment and risk management plan DIR 012/2002, available at www.ogtr.gov.au. Bollgard[®] II cotton is only discussed in this document in so far as it relates to Roundup Ready[®] MON 88913/Bollgard[®] II cotton.

61. Roundup Ready[®] MON 88913 cotton and Roundup Ready[®] MON 88913/Bollgard[®] II cotton both contain two copies of the *cp4 epsps* (5-enolpyruvylshikimate-3-phosphate synthase) gene, derived from the bacterium *Agrobacterium* species strain CP4. The *cp4 epsps* gene encodes an enzyme (CP4 EPSPS) that is tolerant to inhibition by glyphosate (N-phosphonomethyl glycine), the active ingredient in the herbicides, Roundup[®] and Roundup Ready[®]. Thus the GM plants confer tolerance to the foliar application of herbicides, Roundup[®] and Roundup Ready[®]. Further details on the *cp4 epsps* gene and the CP4 EPSPS protein are provided in Section 3 of this appendix.

62. Roundup Ready[®] MON 88913 cotton and Roundup Ready[®] MON 88913/Bollgard[®] II cotton differ in two ways from the existing Roundup Ready[®] and Roundup Ready[®]/Bollgard[®] II cottons, that are being grown commercially or trialed in Australia. Firstly, both MON 88913 cottons contain two copies of the *cp4 epsps* gene rather than one copy, as in the commercially released Roundup Ready[®] cottons. Secondly, in the MON 88913 cottons, expression of the two copies of the *cp4 epsps* gene is under the control of novel regulatory elements, which were not present in the commercially released Roundup Ready[®] cottons.

63. The novel regulatory elements and the two copies of the *cp4 epsps* gene result in improved expression of the *cp4 epsps* gene in the MON 88913 cottons. These cottons are thus able to tolerate application of Roundup[®] on the sensitive reproductive parts of the plant, which are not tolerant to Roundup[®] in commercially released Roundup Ready[®] cotton. Roundup[®] cannot be applied to Roundup Ready[®] cotton beyond the four leaf growth stage, when the effects of the herbicide on the sensitive reproductive parts of the plant begin to result in yield losses (Monsanto Australia Limited 2001). Therefore, if this narrow window of opportunity for Roundup[®] application at four-leaf stage was missed, weed control by Roundup[®] application at later stages of growth is not possible in Roundup Ready[®] cotton crop. MON 88913 cottons can tolerate application of Roundup[®] at later stages of growth, allowing a wider window for targeted application of Roundup[®] during growth of the cotton crop. This is intended to give growers increased flexibility in timing herbicide application and better weed control.

64. Roundup Ready[®] MON 88913/Bollgard[®] II cotton also contains the insecticidal genes *cry1Ac* and *cry2Ab*, derived from the common soil bacterium *Bacillus thuringiensis* (Bt). These genes encode the proteins Cry1Ac and Cry2Ab, which are highly specific insecticidal proteins that are toxic to lepidopteran caterpillar pests of cotton, including *Helicoverpa armigera* (cotton bollworm) and *H. punctigera* (native budworm). Further details on the *cry1Ac* and *cry2Ab* genes and the Bt proteins are provided in Section 3 of this Appendix and in the risk assessment and risk management plans for DIR 012/2002 (Bollgard[®] II cotton) and DIR 022/2002 (INGARD[®] cotton), available at www.ogtr.gov.au.

65. Roundup Ready[®] MON 88913 cotton does not contain any antibiotic resistance genes. Roundup Ready[®] MON 88913/Bollgard[®] II cotton contains two antibiotic resistance genes. These genes were used as selectable markers in the early laboratory stages of development of the Bollgard[®] II plants, to enable selection of bacteria or plant cells containing the desired genetic modification. The neomycin phosphotransferase type II (*nptII*) gene confers resistance to the antibiotics kanamycin and neomycin. The aminoglycoside adenylyltransferase (*aad*) gene confers resistance to spectinomycin and streptomycin, but is controlled by a bacterial promoter that does not function in plants, so the protein is not expressed in the GM cotton. The antibiotic resistance genes are discussed in more detail in Section 3 of this Appendix. Potential hazards relating to transfer of these genes to bacteria are discussed in Appendix 5.

66. Roundup Ready[®] MON 88913/ Bollgard[®] II cotton contains the *uidA* gene from *Escherichia coli*, which codes for the bacterial enzyme β -glucuronidase (GUS). The GUS enzyme is a visual marker that allows the detection of genetically modified tissues in the laboratory using a simple biochemical stain. More information about the *uidA* gene and the GUS protein is provided in Section 3 of this Appendix.

SECTION 2 THE PARENT ORGANISM

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

SECTION 3 THE INTRODUCED GENES AND THEIR PRODUCTS

Section 3.1 The *cp4 epsps* herbicide tolerance gene and encoded protein

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

69. In plants the native *epsps* (5-enolpyruvylshikimate-3-phosphate synthase) gene encodes an enzyme (EPSPS) critical for the biosynthesis of aromatic amino acids (tryptophan, tyrosine and phenylalanine), essential building blocks for cellular proteins. During this biosynthetic process the EPSPS enzyme catalyses the addition of the enolpyruvyl moiety of phosphoenolpyruvate to shikimate-3-phosphate. EPSPS performs this function in plants, bacteria, algae and fungi but is absent from mammals, who are not able to synthesise these aromatic amino acids (Bentley 1990; Padgett et al. 1993).

70. The herbicide Roundup[®] functions by inhibiting the activity of the naturally occurring EPSPS enzyme in plants, thus blocking the biosynthesis of aromatic amino acids and eventually leading to cell death (Steinrucken & Amrhein 1980). The *cp4 epsps* gene from *Agrobacterium* is naturally insensitive to the effects of glyphosate (Padgett et al. 1993) as are a number of other microbial EPSPS enzymes (Schulz et al. 1985; Eschenburg et al. 2002) but are still able to function normally in the biosynthesis of aromatic amino acids. Consequently, in GM plants containing the *Agrobacterium cp4 epsps* gene, biosynthesis of aromatic amino acids is not blocked by the presence of glyphosate and the plants are not killed by glyphosate when applied as a herbicide Roundup[®].

71. The *cp4 epsps* gene in the GM cotton has a coding sequence modified to achieve optimal expression in plants (Padgett et al. 1993). Although the gene sequence has been altered, the enzyme produced has exactly the same amino acid sequence as the native *Agrobacterium* enzyme and its activity is unaltered (information provided by the applicant).

72. The *cp4 epsps* gene in the GM cottons is linked to the chloroplast transit peptide (CTP) coding region from an *epsps* gene from the plant *Arabidopsis thaliana* (Klee et al. 1987). The CTP targets the CP4 EPSPS enzyme to the chloroplast, the site of aromatic amino acid biosynthesis. In plants, EPSPS is synthesised as a preprotein (ie. containing the CTP) by free cytoplasmic ribosomes. The precursor is transported into the chloroplast stroma and proteolytically processed to yield the mature enzyme (della-Cioppa et al. 1986). Once cleaved, chloroplast transit peptides are rapidly degraded (Bartlett et al. 1982; della-Cioppa et al. 1986).

73. Two copies of the *cp4 epsps* gene are present in the GM cottons and each copy is under the control of different promoters. A promoter is a region of DNA linked to a gene that determines whether a gene is expressed and to what extent in which plant tissues. One copy is controlled by the enhanced 35S promoter from the Cauliflower Mosaic Virus (CaMV), the other is controlled by the 35S promoter from the Figwort Mosaic Virus (FMV). The 35S promoters direct the *cp4 epsps* genes to be expressed in all plant tissues throughout the plant lifecycle. This kind of expression is called 'constitutive' expression. The GM cottons also contain additional promoter elements and non-coding sequences for improved gene expression. These additional elements are derived from another plant species.

74. Also required for gene expression in plants is a mRNA termination region, including a polyadenylation signal. The mRNA termination regions for the two copies of the *cp4 epsps* gene in the GM cottons are also derived from another plant species.

75. The origin and identity of the additional promoter and terminator elements controlling the expression of the *cp4 epsps* genes has been declared as CCI. However the CCI was made available to the prescribed expert groups which were consulted in the preparation of the risk assessment and risk management plan.

76. Potential hazards relating to the toxicity and allergenicity of the CP4 EPSPS protein are discussed in Appendices 2 and 3.

Section 3.2 The *cryIAc* and *cry2Ab* insecticidal genes and encoded proteins

77. The *cryIAc* and *cry2Ab* genes are derived from *Bacillus thuringiensis*, a ubiquitous soil bacterium. The Cry (for crystalline) proteins (also called Bt proteins or Bt toxins) are a diverse family of insecticidal proteins produced by various subspecies of *B. thuringiensis*. The *cryIAc* and *cry2Ab* genes are derived from *B. thuringiensis* variety *kurstaki* (B.t.k.). Cry1Ac and Cry2Ab encode Bt toxins that are highly specific to lepidopteran insects (moths and butterflies)(Widner & Whiteley 1989; Dankocsik et al. 1990; Macintosh et al. 1990).

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

79. Expression of the *cryIAc* and *cry2Ab* genes in Bollgard[®] II cotton is controlled by the CaMV enhanced 35S promoter. The mRNA termination region for the *cryIAc* gene is provided by the 3' non-translated region of the soybean alpha subunit of the beta-conglycinin gene and for the *cry2Ab* gene by the 3' non-translated region of the nopaline synthase (*nos*) gene from *A. tumefaciens*.

80. More detailed information about the *cryIAc* and *cry2Ab* genes present in Bollgard[®] II cotton, and the Bt toxins, can be found in the risk assessment and risk management plans for DIR 012/2002 and DIR 022/2002, available at www.ogtr.gov.au.

Section 3.3 The *uidA* reporter gene and encoded protein

81. Roundup Ready[®] MON 88913/ Bollgard[®] II cotton plants contain the *uidA* reporter gene. This gene was used as a marker in the laboratory for selecting successfully modified Bollgard[®] II cotton cells during the gene transfer process. The *uidA* gene, from the common soil bacterium *Escherichia coli*, codes for the enzyme β -glucuronidase (GUS). The GUS enzyme converts a colourless substrate into a blue colour in a simple laboratory assay and is used as a reporter or 'marker' to detect tissues that have been successfully genetically modified. Exposure of plant tissues containing the GUS enzyme to this substrate facilitates measurement of the expression of the *uidA* gene (Jefferson et al. 1986).

Section 3.4 The *nptII* and *aad* antibiotic resistance marker genes and encoded proteins

82. Roundup Ready[®] MON 88913 cotton does not contain any antibiotic resistance genes. Roundup Ready[®] MON 88913/Bollgard[®] II cotton contains the *nptII* and *aad* antibiotic resistance marker genes from *E. coli*. These genes were used in the initial laboratory stages of development of

Bollgard® II cotton plants, to enable selection of cells containing the desired genetic modification. Both genes are in common use as selectable markers in the production of GM plants.

83. The *nptII* gene was isolated from the bacterial Tn5 transposon (Beck et al. 1982). It encodes an enzyme, neomycin phosphotransferase type II (NPTII), which confers resistance to the aminoglycoside antibiotics kanamycin and neomycin. The NPTII enzyme uses ATP to phosphorylate kanamycin and neomycin, thereby inactivating the antibiotic and preventing it from killing the NPTII producing cell. The *nptII* gene functioned as a selectable marker in the initial laboratory stages of cotton plant cell selection following genetic modification, allowing modified cells to grow while inhibiting the growth of non-modified cells.

84. The NPTII enzyme 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).

85. Expression of the *nptII* gene in Roundup Ready® MON 88913/ Bollgard® II cotton plants is controlled by the CaMV 35S promoter and is linked to the mRNA termination region of the *nos* gene from *A. tumefaciens*.

86. The *aad* gene was used in the laboratory, prior to production of the genetically modified plants, to select for bacteria carrying the plasmid with the modified DNA. This gene was isolated from the bacterial Tn7 transposon and confers resistance to the antibiotics spectinomycin and streptomycin (Davies & Benveniste 1974). The *aad* gene is not expressed in the GM cotton plants because it is under the control of its native bacterial promoter, which is not active in plants and regulatory elements necessary for its expression in plants have not been added to the gene.

87. Potential hazards relating to the toxicity and allergenicity of the NPTII protein is discussed in Appendices 2 and 3, and those of antibiotic resistance gene transfer in Appendix 5.

SECTION 4 METHOD OF GENE TRANSFER

88. The Roundup Ready® MON 88913 cotton event was produced by *Agrobacterium*-mediated transformation of a Coker variety of cotton. Coker varieties of cotton are used for genetic modification because of their positive response to the tissue culture system used in producing GM plants. The proposed dealing aims to transfer the Roundup Ready® MON 88913 trait into cotton varieties suitable for Australian conditions by conventional crossing.

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

90. 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).

91. 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.

92. In this instance, a conventional, disarmed, binary vector was used to introduce the *cp4 epsps* genes into cotton variety Coker using standard *Agrobacterium* transformation protocols. (Details of the plasmid map are declared as CCI.). Genetically modified plants were screened for tolerance to glyphosate herbicide in the laboratory and under field conditions in the United States.

93. Bollgard[®] II cotton was produced by inserting the *cry2Ab* and *uidA* genes into the genomic DNA of INGARD[®] cotton variety DP50B (containing the *cry1Ac* and *nptII* genes). Genes were delivered into the cotton meristematic cells by microprojectile bombardment (transformation event 15985) (McCabe & Martinell 1993). This technique is a well-established method of plant transformation that uses compressed air to ‘shoot’ tiny tungsten or gold particles coated with the genes to be inserted into plant cells. The introduced genes become incorporated into the genome of the bombarded plant cells. The *uidA* gene is used as marker to identify plant tissue that contains the introduced gene of interest.

94. Roundup Ready[®] MON 88913/ Bollgard[®] II cotton will be produced by conventional cross breeding of Roundup Ready[®] MON 88913 GM cotton with GM Bollgard[®] II cotton.

SECTION 5 CHARACTERISATION OF THE INSERTED GENETIC MATERIAL AND STABILITY OF THE GENETIC MODIFICATION

95. Preliminary data, obtained using Southern blotting, indicate that all inserted genetic elements, including coding regions, promoters and non-coding regions, are present as single, stably integrated inserts in the Roundup Ready[®] MON 88913 cotton and that no unnecessary vector sequences are present (information supplied by the applicant).

96. Roundup Ready[®] MON 88913 cotton has been taken through seven back-cross generations without loss or change in the glyphosate tolerant phenotype or loss or rearrangement of the inserted genetic elements. The inserted elements are inherited in a normal Mendelian manner for single, dominant genes and gene-negative segregants do not retain the glyphosate tolerant phenotype (information supplied by the applicant).

97. Bollgard[®] II cotton contains one complete copy of the *cry1Ac*, *cry2Ab*, *nptII* and *uidA* genes, stably integrated at one location in the cotton genome. Detailed information on the characterisation of the inserted genetic material and stability of the genetic modification in Bollgard[®] II cotton is provided in the risk assessment and risk management plan for DIR 012/2002 (available at www.ogtr.gov.au).

SECTION 6 EXPRESSION OF THE INTRODUCED PROTEINS

98. The applicant expects that the constitutive viral promoters (CaMV and FMV 35S promoters) that control the expression of the *cp4 epsps* gene in Roundup Ready[®] MON 88913 cotton will provide

gene expression throughout most or all parts of the cotton plant. Roundup Ready[®] MON 88913 cotton has improved expression of the *cp4 epsps* genes, compared to commercially released Roundup Ready[®] cotton, including expression in the reproductive parts of the plant. In comparison, expression of the CP4 EPSPS protein in Roundup Ready[®] cotton is lower in reproductive tissues such as stigmas, anthers and floral buds than in vegetative tissue such as leaves (Pline et al. 2002a).

99. The reproductive structures of cotton plants are very sensitive to the effects of glyphosate (Pline et al. 2002a; Pline et al. 2002b) and, despite the presence of the *cp4 epsps* gene, Roundup Ready[®] cotton crops sprayed with glyphosate beyond the four true leaf stage of growth exhibit reduced pollination and increased boll abortion (Monsanto Australia Limited 2001). Tolerance to glyphosate in the reproductive parts of Roundup Ready[®] MON88913 cotton plants is intended to target glyphosate application over the top of the cotton crop at later stages of growth.

100. Preliminary data on the level of expression of the CP4 EPSPS protein in Roundup Ready[®] MON 88913 cotton has been declared as CCI. The applicant proposes to gather data on expression of the CP4 EPSPS protein in Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton as part of the proposed field trials under Australian conditions.

101. Expression levels of the Cry1Ac, Cry2Ab, GUS and NPTII proteins in Bollgard[®] II cotton have been extensively studied. Detailed information on the expression levels of these proteins in leaves, seeds and pollen of Bollgard[®] II cotton is provided in the risk assessment and risk management plan for DIR 012/2002, available from www.ogtr.gov.au.

102. Detailed information on the levels of expression of the introduced CP4 EPSPS protein and the plant parts in which it is expressed under Australian field conditions and details of molecular characterisation of the insertion sites of the introduced gene constructs in the GM cottons will be required before any application for larger scale or commercial release of Roundup Ready[®] MON 88913 cotton could be assessed.

SECTION 7 PLEIOTROPIC EFFECTS OF THE GENETIC MODIFICATION

103. A single plant gene can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Single gene inserted into a plant by genetic modification can also be pleiotropic and it is necessary to evaluate genetically modified plants for unintended, pleiotropic effects of the inserted genes, such as changes in agronomic characteristics.

104. No unintended or secondary effects have been observed in greenhouse or field trials of Roundup Ready[®] MON 88913 cotton in the United States, including no effects on the fertility of the GM cotton plants (information supplied by the applicant).

105. The agronomic performance of commercially released Roundup Ready[®] and Roundup Ready[®]/Bollgard[®] II cotton (expressing the same introduced proteins as the MON 88913 cottons) has been thoroughly analysed during numerous field trials and commercial releases of these GM cottons in Australia and elsewhere in the world. Such analysis has been closely monitored for differences between the GM and parental non-GM cotton varieties. Field trials in the US in 1998 and 1999 (Hamilton & Reed 1999; Hamilton et al. 2000) demonstrated the agronomic equivalence of Roundup Ready[®] cotton and non-GM cotton lines in terms of germination, growth habit, plant

morphology and disease susceptibility. Fibre characteristics (length, strength, diameter) of the GM cotton are also equivalent to that of non-GM cotton (Cotton Seed Distributors 2002).

106. The applicant proposes to conduct evaluation of agronomic and phenotypic characteristics of Roundup Ready[®] MON 88913 cotton and Roundup Ready[®] MON 88913/Bollgard[®] II cotton as part of the proposed trials.

APPENDIX 2 HUMAN HEALTH AND SAFETY

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

108. It should be noted that since the commercial release of the similar GM cottons, Roundup Ready[®] and Roundup Ready[®]/Bollgard[®] II cotton, which express the same introduced proteins as Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton, there have been no reported adverse toxic or allergic effects on human health resulting from occupational exposure, from ingestion of foods derived from the oil or linters of these GM cottons or from the use of other products containing their oil, lint or linters. However, expression levels of the introduced proteins in Roundup Ready[®] MON 88913/Bollgard[®] II cotton have not been determined.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

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

110. Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cottons differ from conventional cotton in the expression of one and five additional proteins, respectively. These are the CP4 EPSPS protein (in both GM cottons) and the Cry1Ac, Cry2Ab, GUS and NPTII proteins (in Roundup Ready[®] MON 88913/Bollgard[®] II cotton only) (see Appendix 1 for details of protein expression in the GM cottons). The potential for these cottons to be toxic or allergenic to humans due to either expression of the novel gene products or because of unintended effects of the genetic modification is considered here.

111. Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cottons express the same proteins as commercially released Roundup Ready[®] and Roundup Ready[®]/Bollgard[®] II cottons. The only difference between the MON 88913 cottons and the commercially released Roundup Ready[®] cottons is expected to be the level of expression of the CP4 EPSPS protein and reproductive tolerance to glyphosate. Consequently, some reference to data derived from analysis of Roundup Ready[®], Bollgard[®] II and Roundup Ready[®]/Bollgard[®] II cotton is made throughout this appendix. However, although the MON 88913 cottons express the same introduced proteins as commercially released Roundup Ready[®] cottons, the site of insertion and level of expression of the introduced genes will be different. There is a possibility, although considered low, that it could potentially alter some aspect of plant metabolism that impacts on toxicity or allergenicity.

112. The Australian Pesticides and Veterinary Medicines Authority (APVMA) is responsible for the use and safety of herbicides in Australia. Glyphosate is not currently registered for use on cotton beyond the four-leaf stage of growth. A research permit from the APVMA for use of glyphosate after this stage on Roundup Ready[®] MON 88913 cotton is not required for the first year of the proposed

field trial as less than one hectare of cotton plants per state are proposed to be sprayed with glyphosate. However, APVMA permit or approval will be required for such use of glyphosate in the subsequent years of the proposed trials or any further larger scale release. As part of their assessment of this use, the APVMA consider any potential human health effects, for example risks arising through occupational exposure or residues in food and the environment. Thus risks associated with the use of glyphosate are not considered in the risk assessment of these GM cottons.

SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

113. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/ Bollgard[®] II cotton on human health and safety, the following factors were considered:

- the inherent toxicity and allergenicity of conventionally bred cotton;
- the potential exposure to these GM cottons, to their products and to the new proteins which are expressed in the cottons, the CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII proteins;
- the potential exposure to the CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII proteins from other sources in the environment; and
- the potential toxicity and allergenicity of the new proteins expressed in the GM cottons.

Section 2.1 Toxicity and allergenicity of conventionally bred cotton

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

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

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

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

Section 2.2 Exposure of people to GM cottons

118. The applicant proposes to retain the cottonseed produced in the field trials. Seed will be stored or planted in further trials authorised by the same licence. As cottonseed produced in the proposed field trials will not be used in human food or animal feed, there will be no opportunity for human exposure to the GM cottons through food. The applicant does however propose to sell the lint produced in the release for use in fabric, upholstery and other non-food products. Hence, exposure of people to the GM cotton will be by means of:

- wearing cotton clothing or using household items made from cotton lint;
- working with cotton (e.g. on cotton farms, in cotton processing facilities); or
- living in or near the areas where cotton is grown.

119. As there will be no opportunity for humans to consume food products from this proposed release of GM cottons, hazards to humans through food do not warrant detailed discussion here. If, in future applications, the applicant intended to use cotton products (eg. cotton linters and cottonseed oil) from these GM cottons for human food, approval will need to be sought from Food Standards Australia New Zealand (FSANZ, formally ANZFA).

120. However, cottonseed oil and cotton linters are highly refined and processed, with no detectable DNA (genetic material) or proteins (Leffler & Tubertini 1976; Sims et al. 1996). Oil and linters derived from the currently commercially released Roundup Ready[®] and Roundup Ready[®]/Bollgard[®] II cottons cannot be distinguished from those derived from non-GM cotton. FSANZ considers that food products (oils and linters) for human consumption that are derived from Roundup Ready[®] cotton and from Bollgard[®] II cotton are as safe as those derived from conventional cotton varieties (ANZFA 2002; ANZFA 2000; ANZFA 2001b).

2.2.1 Exposure to GM cotton products through wearing clothing and using household products made from cotton lint

121. Cotton fabrics, used in clothing, upholstery, towels and other household products, are made from the cotton lint (long fibres) which surrounds the cottonseed. 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 pharmaceutical's, cosmetics, toothpaste, lacquers, paints and variety of plastics (Gregory et al. 1999). Cotton fibre is widely used in pharmaceutical and medical applications because of its very low allergenicity.

122. Processed cotton lint, linters and oil contain no detectable DNA or protein (Leffler & Tubertini 1976; Sims et al. 1996). Fibre characteristics (length, strength, fineness) of commercially released Roundup Ready[®] cotton are the same as for non-GM varieties (Cotton Seed Distributors 2002). The MON 88913 cottons are expected to differ from Roundup Ready[®] cotton only in the level of expression of the CP4 EPSPS protein (which is not present in lint) and the applicant expects fibre characteristics to be equivalent to non-GM varieties. Therefore the safety of wearing cotton clothing or using other products made from MON 88913 cottons is not likely to be different from that of current Roundup Ready[®] or Roundup Ready[®]/Bollgard[®] II cotton or non-GM cotton.

2.2.2 Exposure to GM cotton through working with cotton and living near cotton plantations

123. 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. A comprehensive review of conventional cotton, including information on its toxicity, allergenicity and pathogenicity, is provided in the document ‘The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia’, available at www.ogtr.gov.au.

124. Humans working with cotton plants would be exposed primarily to the outer waxy cuticle layer at the plant surface, to the seed coat or to the cotton fibres, all of which are essentially free of protein. Exposure to proteins (including the new proteins expressed in the GM cottons) or to other cellular components of the cotton plants will only occur if plant cells are ruptured.

125. Even if exposure to the introduced proteins in Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton occurs, these proteins are not toxic or allergenic to humans (see Section 2.4). The introduced proteins present in the MON 88913 cottons (the CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII proteins) are the same as those present in commercially released Roundup Ready[®] and Bollgard[®] II cottons. There have been no confirmed reports of adverse effects on health through occupational exposure from people working with GM cottons expressing these proteins or conventional Bt sprays containing the Cry toxins. As the level of expression of CP4 EPSPS protein in Roundup Ready[®] MON 88913 cotton is considered to be higher than the commercially approved Roundup Ready[®] cotton, the applicant proposes to collect data on protein expression as part of the proposed field trials under Australian environmental conditions.

126. Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002), therefore limiting possible exposure to cotton pollen as a potential airborne allergen. The introduced Cry1Ac, Cry2Ab, GUS and NPTII proteins are expected to be expressed at very low levels in the pollen of the GM cottons, based on expression of the Cry1Ac protein in INGARD[®] cotton pollen (relative to that in other plant tissues) and the similarity of the promoter elements controlling the expression of the introduced genes (see Appendix 1). The CP4 EPSPS protein may be expressed at higher levels in pollen of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton. However, this protein has no known toxicity or allergenicity (see Section 2.4). As noted in Appendix 1 (Section 6), the applicant proposes to gather data on expression of the CP4 EPSPS protein in Roundup Ready[®] and Roundup Ready[®]/Bollgard[®] II as part of the proposed field trials under Australian conditions.

127. The primary processing of cotton at cotton gins, and the bulk handling of cottonseed and cotton fibre, can create and stir up fine dust and lint particles. Use of personal protective equipment by exposed workers is commonplace in such facilities, to prevent respiratory irritations. Fibre characteristics (length, strength, fineness) of Roundup Ready[®] cotton are the same as for non-GM cotton varieties (Cotton Seed Distributors 2002). The fibre characteristics of MON 88913 cottons are expected to be equivalent to Roundup Ready[®] cotton and non-GM cotton. The cotton lint derived from the MON 88913 cottons is no more likely to induce adverse responses in workers than is conventional cotton. However, the applicant proposes to assess the fibre characteristics of the MON 88913 cottons as part of the proposed trials.

Section 2.3 Other sources of CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII in the environment

128. The CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII proteins are widespread in the environment, through the presence of the bacteria to which they are native. All five proteins can be found in or on fresh food.

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

130. The native Cry1Ac and Cry2Ab proteins are naturally produced by the bacterium *Bacillus thuringiensis* variety *kurstaki* (Btk). Related Cry toxins are also produced by other varieties of *Bacillus thuringiensis*. Bt spores and their crystal (Cry) toxins are found widely in soils, on plant leaves and in grain stores (Meadows 1993).

131. The presence of Bt toxins in food has increased over the past 30 years due to the commercial use of Btk microbial sprays to protect food crops, including ‘organic’ crops, from insect attack. Residues of Btk proteins, including Cry1Ac and Cry2Aa proteins (to which the Cry2Ab protein is closely related), are present on a wide variety of foods, including fresh foods such as lettuce and tomato, with no reported toxic or allergic responses (ANZFA 1999). The World Health Organisation’s (WHO) International Program on Chemical Safety report on environmental health criteria for Bt concluded that ‘Bt has not been documented to cause any adverse effects on human health when present in drinking water or food’ (International Programme on Chemical Safety 1999). The Cry1Ac and Cry2Ab proteins produced by Bollgard® II cotton lines are almost identical to those found in nature and in the Btk microbial insecticide formulations (Adang et al. 1985).

132. The GUS protein is derived from *Escherichia coli* and is, therefore, already present in the gut of many animals, including humans. GUS enzyme activity has been detected in numerous microbial, plant and animal species, including species used as raw food (Gilissen et al. 1998). The GUS protein used in GM plants is 99.8 % homologous to the *E. coli* GUS protein.

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

Section 2.4 Toxicity and allergenicity of the introduced proteins

134. There will be no opportunity for people to consume food products (oil and linters) of the GM MON 88913 cottons, nor will cottonseed be fed to cattle. However, it is worth noting that the introduced proteins present in the MON 88913 cottons are the same as those present in commercially released Roundup Ready® and Bollgard® II cottons. Studies using the purified forms of the introduced proteins present in Roundup Ready® and Bollgard® II cotton have been conducted. Detailed

descriptions of the results of these studies are available in the risk assessment and risk management plans for DIR 012/2002, DIR 022/2002 and DIR 023/2002. The key results are summarised here.

2.4.1 Toxicity

THE CP4 EPSPS PROTEIN

135. Purified CP4 EPSPS protein, at acute doses of up to 572 mg/kg body weight, produced no adverse effects in mice. This is more than a thousand times the anticipated potential consumption of CP4 EPSPS in commercial food derived from all GM food crops expressing this enzyme under development by Monsanto at that time (soybean, potato, tomato, corn) (Harrison et al. 1996). FSANZ has concluded that foods derived from Roundup Ready[®] cotton expressing the same CP4 EPSPS proteins are as safe as those derived from conventional varieties (ANZFA 2000). Likewise, the US EPA has issued an exemption from the requirement for tolerance for residues for the CP4 EPSPS protein, and Roundup Ready[®] cotton has been approved for use in food in a number of other countries (see Chapter 1, Section 2.4).

THE CRY1AC AND CRY2AB PROTEINS

136. Purified Cry1Ac protein, at acute doses of up to 4300 mg/kg body weight, produced no adverse effects in mice (Naylor 1993a; Naylor 1993b). Likewise, acute oral toxicity studies in mice with purified Cry2Ab protein at doses of up to 1450 mg/kg have not shown any adverse effects (Bechtel 1999).

137. Multiple studies on the acute oral toxicity of Bt microbial preparations, containing Cry1Ac and Cry2Aa (to which Cry2Ab is very similar), in mammals such as rats and rabbits have revealed no adverse effects at very high doses (Carter & Liggett 1994; McClintock et al. 1995; Barbera 1995; Spencer et al. 1996). Two separate studies on humans found no observable health effect of an oral dose of 1000 mg of Bt microbial spores per day for 3 or 5 days (McClintock et al. 1995; Betz et al. 2000).

138. The US Environment Protection Agency (EPA) considers Cry1Ac protein to be non-toxic to mammals and has established an exemption from residue tolerance requirements (EPA 2000). In Australia, the APVMA has also determined that a maximum residue limit (MRL) for human food and animal feed is not necessary for the Cry1Ac protein expressed in GM cotton, indicating that it is of no toxicological significance (see The MRL Standard, table 5 at: www.apvma.gov.au/residues/mrl_standard.shtml). FSANZ has concluded that oil and linters derived from Bollgard[®] II cotton are as safe as those derived from conventional cotton varieties (ANZFA 2002).

THE GUS PROTEIN

139. Acute oral toxicity studies in mice, with purified GUS protein at doses of up to 100 mg/kg did not show any adverse effects (Naylor 1992). FSANZ has concluded that food derived from glyphosate-tolerant sugarbeet, which expresses the GUS protein, and from Bollgard[®] II cotton, is safe for human consumption (ANZFA 2002; ANZFA 2001a). The US EPA does not consider GUS to be toxic for mammals and has approved its exemption from the requirement to establish tolerance levels.

THE NPTII PROTEIN

140. An acute oral toxicity study in mice, in which the purified NPTII protein was fed at doses of up to 5000 mg/kg of body weight (2500 mg/kg administered twice, four hours apart), did not show any adverse effects (Berberich et al. 1993). The US FDA has concluded that NPTII does not possess any properties that would distinguish it toxicologically from other phosphorylating enzymes in the food supply, and which are present in all plants and animals. NPTII is approved as an additive in food for human consumption in the US (FDA 1994). The US EPA has also established an exemption for NPTII from the requirement for a residue tolerance limit when used as a plant pesticide inert ingredient (EPA 1994).

2.4.2 Allergenicity

141. 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 (Metcalf et al. 1996; Taylor & Lehrer 1996). For example, food allergens are usually present as a major component of the ingested food and are resistant to heat, protease digestion and to the acid conditions of the stomach (Astwood et al. 1996; Metcalf et al. 1996; Taylor & Lehrer 1996; Kimber et al. 1999). The allergenic proteins of many major sources of allergens, including food allergens, have been characterised by molecular means, allowing comparisons to be made as a useful step in assessment of allergenic potential (Metcalf et al. 1996).

142. None of the introduced proteins in Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton are derived from known allergens. Nevertheless, as noted in Section 2.2, GM cotton seed produced in the proposed field trials will not be used in human food or animal feed.

THE CP4 EPSPS PROTEIN

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

THE CRY1AC AND CRY2AB PROTEINS

144. The Cry1Ac protein is heat labile and rapidly degraded, in under 30 seconds, under simulated mammalian gastrointestinal conditions (Fuchs et al. 1993a). Neither the Cry1Ac or Cry2Ab proteins display characteristics common to known food allergen proteins. Searches of allergen sequence databases have shown no significant matches of the Cry1Ac or Cry2Ab proteins to known allergens (Metcalf et al. 1996).

145. While there have been reports in the US claiming allergic reactions to Bt microbial products in topical insecticidal sprays, these are not due to the Cry1Ac or Cry2Ab proteins. A survey conducted among farm workers who picked vegetables treated with Bt microbial products indicated that exposure to Bt products may lead to allergic skin sensitisation, however there was no clinical allergic disease in any of the workers. Most reactions in these workers were shown to be due to other constituents of the Bt sprays, and there was no evidence of antibodies specific to the endotoxin proteins of the Bt sprays (Bernstein et al. 1999). The US EPA have also determined that reports of

reactions to Bt microbial products have been due to non-Cry proteins produced during fermentation or to other ingredients added to the insecticidal formulations (EPA 2001).

THE GUS PROTEIN

146. The GUS protein is unlikely to be a major allergen and does not display the characteristics common to known allergens or homology to known allergens. Exposure of GUS to simulated mammalian digestive systems results in rapid denaturation (ANZFA 2001a).

THE NPTII PROTEIN

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

SECTION 3 CONCLUSIONS REGARDING TOXICITY OR ALLERGENICITY

148. It is considered that the risk of Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton being toxic or allergenic for humans is very low because:

- cotton lint used in clothing and household items contains no proteins or DNA and cotton products from GM cottons can not be distinguished from non-GM products;
- cotton pollen is not wind-dispersed and therefore unlikely to be an air-borne allergen;
- none of the GM cotton materials from the release will be used in human food or animal feed;
- exposure to the introduced proteins through working with cotton plants is very low;
- humans are commonly exposed to the CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII proteins, as these proteins are naturally widespread in the environment;
- feeding studies indicate that the introduced proteins are not toxic;
- evidence indicates that the introduced proteins are not allergenic, nor do they have properties of known allergenic proteins;
- there have been no reported toxic or allergic effects from similar GM cottons expressing the same proteins that have been extensively field trialed and are commercially released in Australia; and
- although dust and lint from cotton can be created at processing facilities, the use of protective equipment prevents respiratory irritation, and fibre characteristics of the GM cottons are likely to be the same as for non-GM cotton.

149. FSANZ approval will be required before the GM materials could be used for food.

150. As a condition of licence, Monsanto must 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.

151. As a condition of licence, Monsanto is required to determine the expression levels of the introduced proteins in Roundup Ready[®] MON 88913/Bollgard[®] II cotton.

APPENDIX 3 TOXICITY TO NON-TARGET ORGANISMS

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

153. It should be noted that since the commercial release of the similar GM cottons, Roundup Ready[®] and Roundup Ready[®]/Bollgard[®] II cotton, which express the same introduced proteins as Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton, there have been no reported adverse effects on non-target organisms. However, expression levels of the introduced proteins in Roundup Ready[®] MON 88913/Bollgard[®] II cotton have not been determined.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY HAZARD

154. Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cottons differ from conventional cotton in the expression of one and five additional proteins, respectively. These are the CP4 EPSPS protein (in both GM cottons) and the Cry1Ac, Cry2Ab, GUS and NPTII proteins (in Roundup Ready[®] MON 88913/Bollgard[®] II cotton only). The potential for these cottons to be toxic to organisms, other than the target pests of Bollgard[®] II cotton (lepidopteran caterpillars), is considered. This could occur either due to expression of the novel gene products or because of unintended effects of the genetic modifications.

155. If Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/Bollgard[®] II cotton is toxic for non-target organisms, the potential hazards could include adverse impacts on:

- wildlife, including mammals, fish and birds;
- invertebrates, including beneficial insects (pollinators, parasitoids or predators of insect pests); and
- microbial organisms, particularly soil microorganisms.

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

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

157. The use of Roundup Ready[®] herbicide (a formulation of glyphosate) on GM cotton crops, in Australia is registered by the APVMA. As part of their assessment of this use, the APVMA consider any potential health and environmental effects, such as toxicity to non-target organisms and herbicide residues in crops. Thus risks associated with the use of glyphosate are not considered in the risk assessment of these GM cottons. It should be noted, however, that the cultivation of Roundup Ready[®] cotton in Australia has led to a shift in herbicide use (away from residual or environmentally persistent

herbicides) rather than an increased use (Australian Cotton Cooperative Research Centre 2002a, data from Monsanto).

SECTION 2 LIKELIHOOD OF THE TOXICITY HAZARD OCCURRING

158. In assessing the likelihood of adverse impacts due to toxicity of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton, a number of factors were considered including:

- the inherent toxicity of conventionally bred cotton;
- the potential exposure to the introduced proteins from other sources in the environment;
- information about the likely routes of exposure to Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton and to the introduced proteins, for example, through direct contact with the crop or through contact with soil in which the crop is grown; and
- the potential toxicity of the new proteins expressed in the cotton for particular species, including mammals, fish and birds, non-target invertebrates and soil microorganisms.

159. Potential non-target effects of the insecticidal proteins expressed in Roundup Ready[®] MON 88913/Bollgard[®] II cotton have been considered in detail in the risk assessment and risk management plans for Bollgard[®] II cotton (DIR 012/2002), and INGARD[®] cotton (DIR 022/2002), available at www.ogtr.gov.au, and are only presented here in summary.

Section 2.1 Toxicity of conventionally bred cotton

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

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

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

163. 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 pesticides that could be found in the cotton trash and stubble.

Section 2.3 Other sources of CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII in the environment

164. As discussed in Appendix 2, the CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII proteins are widespread in the environment. The genes for these proteins have been derived from common soil bacteria, and are hence already a natural component of the soil.

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

166. The native Cry1Ac and Cry2Ab proteins are naturally produced by the bacterium *Bacillus thuringiensis* variety *kurstaki* (Btk). Related Cry toxins are also produced by other varieties of *Bacillus thuringiensis*. Bt spores and their crystal toxins are found widely in both the agricultural and natural environment, including in soils, on plant leaves, in grain stores and in dead insects (Meadows 1993).

167. The presence of Bt toxins in agricultural situations has increased over the past 30 years due to the commercial use of Btk microbial sprays to protect food crops, including ‘organic’ crops, from insect attack. Residues of Btk proteins, including Cry1Ac and Cry2Aa proteins (to which the Cry2Ab protein is closely related), are present on a wide variety of foods, including fresh foods such as lettuce and tomato, with no reported toxic or allergic responses (ANZFA 1999). The World Health Organisation’s (WHO) International Program on Chemical Safety report on environmental health criteria for Bt concluded that ‘Bt has not been documented to cause any adverse effects on human health when present in drinking water or food’ (International Programme on Chemical Safety 1999).

168. The GUS protein is derived from *Escherichia coli* and is, therefore, already present in the gut of many animals, including humans. GUS enzyme activity has been detected in numerous microbial, plant and animal species (Gilissen et al. 1998). The GUS protein used in GM plants is 99.8 % homologous to the *E. coli* GUS protein.

169. 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 hazards for stock and wildlife, including mammals, birds and fish

2.4.1 Exposure of stock and wildlife to cotton

170. None of the cotton plants from the release, or their by-products, will be used as stockfeed. Most mammals avoid feeding on cotton due to the presence of gossypol and other components of

cotton tissues and the morphology of the plant (OGTR 2002). Cotton tissue, particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritive factors, including gossypol and cyclopropenoid fatty acids. In the field, seed cotton is present as large lint-covered seeds that are unattractive to avian species (OGTR 2002), so birds are not likely to be exposed to the introduced proteins in the seeds of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton.

171. Cottonseed or pollen is not expected to enter aquatic habitats in any significant quantity, limiting exposure of aquatic organisms. Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on farm to minimise the entrance of pesticide residues into natural waterways. The licence for the release includes a requirement to separate cotton trials from natural waterways by at least 50 m.

2.4.2 Toxicity of Roundup Ready[®] MON 88913 cotton and Roundup Ready[®] MON 88913/Bollgard[®] II cotton to stock and wildlife

172. As noted above, none of the cotton plants, or their by-products, could be used as stockfeed. However, it is worth noting that the introduced proteins present in the MON 88913 cottons are the same as those present in commercially released Roundup Ready[®] and Bollgard[®] II cottons. Studies using the purified forms of the introduced proteins present in Roundup Ready[®] and Bollgard[®] II cotton have been conducted. Detailed descriptions of the results of these studies are available in the risk assessment and risk management plans for DIR 012/2002, DIR 022/2002 and DIR 023/2002. The key results are presented in Appendix 2, Section 2.4 as well as summarised here.

173. Acute oral toxicity studies in a range of mammalian species (mice, rats and rabbits) with each of the CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII proteins have not demonstrated any adverse effects. Likewise, in trials with rats, quail or catfish fed cottonseed meal at 5 to 20 % of the diet, no significant differences in weight gain, feed conversion or gross necroscopy were found for animals fed Roundup Ready[®] cottonseed meal compared to those fed non-GM cottonseed meal (Canadian Food Inspection Agency 1997).

Section 2.5 Potential toxicity hazard for invertebrates

2.5.1 Exposure of invertebrates to cotton

174. Non-target invertebrates may be exposed to Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton and to the introduced proteins directly, through feeding on the plants and potentially, via the soil, when cotton tissues break down and are incorporated into the soil. Exposure may also occur indirectly, through eating other organisms, including the lepidopteran target organisms of Roundup Ready[®] MON 88913/Bollgard[®] II cotton, that feed on the plants.

175. Relative exposure will be greatest for herbivorous species feeding on the cotton plants. Sap feeders, such as aphids, will have minimal exposure to the introduced proteins as the sap is primarily composed of sugars and mineral salts dissolved in water. However, species feeding on lepidopteran larvae may be exposed to both the full-length Cry1Ac and Cry2Ab proteins and the activated core toxins, since their lepidopteran prey may have ingested GM 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.

176. Pollinator species and various insects that feed on pollen will also have low exposure to the introduced Cry1Ac, Cry2Ab, GUS and NPTII proteins, because of their expected much lower expression in pollen, relative to that in other plant tissues, based on expression of the Cry1Ac protein in INGARD[®] cotton pollen and the similarity of the promoter elements controlling expression of the introduced genes. The levels of CP4 EPSPS protein in pollen may be higher due to the presence of different promoter elements (see Appendix 1).

177. Non-target lepidopteran species may be exposed to the GM cotton and may be affected by the Bt toxins. However, cotton is not the preferred food source for non-target lepidopteran species, and their populations would be sustained on other types of plants found around field trial locations.

2.5.2 Toxicity of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton for invertebrates

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

179. Most interest in toxicity of GM cottons for invertebrates has concentrated on the insecticidal Cry1Ac and Cry2Ab proteins, which are expressed in Roundup Ready[®] MON 88913/Bollgard[®] II cotton. Potential non-target effects of the Cry1Ac and Cry2Ab proteins have been considered in detail in the risk assessment for the commercial release of Bollgard[®] II cotton (DIR 012/2002) and in the risk assessment for continued commercial release of INGARD[®] cotton (expressing only the Cry1Ac protein), DIR022/2002, available at www.ogtr.gov.au. A summary is presented below.

STUDIES CONDUCTED UNDER CONTROLLED CONDITIONS

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

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

- the larval and adult honey bee (*Apis mellifera* L.), a beneficial insect pollinator (Maggi 1993a; Maggi 1993b);
- parasitic Hymenoptera (*Nasonia vitripennis*), a beneficial parasitoid of the housefly (*Musca domestica*) (Palmer & Beavers 1993c; Sims 1994);

- ladybird beetles (*Hippodamia convergens*), a beneficial predatory insect which feeds on aphids and other plant bugs commonly found on stems and foliage of weeds and cultivated plants (Palmer & Beavers 1993b; Sims 1994);
- green lacewing larvae (*Chrysopa carnea*), a beneficial predatory insect commonly found on cotton and other cultivated crops (Palmer & Beavers 1993a; Sims 1994); and
- springtails (*Folsomia candida* and *Xenylla grisea*), scavenging soil insects important in nutrient cycling (Sims & Martin 1996).

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

183. The toxicity of the Cry2A class of Bt toxins, to which Cry2Ab belongs, has been tested against at least 35 insect species representing ten orders. Bt toxins of this class are specifically toxic to lepidopteran and in some cases, dipteran, insects. The Cry2Ab toxin expressed by Bollgard[®] II cotton has specific toxicity for lepidopterans (Widner & Whiteley 1990; Widner & Whiteley 1989; Dankocsik et al. 1990).

184. Data from feeding studies indicate that Cry2Ab is not toxic for larval or adult honey bees (*Apis mellifera* L.) (Maggi 2000a; Maggi 2000b). Data supplied by Monsanto indicate that Cry2Ab has no significant impact on the mortality of green lacewing (*Chrysoperla carnea*), a ladybird beetle (*Hippodamia convergens*), a parasitic wasp (*Nasonia vitripennis*), a collembolan (*Folsomia candida*) or an earthworm (Palmer & Krueger 2000a; Palmer & Krueger 2000b; Palmer & Krueger 2000c; Palmer & Krueger 2000d).

STUDIES CONDUCTED IN THE FIELD

185. The effects of INGARD[®] cotton (expressing Cry1Ac protein) on non-target arthropod populations were studied over two seasons in cotton fields near Dalby, QLD (Addison 2001a; Addison 2001b). 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.

186. 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.

187. An American study compared 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). 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.

188. 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).

189. 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. While such indirect impacts have been demonstrated in several studies (see Groot 2002), the impacts relate to altered arthropod community structure in the agricultural field in which the GM insecticidal plant was cultivated and it is likely that affected predator species simply dispersed to nearby non-GM habitats (Groot & Dicke 2002). Rigorous evaluation of potential risks posed by GM insecticidal cottons to the structure and function of multi-trophic ecological communities in natural ecosystems that may occur near GM insecticidal cotton crops therefore remains challenging. It is likely, nevertheless, that if any such impacts were to be documented, they would derive from arthropod herbivores that ingest GM insecticidal cotton tissues expressing Cry1Ac and Cry2Ab because, as noted elsewhere in this appendix, these Cry toxins are specific to lepidoptera and non-target lepidoptera are likely to have alternative and more preferred host plants.

190. There remains some doubt over the potential impact of Cry2Ab expressed in insecticidal GM cottons on particular classes of Diptera. An independent research organisation will conduct studies to obtain further information on this issue.

Section 2.6 Potential toxicity hazard for microorganisms

2.6.1 Exposure of microorganisms to cotton

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

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

193. 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. Generally in Australia cotton is grown in alkaline soil, with a pH ranging from 7.5 – 8.5 (Australian Cotton Cooperative Research Centre 2002b), in which Bt endotoxins would desorb from clay soils and be degraded by soil microorganisms (Tapp & Stotzky 1998). Thus the Cry1Ac and Cry2Ab proteins are not likely to accumulate in agricultural soils as a result of successive seasons of cultivation of GM cottons expressing these proteins.

2.6.2 Toxicity of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton for microorganisms

194. The direct effects of the CP4 EPSPS, GUS and NPTII enzymes have not been tested on microorganisms. EPSPS enzymes are present in all plants, bacteria, algae and fungi and the CP4 EPSPS enzyme expressed by the MON 88913 cottons is derived from a common soil bacterium. The GUS enzyme is also derived from a common bacterium and is already present in soil and water ecosystems. Thus the expression of a bacterial EPSPS or GUS enzyme in plants is not likely to have any adverse effect on soil microorganisms.

195. NPTII is a phosphorylating enzyme which does not possess any properties that distinguish it toxicologically from other phosphorylating enzymes present in microorganisms, plants and animals (FDA 1994). The function of this enzyme is the phosphorylation (inactivation) of the antibiotic neomycin (and the related kanamycin). In the environment, this enzyme is not likely to be active outside of living cells, as it requires specific chemical conditions for activity, including the availability of specific co-factors. Although antibiotic production by non-pathogenic bacteria has been implicated in suppression of some plant diseases (Brimecombe et al. 2001), no evidence for the involvement of neomycin or kanamycin has been found in a search of the scientific literature. Neither are these antibiotics used in agriculture for controlling soil borne disease. Thus the presence of NPTII in soil is not expected to impact on microbial populations or plant disease susceptibility. Furthermore, expression of NPTII in a variety of crop plants (for example, canola, corn, cotton, and tomato), over several years of agronomic performance testing and commercial cultivation, has not been linked to any increased occurrence of disease.

196. Potential effects of the Cry1Ac protein on microorganisms have been considered in detail in the risk assessment for the continued commercial release of INGARD[®] cotton, DIR 022/2002, available at www.ogtr.gov.au. In a preliminary experiment, Gupta et al. (2002) examined the decomposition of INGARD[®] cotton and non-GM cotton plant residues, finding that the GM material decomposed more slowly. Fungal colonisation and total microbial activity was greater on INGARD[®] cotton residues, however total carbon usage by rhizosphere microorganisms (as measured on added ¹⁴C-substrate) was reduced.

197. The effect on soil microorganisms of a truncated Cry 1Ac toxin, either purified or in GM cotton tissue, have been examined (Donegan et al. 1995; Donegan & Seidler 1998). Numbers and types of protozoans, bacteria and fungi, as well as substrate utilisation tests and DNA fingerprinting of eubacterial ribosomal sequences, were used to analyse the composition of microbial soil community. The addition of purified Cry1Ac toxin to soil did not cause any detectable effects. Similarly, there was

no difference between soils to which non-GM leaves were added with or without Cry1Ac toxin. Leaves of GM cotton produced a short-term stimulatory effect on soil bacterial and fungal populations, relative to leaves from the non-GM cotton line. It was speculated that this may reflect faster decomposition and nutrient release from the transgenic leaves compared to the non-GM leaves, possibly due to some unknown and unintended effect of the genetic modification (or the tissue culture process involved) on the plant characteristics, separate from expression of the truncated Cry1Ac protein.

198. It should be noted that the licence has a requirement to conduct research on the potential effect on soil biota of the new proteins expressed in GM insecticidal cottons, including information on the biodegradability of the introduced proteins.

SECTION 3 CONCLUSIONS REGARDING TOXICITY TO NON-TARGET ORGANISMS

199. It is considered that the risk of Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/Bollgard[®] II cotton being toxic to non-target organisms (other than Lepidoptera) is low because:

- the introduced proteins are already widespread in the environment, through the presence of the bacteria from which they are derived;
- exposure of stock and wild life to these GM cottons is low;
- the toxicity of Cry1Ac and Cry2Ab is highly specific to lepidopteran insect larvae;
- the CP4 EPSPS, GUS and NPTII enzymes are not known to be toxic to any organisms;
- laboratory and field studies suggest that populations of key non-target invertebrates are unlikely to be affected by the Bt toxins. Indeed it is likely that their populations would be favored by decreases in the use of broad-spectrum insecticides; and
- the Cry1Ac and Cry2Ab toxins are unlikely to have adverse affects on soil biota and soil microorganisms.

200. Further information on the potential impacts of Cry proteins from GM cottons on soil biota is required. The licence contains a requirement to conduct research on the potential effect on soil biota of the new proteins expressed in GM insecticidal cottons, including information on the biodegradability of the introduced proteins.

201. The licence holder is required to report any adverse effects on human health and safety or the environment (for example, any indication of toxicity of the GM cottons for non-target organisms). Refer to Appendix 7 for the details of licence conditions.

202. The licence holder is required to determine the expression levels of the introduced proteins in Roundup Ready[®] MON 88913/Bollgard[®] II cotton.

APPENDIX 4 WEEDINESS

203. 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. In this Appendix, risks posed by the proposed dealing to the environment are considered in relation to the potential for the GMOs to become a problematic weed.

204. 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

205. Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton differ from conventional cotton in the expression of one and five additional proteins, respectively. These are the CP4 EPSPS protein (in both GMOs) and the Cry1Ac, Cry2Ab, GUS and NPTII proteins (in Roundup Ready[®] MON 88913/Bollgard[®] II cotton only) (see Appendix 1).

206. The possibility was considered that Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/Bollgard[®] II cotton might have the potential to be harmful to the environment, because of inherent weediness or increased potential for weediness, either due to expression of the novel gene products or as a result of unintended effects of the genetic modification.

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

208. The use of Roundup Ready[®] herbicide (a formulation of glyphosate) on GM cottons in Australia is registered by the APVMA. As part of their assessment of this use, the APVMA consider any potential environmental effects, such as development of glyphosate-resistant weeds, levels of glyphosate in the environment and drift from spray applications. Therefore, risks associated with the use of glyphosate are not considered in the risk assessment of these GM cottons. The risk of development of glyphosate-resistant weeds is also addressed briefly in Appendix 6.

SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

209. In assessing the likelihood of adverse impacts due to weediness of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton, a number of factors were considered, including:

- the inherent weediness of conventionally bred cotton;
- the means of dispersal of cottonseed into the environment;
- the potential weediness of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton;

- the potential selective advantage conferred by the introduced CP4 EPSPS, Cry1Ac, Cry2Ab, GUS and NPTII proteins; and
- data gathered from the environmental monitoring program for currently commercially released Roundup Ready[®] and Roundup Ready[®]/INGARD[®] cotton.

Section 2.1 Inherent weediness of conventional cotton

210. Attributes of non-GM cotton associated with potential weediness are discussed in the document ‘The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia’ (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at www.ogtr.gov.au. In summary, the document concludes that non-GM cotton is not a problematic weed in Australia, because factors including soil moisture, nutrient limitation, and roadside management practices limit the establishment and/or persistence of cotton seedlings. Further information on the weediness of non-GM cotton is included here to establish a baseline for comparison with the GM cottons being considered.

211. 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).

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

Section 2.2 Potential weediness of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton

213. Many of the characteristics associated with weediness are also important agronomic characteristics. Consequently these are assessed as part of the agronomic evaluations during the development of new cotton varieties, including GM varieties. Such characteristics have been assessed for commercially released Roundup Ready[®] and Roundup Ready[®]/Bollgard[®] II cotton. The MON 88913 cottons differ from these Roundup Ready[®] cottons only in improved expression of the CP4 EPSPS protein and reproductive tolerance to glyphosate. Therefore, data gathered in the evaluation of the commercially released cottons can be considered in evaluation of the potential weediness of the MON 88913 cottons. Although the MON 88913 cottons express the same introduced proteins as commercially released Roundup Ready[®] cottons, the site of insertion and level of expression of the introduced genes will be different. Therefore, there may be some possibility for, although considered low, pleiotropic effects of the genetic modification, which could potentially alter some aspect of the cotton biology that may affect weediness.

214. No unintended or secondary effects on agronomic characteristics, including no effects on fertility, have been observed in greenhouse or field trials of Roundup Ready[®] MON 88913 cotton in the United States (information supplied by the applicant). The applicant proposes to conduct continued

evaluation of agronomic characteristics of the MON 88913 cottons in Australia as part of the proposed field trials.

215. Seed survival, germination, vigour, yield and disease susceptibility of Roundup Ready[®] and Bollgard[®] II cotton varieties currently being grown have been evaluated in both controlled environments and field releases and are within the range of current non-GM cotton varieties (Moser 2000; Allen et al. 2000; Monsanto Australia Limited 2001; Cotton Seed Distributors 2002). These data suggest that the introduced genes present in Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton are not likely to lead to any unintended effects on characteristics typically associated with weediness.

216. INGARD[®] cotton (expressing the Cry1Ac and NPTII proteins) has been in commercial release since 1996 and Roundup Ready[®] and Roundup Ready[®]/INGARD[®] cottons since 2000. Bollgard[®] II and Roundup Ready[®]/Bollgard[®] II cottons have been in commercial release since 2002. Since their commercial release, cottonseed from these GM cottons has been used as stockfeed in Australia. Over this period there has been no evidence that GM cotton has become weedier than non-GM cotton at latitudes south of 22°S (see Section 2.5). Surveys of volunteer cotton in southern Australia and experimental research on the weedy potential of GM cotton in northern Australia consistently suggest that major factors limiting cotton establishment and survival include water and nutrient availability, herbivory by non-lepidopteran species (vertebrate and invertebrate), plant competition, frost and fire (Eastick 2002; Farrell & Roberts 2002).

217. The potential weediness of GM cottons expressing Bt toxins has been considered in the risk assessment for applications DIR 012/2002 and DIR 022/2002 available at www.ogtr.gov.au, and is only presented here in summary. The results of this research strongly indicate that the likelihood of GM cotton establishing and persisting as a weed in southern Australia at higher levels than the very low rate of establishment of non-GM cotton is negligible, because environmental variables that are unaffected by the genetic modifications are the key limitations on cotton populations. For these reasons, it is also expected that the likelihood of GM cotton establishing as a weed in northern Australia is low, however this is yet to be determined conclusively (see Section 2.5).

218. The provision of additional information on agronomic characteristics indicative of potential weediness of GM cottons in northern Australia is a condition of the Licence

Section 2.3 Environmental monitoring program for Roundup Ready[®] and Roundup Ready[®]/INGARD[®] cotton

219. As part of the proposed licence for continued commercial release of Roundup Ready[®] and Roundup Ready[®]/INGARD[®] cotton, conditions require continuation of monitoring for volunteer cotton in non-agricultural situations for an additional growing season, to meet the expectations of the original three-year program. Original program was developed through consultation with the Genetic Manipulation Advisory Committee (GMAC), the cotton industry, scientists, cotton growers and other interested parties, was designed to assess the potential for these GM cottons to spread and establish populations outside the agricultural environment. This program started in 2001. It comprises three core components:

- monitoring for the incidence of volunteer GM cotton in non-agricultural situations (e.g. roadsides and non-crop areas);

- monitoring for the incidence of volunteer GM cotton in non-GM crops; and
- monitoring for any shifts in weeds resistant to glyphosate associated with the use of these GM crops (see Appendix 6).

220. The data gathered to date were reviewed as part of the risk assessment for application DIR 023/2002, available at www.ogtr.gov.au, and are summarised here.

221. Both GM and non-GM cotton volunteers are found on roadsides along transportation routes and on farms in which cottonseed has been used as stockfeed. In both situations, GM and non-GM volunteers tend to establish in disturbed environments with increased water availability and limited competition from other vegetation. Cotton volunteers appear to have negligible ability to invade non-disturbed habitats (eg. native bush), irrespective of whether or not they are genetically modified. Survival beyond one year appears to be limited and there is no indication that cotton has become a problematic weed in either roadside or farm environments. There were no indications that GM cotton had enhanced survivorship or reproductive potential in any situation.

222. The environmental monitoring program for Roundup Ready[®] and Roundup Ready[®]/INGARD[®] cotton demonstrates that, for regions of Australia south of latitude 22° South, the establishment and persistence of these GM cottons occurs infrequently and does not occur at levels disproportionate to the amount of cottonseed entering the environment. That is, for southern Australia, there is no indication that either Roundup Ready[®] or Roundup Ready[®]/INGARD[®] cotton is weedier than the negligible weediness of non-GM cotton. Moreover, where these volunteers have persisted, they can be managed easily either mechanically or with herbicides other than the glyphosate. Similarly, results of the environmental monitoring program suggest that the weediness of GM cottons in northern Australia is also likely to be very low.

Section 2.4 Potential selective advantage conferred by the introduced proteins

2.4.1 CP4 EPSPS

223. The CP4 EPSPS protein could only confer a selective advantage in the presence of glyphosate herbicide application. In an agricultural setting, Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/ Bollgard[®] II cotton will have increased fitness where glyphosate is applied for weed control. However, glyphosate is not generally used to control established volunteer cotton plants in agricultural systems, as it has limited effectiveness on established cotton (ie. beyond the seedling stage). Cultivation or alternative herbicides are the main control strategies employed (Cotton CRC, 2002).

224. Glyphosate may be used to control weeds on roadsides. In this situation, spraying is often limited to around fixtures such as signs and guideposts, while slashing is used in accessible areas. Evidence suggests that cotton is not a significant weed in these situations and that resistance to glyphosate is not likely to change this (see Section 2.5).

2.4.2 Cry1Ac and Cry2Ab

225. A detailed discussion of the potential of the Cry1Ac protein to influence weediness is provided in the risk assessment document for DIR 022/2002, available at www.ogtr.gov.au. A detailed assessment of the potential of both the Cry1Ac and Cry2Ab proteins together to influence weediness is

provided in the risk assessment for DIR 012/2002. The Cry proteins could confer a selective advantage in areas where lepidopteran insect predation limits one or more of the key life stages of cotton.

226. An investigation into the weediness potential of GM insecticidal cottons has been conducted over two growing seasons in northern Australia (Eastick 2002). Habitats examined were bushland, roadsides, cattle areas and waterways. Data gathered demonstrate that for each of cotton's life stages, the performance of insecticidal cotton is not significantly different to that of conventional cotton. There was no evidence that insecticidal genes enhanced survival. Only limited data was obtained on reproductive capacity, due to low germination, growth and survival in most situations, however INGARD[®] cotton (expressing the Cry1Ac protein) produced significantly higher numbers of bolls at one of the 13 sites studied (an artificial waterway site) in one of the two seasons.

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

2.4.3 GUS

228. The GUS protein is an enzyme involved in bacterial carbohydrate and energy metabolism and is very unlikely to confer any selective advantage to cotton that might result in weediness (Gilissen et al. 1998).

2.4.4 NPTII

229. The NPTII protein could confer a selective advantage to GM cotton plants in the presence of a high concentration of neomycin or kanamycin. However, antibiotics are not applied to cotton crops and are not likely to be present in any environment where cotton grows. Thus the expression of NPTII is highly unlikely to confer any selective advantages on the MON 88913 cottons.

2.4.5 CP4 EPSPS and Cry1Ac, Cry2Ab in combination

230. The potential for weediness of Roundup Ready[®] MON 88913/Bollgard[®] II cotton is not likely to be greater than that for the two parental GM varieties, Roundup Ready[®] MON 88913 cotton and Bollgard[®] II cotton. The herbicide tolerance and the insecticidal genes operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the two genes, their proteins or their metabolic pathways, and no reason to expect that this is likely to occur.

231. Agronomic characteristics of commercially released Roundup Ready[®]/Bollgard[®] II cotton are similar to those of non-GM cotton (Dr G Constable, Program Leader, CSIRO Cotton Research Unit, personal communication; data supplied by Monsanto). Expression of the introduced proteins is also similar in combined trait cotton plants to that in the respective single trait cottons. These data suggest that no unintended effects are likely to occur as a result of the combined genetic modifications in Roundup Ready[®] MON 88913/Bollgard[®] II cotton.

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

Section 2.5 Spread of GM cottons beyond the release sites

233. The proposed dealing includes cultivation of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton and retention of all cottonseed for storage or planting in field trials authorised by the same licence. Cottonseed produced in the proposed field trials will not be used for human food or stockfeed.

234. After harvest, seed cotton may be dispersed beyond the limits of the farms where it is grown during transportation to ginning facilities. The escape of seed cotton during transportation presents an opportunity for the GM cotton to spread in the environment. Specific licence conditions have been imposed to require harvested material to be securely wrapped before transporting away from the release sites so as to prevent seed cotton escaping into the environment.

235. Cotton is primarily a self pollinating plant, with pollen that is unlikely to be dispersed by wind (OGTR 2002). However, insect vectors may transfer pollen to other cotton plants. Pollen dispersal studies have shown that outcrossing is localised around the pollen source and decreases significantly with distance (Umbeck et al. 1991; Llewellyn & Fitt 1996). The applicant proposes to limit outcrossing to non-GM cotton using pollen traps and isolation from all other cotton crops (information provided by the applicant). (Pollen flow is also discussed in Appendix 5, Section 1 in relation to gene transfer.)

236. Licence conditions have been imposed to limit dispersal of seed and pollen.

237. Licence conditions have been imposed to establish a 400 m wide area of land (called a ‘Research Zone’) around Locations in excess of 1 hectare for the purpose of conducting research to inform the ongoing review of the data on spread and persistence of the GMOs in the environment and validate the efficacy of containment measures.

Section 2.6 Persistence of the GM cotton at the release sites

238. Following harvest of the seed cotton from the release sites, the remaining plant material will be slashed and incorporated into the soil. Some seed may fall to the ground during harvesting and also be incorporated into the soil. A soil seed bank represents an opportunity for the GMO to persist in the environment. Cotton has little dormancy, meaning seed will germinate with the arrival of favourable soil moisture and temperature conditions. GM cotton volunteers are expected to germinate in the wet season following harvest.

239. Licence conditions have been imposed to require post harvest monitoring, and destruction of cotton volunteers to ensure that the GM cottons do not persist in the environment at the release sites.

SECTION 3 CONCLUSIONS REGARDING WEEDINESS

240. It is concluded that the risk of Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/Bollgard[®] II cotton establishing as environmental weeds in cotton

growing regions of New South Wales and Queensland and the proposed release areas in northern Australia is low because:

- cotton does not possess characteristics commonly associated with weediness, and is not known to be a problematic weed in any environment;
- cotton has a low potential for dispersal by natural means;
- the introduced genes in the GM cottons are unlikely to affect these characteristics;
- there is a low possibility for the genetic modification to potentially have pleiotropic effects that could alter traits affecting weediness, therefore, applicant proposes to collect data on agronomic characteristics associated with weediness during the release.
- major constraints on weediness of GM and non-GM cotton are water availability, nutrient availability, plant competition, herbivory by non-lepidopteran species, frost and fire;
- the combination of herbicide tolerance and insect resistance in Roundup Ready[®] MON 88913/Bollgard[®] II cotton is unlikely to increase the weedy potential of this GMO above the low potential for either Roundup Ready[®] MON 88913 or Bollgard[®] II cotton individually, to become problematic weeds;
- surveys carried out since the commercial release of Roundup Ready[®] and Roundup Ready[®]/INGARD[®] cotton in 2000 indicate that these GM cottons have not become problematic weeds;
- INGARD[®] cottonseed has been used as stockfeed in northern Australia since 1996, and Roundup Ready[®]/INGARD[®] cotton since 2000, with no indication that they have become more weedy than non-GM cotton; however
- preliminary experimental data suggest that insecticidal cotton may be weedier than non-GM cotton in certain nutrient-rich habitats such as artificial waterways and stock feeding areas.

241. However, further information on the impact of the genetic modifications on agronomic characteristics of GM cottons in Australian field conditions that may be indicative of potential weediness (eg. dormancy of seeds, competitive ability, susceptibility to natural enemies and spread within the environment) will be required before the Regulator could determine this conclusively. This information will be required before a larger scale or less stringently controlled release could be considered.

APPENDIX 5 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

242. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan. This Appendix considers potential hazards that may be posed through the transfer of the introduced genes from Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/Bollgard[®] II cotton to other organisms.

243. 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.

244. The likelihood of hazards arising from gene transfer is dependent on a number of factors that form a necessary chain, including:

- opportunity for gene transfer to occur such that the recipient organism is exposed to the genetic material in the form of pollen, plant cells or DNA; and
- incorporation of the genetic material into the genome of the recipient organism at a site and in a configuration that allows the gene to be functional; and
- persistence of the transferred genetic material such that the newly modified organism is able to survive, reproduce and maintain the genetic modification; and
- significance of the transferred genetic material such that its presence and/or expression in the recipient organism will result in an adverse impact on human health and safety, or the environment.

245. A detailed assessment of the likelihood of gene transfer from GM cottons to other organisms is presented in the applications, DIR 005/2001, 006/2001, 008/2001, 009/2002, 012/2002, 015/2002, 016/2002, 017/2002, 021/2002 and 022/2002.

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

- Section 1 details the nature and likelihood of genes introduced to Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton transferring to other plants, including other cotton crops;

- Section 2 details the nature and likelihood of genes introduced to Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton transferring to microorganisms; and
- Section 3 details the nature and likelihood of genes introduced to Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton transferring to animals, including humans.

247. Section 4 draws together the conclusions from these sections.

SECTION 1 GENE TRANSFER FROM ROUNDUP READY[®] MON 88913 AND ROUNDUP READY[®] MON 88913/BOLLGARD[®] II COTTON TO OTHER PLANTS

Section 1.1 Nature of the gene transfer hazard

248. Transfer of the introduced genes or regulatory sequences to cultivated cotton plants would present the same hazards and have the same potential impacts as the presence of the genes in the GM insecticidal cotton (see Appendices 2 - 6).

249. Feral or naturalised cotton is the same species as cultivated cotton, however the morphological features in these populations suggest they are not derived from modern elite cultivars that have escaped from cultivation, but rather are derivatives of primitive cultigens introduced before 1900 (information provided by CSIRO). If gene transfer to feral cotton occurred, this may increase the likelihood that the genes would persist in the environment. The flow on impacts of such a transfer could depend on whether the introduced genes (*cp4 epsps*, *cryIAC*, *cry2Ab*, *nptII* and *gus*) conferred any selective advantage to the feral cotton in its environment. (Refer to Appendix 4, Section 2.2 for discussion on this issue.)

250. 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.

1.1.1 Potential hazards from the introduced genes

THE *CP4 EPSPS* (HERBICIDE TOLERANCE) GENE

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

THE *CRYIAC* AND *CRY2AB* (INSECTICIDAL) GENES

252. Plants expressing these genes could become toxic to lepidopteran insects. This could confer a selective advantage on the plants or adversely affect survival of lepidopteran insects and consequently other organisms linked to lepidopteran insects through food webs.

THE *UIDA* (REPORTER) GENE

253. Plants expressing this gene would express the bacterial β -glucuronidase enzyme. However, this is unlikely to confer any selective advantage on those plants. Expression of the *uidA* gene enabled selection of plant cells containing the genetic modification in the laboratory.

THE *NPTII* AND *AAD* (ANTIBIOTIC RESISTANCE) GENES

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

PROMOTERS AND OTHER REGULATORY SEQUENCES

255. If these sequences were to be transferred to other plants without the associated genes of Roundup Ready[®] MON 88913 and Roundup Ready[®] MON88913/Bollgard[®] II 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.

256. 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.

257. 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 occurs naturally in all plant genomes and 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 or sequence transfer occurring within the genome of a plant species.

Section 1.2 Likelihood of a hazard arising through gene transfer from Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/Bollgard[®] II cotton to other plants

258. 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 Transfer to cultivated cotton and other trial cotton crops

259. 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”, available at

www.ogtr.gov.au, that was produced in order to inform the risk assessment processes for licence applications involving GM cotton.

260. *Gossypium barbadense* (pima cotton) is also used for commercial cotton production, but only to a very minor extent in Australia (Lake Tandou and Bourke, NSW). *G. hirsutum* and *G. barbadense* are closely related and hybridisation between the two species can occur, yielding fertile progeny. Hybrid progeny exhibit characteristics intermediate to the parents but typically with lower capacity to produce fruit. After several generations, progeny of the hybrids revert to the characteristics of one or other of the parents. *G. barbadense* and hybrids are not more weedy or difficult to control than is *G. hirsutum* (personal communication, Warwick Stiller & Greg Constable, CSIRO).

261. Transfer of the introduced genes or regulatory sequences to other cotton plants including other GM cotton trial crops growing in cultivation would present the same hazards as the presence of the genes in Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton (see Appendices 2 - 6).

262. For this proposed release, the likelihood of the hazard will be further minimised because licence conditions have been imposed to limit cross-pollination to plants outside the release sites (see Chapter 2 and Appendix 7 for details).

263. As part of the OGTR's ongoing commitment to the review of data, specific research conditions have been imposed in the licence. The research is intended to confirm previous research on gene flow undertaken prior to the implementation of the Act and the commercial release of GM cottons and validate the containment measures imposed during field trials of new GM cottons. In order to facilitate this review and data collection, the Regulator has imposed a condition to establish 400 m research zones around Locations in excess of 1 hectare. This research program will be developed in consultation with the OGTR.

1.2.2 Transfer to volunteer and naturalised/feral cotton

264. 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 cotton populations is thought to be unlikely because of the geographic distances between these naturalised populations and the cotton growing regions of NSW and QLD. However herbarium records of *G. hirsutum* and *G. barbadense* suggest that naturalised populations may occur, or may have occurred in the past, in central and south eastern Queensland. The remnants of these populations, which may be within pollinating distance of cotton crops, has not been confirmed. As part of the licence conditions for DIR 022/2002 (INGARD[®] cotton), Monsanto is required to conduct a survey of naturalised cotton populations in Queensland, in location suggested by herbarium records.

265. Transfer of the introduced genes or regulatory sequences to non-cultivated cotton plants would present the same hazards as the presence of the genes in Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton (see Appendices 2 - 6).

266. Licence conditions have been imposed to limit cross-pollination to plants outside the release sites (see Chapter 2 and Appendix 7 for details).

267. Licence conditions have been imposed to establish a 400 m wide area of land (called a 'Research Zone') around Locations in excess of 1 ha for the purpose of conducting research to inform

the ongoing review of data on gene flow and to validate the efficacy of containment measures (see section 1.2.1 above for details).

1.2.3 Transfer to native cottons and other plant species

268. 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 populations.

269. The centre of native *Gossypium* diversity in Australia is in northern Western Australia and the Northern Territory. Most of the Australian *Gossypium* species have limited distributions and occur at considerable geographic distance from cultivated cotton fields. Thus gene transfer from Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton to native cottons is prevented not only by genetic incompatibility but also by geographic constraints to cross-pollination (OGTR 2002).

270. The failure of cross-pollination due to well established genetic incompatibility also prevents gene transfer from Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton to other plant species.

SECTION 2 GENE TRANSFER FROM ROUNDUP READY[®] MON 88913 OR ROUNDUP READY[®] MON 88913/ BOLLGARD[®] II COTTON TO MICROORGANISMS

Section 2.1 Nature of the gene transfer hazard

271. 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).

272. The potential hazards associated with the introduced genes of Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II 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.

2.2.1 Potential hazards from the introduced genes

THE *CP4 EPSPS* (HERBICIDE TOLERANCE) GENE

273. Microorganisms expressing this gene could gain the capacity to synthesise aromatic amino acids and other aromatic compounds in the presence of glyphosate. However this would not have a significant effect on microbial communities, since the ability to rapidly degrade glyphosate is

widespread among microorganisms, and soil microorganism populations are not significantly effected by glyphosate application (Malik et al. 1989).

THE *CRY1AC* AND *CRY2AB* (INSECTICIDAL) GENES

274. 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.

THE *UIDA* (REPORTER) GENE

275. The *uidA* gene present in the GM plants has been modified for optimal expression in plants by the introduction of an intron and consequently a functional GUS protein would not be expressed from this gene in bacteria. Even if a functional GUS protein was expressed by microorganisms, this would not have a significant effect on microbial communities as the enzyme is involved in bacterial carbohydrate and energy metabolism and is widespread among bacterial species (Gilissen et al. 1998).

THE *NPTII* AND *AAD* (ANTIBIOTIC RESISTANCE) GENES

276. 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.

277. 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.

PROMOTERS AND OTHER REGULATORY SEQUENCES

278. If these sequences were to be transferred to microorganisms without the associated genes of Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II 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.

279. 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.

280. 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

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

282. All of the introduced genes in Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/ Bollgard[®] II cotton are already widespread in the environment, being derived from common soil bacteria. The regulatory sequences are derived from common plant viruses, bacteria or plants.

THE *CP4 EPSPS* (HERBICIDE TOLERANCE) GENE

283. All plants, bacteria and fungi carry *epsps* genes. The difference between the *cp4 epsps* gene in Roundup Ready[®] MON 88913 and Roundup Ready[®] MON 88913/ Bollgard[®] II cotton and other *epsps* genes, and the encoded enzymes, is in their DNA and amino acid sequences, not in their physiological functions (see Appendix 1 for details). The *cp4 epsps* gene is derived from the common soil bacterium *Agrobacterium* species strain CP4 (Barry et al. 1992; Padgett et al. 1996), which is found in soil and on plants. The encoded CP4 EPSPS enzyme is naturally insensitive to glyphosate (Padgett et al. 1993), as are a number of other microbial EPSPS enzymes (Schulz et al. 1985; Eschenburg et al. 2002). Thus insensitivity to glyphosate is already widespread in microbial populations.

THE *CRYIAC* AND *CRY2AB* (INSECTICIDAL) GENES

284. The *cryIac* and *cry2Ab* insecticidal genes expressed in Roundup Ready[®] MON 88913/ Bollgard[®] II cotton occur naturally in the common soil bacteria *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).

285. 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 *cryIac* 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 bacteriophage (Glare & O'Callaghan 2000).

THE *UIDA* (REPORTER) GENE

286. GUS enzyme activity is found in plants, vertebrates, invertebrates and many common soil microorganisms (Gilissen et al. 1998). *uidA* genes are thus widespread in microbial populations.

THE *NPTII* AND *AAD* (ANTIBIOTIC RESISTANCE) GENES

287. 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 Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton to microorganisms occurring

288. 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, as discussed in previous sub-sections.

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

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

BACTERIA

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

292. 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.

293. 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 (e.g. Schlüter et al. 1995; Coghlan 2000). Transfer of plant DNA to bacteria has been demonstrated only under highly artificial laboratory conditions, between homologous sequences and under conditions of selective pressure (Mercer et al. 1999; Gebhard & Smalla 1998; De Vries & Wackernagel 1998; De Vries et al. 2001) and even then only, at a very low frequency.

294. Using antibiotic selection to detect extremely rare events, *Acinetobacter* 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 *Acinetobacter* sp. (Nielsen et al. 2000; De Vries et al. 2001) or in *Pseudomonas stutzeri* (De Vries et al. 2001).

RELEASE AND PERSISTENCE

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

BACTERIAL COMPETENCE AND DNA UPTAKE

296. 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).

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

DNA INTEGRATION

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

EXPRESSION AND SELECTION

299. 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 (e.g. 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 *cryIAc*, *cry2Ab* and *cp4 epsps* genes were modified to enhance expression in plants); and
- processing of an encoded 'pro-protein' may be required for production of a functional product (e.g. cleavage of the Chloroplast Transit Peptide from plant EPSPS pro-proteins, see Appendix 1).

300. 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 to 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).

VIRUSES

301. 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 risk of adverse effects arising from gene transfer is reduced because with highly similar sequences the likelihood of any recombinants expressing novel properties is low.

302. 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).

FUNGI

303. 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).

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

SECTION 3 GENE TRANSFER FROM ROUNDUP READY[®] MON 88913 OR ROUNDUP READY[®] MON 88913/ BOLLGARD[®] II COTTON TO ANIMALS

Section 3.1 Nature of the gene transfer hazard

305. The potential hazards associated with the introduced genes in Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II 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.

3.1.1 Potential hazards from the introduced genes

THE *CP4 EPSPS* (HERBICIDE TOLERANCE) GENE

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

THE *CRY1AC* AND *CRY2AB* (INSECTICIDAL) GENES

307. 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.

THE *UIDA* (REPORTER) GENE

308. Animals could express the β -glucuronidase enzyme. This is not likely to lead to any significant effects, since this enzyme is naturally present in animals and their intestinal fauna.

THE *NPTII* AND *AAD* (ANTIBIOTIC RESISTANCE) GENES

309. 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.

PROMOTERS AND OTHER REGULATORY SEQUENCES

310. If these sequences were to be transferred to animals without the associated genes of Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II 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.

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

312. 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.

Section 3.2 Likelihood of hazard arising through gene transfer from Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton to animals (including humans)

313. 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 previous sub-sections.

3.2.1 Humans

314. 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.

315. No products from the GM cottons in the proposed field trials will be used for human food. Thus, the likelihood of gene transfer to humans is negligible. It is worth noting that cotton oil and linters are the only fraction of cotton plants used in human food. Since these products are free of DNA, even if products of the GM cottons were used in food, humans would not be exposed to GM cotton DNA via the digestive system, excluding the possibility of gene transfer to human cells in the gut.

3.2.2 Animals

316. GM cottonseed from the proposed field trials will not be fed to livestock. If GM cottonseed were fed to animals, the most significant route for entry of foreign DNA, as with humans, would be through food as it passes through the gastrointestinal tract. Thus, the likelihood of gene transfer to animals is negligible. The fate of DNA in the digestive tract of various animals has been studied and is discussed in the risk assessment for DIR 022/2002. This risk assessment concluded that the likelihood of transfer via food 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

317. It is considered that although some gene transfer from Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton to cultivated cotton (of both *G. hirsutum* and *G. barbadense*) including other GM cotton trial crops is likely, the risks posed are low because:

- the applicant proposes to surround the trial sites by pollen traps and isolate the trials from feral cotton and all other cotton crops to minimise gene flow and persistence; and
- gene transfer poses no risks in addition to the low risks posed by Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton.

318. Licence conditions have been imposed to limit gene transfer to cultivated cotton (See Chapter 2, Appendix 7). Additional licence conditions have been imposed to establish a 400 m wide area of land (called a ‘Research Zone’) for the purpose of conducting research to inform the ongoing review of the efficacy of containment measures.

319. Although transfer of the introduced genes from Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton to naturalised/feral cotton (both *G. hirsutum* and *G. barbadense*) may increase the likelihood that the genes could spread and/or persist in the environment, it is considered that the likelihood of a hazard arising through gene transfer to naturalised cotton is low, because:

- the applicant proposes to isolate trial sites from naturalised/feral cotton; and
- gene transfer would not pose any risks additional to the low risks posed by Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton.

320. The licence imposes conditions to limit gene transfer to naturalised cotton (See Chapter 2, Appendix 7).

321. It is considered that the risk of gene transfer from Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton to native cotton species is negligible, because:

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

322. It is considered that the risk of gene transfer from Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II 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

323. It is considered that the risk of a hazard arising through transfer of the introduced genes from Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton to microorganisms is negligible, because:

- all of the introduced genes in Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton are already widespread in the environment, and are readily available for transfer from these sources via demonstrated natural mechanisms; and
- gene transfer from plants to bacteria 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

324. Products from the GM cottons will not be fed to animals or entered in the human food supply. The most significant route of entry of foreign DNA into animals and humans is through food. Thus the likelihood of gene transfer to animals or humans is negligible. This is because:

- simulated ruminant digestion studies with model experimental systems indicate that introduced genes and endogenous plant genes are rapidly degraded, representing a considerable barrier to gene transfer; and
- vertebrate animals will not be exposed to the GM cottons.

325. It should be noted that in the extremely unlikely event of such a transfer occurring, human health and safety and the environment are unlikely to be adversely effected.

APPENDIX 6 HERBICIDE TOLERANCE AND INSECTICIDE RESISTANCE

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

327. Regulation of agricultural chemicals, including herbicides and insecticides, is principally the responsibility of the Australian Pesticides and Veterinary Medicines Authority (APVMA) under the *Agricultural and Veterinary Chemicals (Code) Act 1994*. The APVMA operates the national system that evaluates, registers and regulates agricultural and veterinary chemical products. Any changes to a product that is already on the market must also be referred to the APVMA. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits allowing restricted use of a chemical product, for example for a limited period of time or for a limited area.

328. In considering applications for registration or permits, the APVMA also considers a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as the efficacy of herbicides and insecticides, and herbicide and insecticide resistance management. The APVMA can impose conditions on the use of chemical products in registrations and permits. These conditions can include implementation of an insect resistance management or herbicide resistance management plan, and ongoing reporting on compliance and effectiveness.

329. All submissions to the APVMA are treated on their merits and applicants are free to address any issue through the provision of data or through scientific argument. The APVMA can only grant a permit if it is satisfied that the use of a product as specified in the permit:

- would not be an undue hazard to the safety of people exposed to it during its handling or people using anything containing its residues;
- would not be likely to have an effect that is harmful to human beings;
- would not be likely to have an effect that is harmful to animals, plants or things or to the environment;
- would not unduly prejudice trade or commerce between Australia and places outside of Australia;
- would be effective according to criteria that the APVMA has proposed for the product;
- that any requirements prescribed by the regulations have been complied with;
- if the product is not registered, that there are reasonable grounds for an application not having been made or for issuing a permit pending determination of an application for registration; and
- that the applicant is a suitable person to hold the permit, according to criteria determined by the APVMA.

330. As part of its charter, the APVMA also manages a national compliance program in partnership with the States and Territories to ensure that products are used in accordance with their registration and their labels continue to meet the conditions of approval.

331. The APVMA and the OGTR work closely together to ensure thorough coordinated assessments are undertaken and, wherever possible, that timing of assessments and decisions by both agencies coincide. Further information about how the APVMA's assessment and approval processes can be obtained from their website www.apvma.gov.au.

SECTION 1 HERBICIDE TOLERANCE HAZARD

332. Selection for herbicide tolerance has an increased probability of occurring in the long-term if the Roundup Ready[®] MON 88913 or Roundup Ready[®] MON 88913/ Bollgard[®] II cotton was grown on a large scale and without taking any steps to manage this risk..

333. Roundup[®] or Roundup Ready[®] herbicide is not currently registered for use on cotton beyond the four-leaf stage of growth. A research permit from the APVMA for use of glyphosate after this stage on Roundup Ready[®] MON 88913 cotton is not required for the first season of the proposed field trials as less than one hectare of cotton plants per state are proposed to be sprayed with this herbicide. However, an APVMA permit will be required for such use of herbicide in the subsequent years of the proposed trials or any further larger scale release. Further trials or commercial release of Roundup Ready[®] MON 88913 cotton will also require further applications and approvals by the Regulator.

334. As part of the Deed of Agreement between the Australian Government and Monsanto for the commercial release of Roundup Ready[®] and Roundup Ready[®]/INGARD[®] cottons in 2000, Monsanto were required to develop a crop management plan designed to minimise the potential for development of weeds resistant to glyphosate (Monsanto Australia Limited 2001) (refer DIR 023/2002). This plan has been endorsed by the herbicide tolerant cotton subcommittee of the Transgenic and Insect Management Strategy (TIMS) committee of the Australian Cotton Growers Research Association and is enforced by Monsanto under its' Technology User Agreement. Included is a requirement to prevent seed set of weeds that have survived exposure to Roundup[®] herbicide. The requirement to adhere to this herbicide resistance management plan is also continued in the continued commercial release of Roundup Ready[®] cotton as noted in the licence DIR023/2003, issued by the regulator in June 2003.

335. Data collected by Monsanto from growers of Roundup Ready[®] cotton varieties indicates general compliance with this plan. Over three seasons of cultivation of these GM cottons, there has been no indication of a shift in weeds resistant to glyphosate and no change in the level of grower satisfaction with this technology.

SECTION 2 INSECTICIDE RESISTANCE HAZARD

336. Extensive cultivation of Bollgard[®] II cotton varieties, including Roundup Ready[®] MON 88913/Bollgard[®] II cotton, could potentially result in the emergence of resistance to the Cry1Ac and Cry2Ab proteins in the target species (*Helicoverpa armigera* and *H. punctigera*) and other susceptible lepidopteran species feeding on cotton. This would result in a reduction in the efficacy of these cottons for the control of insect pests, and could also have impacts on the use of Bt microbial sprays to control insects in other agricultural systems. Potential adverse effects

include attenuation of the benefits of growing Bollgard® II cotton varieties to the environment and human health.

337. It should also be noted that Bollgard® II cotton was developed with the specific intention of reducing the risk of insects developing resistance to the Cry1Ac or Cry2Ab proteins. The expression of two insecticidal proteins in Bollgard® II cotton, and the fact that the overall insecticidal activity is increased relative to INGARD® cotton, is expected to delay the emergence of resistance insects. INGARD® cotton is currently being phased out in Australia in favour of Bollgard® II cotton.

338. GM insecticidal cotton falls under the Agricultural and Veterinary Chemicals Code (1994) definition of an agricultural chemical product, due to its production of two insecticidal substances, and is thus subject to regulation by the APVMA. In July 2003, the APVMA registered Bollgard® II genes (*cry1Ac* and *cry2Ab*) as insecticidal products in GM cotton. An insecticide resistance management plan for Bollgard® II and Bollgard® II/Roundup Ready® cotton has been developed by the TIMS Committee of the Australian Cotton Growers' Research Association in consultation with the APVMA. The APVMA requires implementation of this plan as a condition of registration. The insecticide resistance management plan is designed to minimise resistance development and requires growers to employ a number of measures designed to achieve this objective. One condition within this resistance management plan specifies that the amount of combined INGARD® and Bollgard® II cotton to be grown by one grower (as defined by the growers trading name) in a valley must not exceed 40% of the total cotton being grown by that grower in that valley. A grower may choose to plant up to 40% Bollgard® II and no INGARD®. These trials will comply with all conditions imposed by the APVMA.

SECTION 3 CONCLUSIONS REGARDING INSECTICIDE AND HERBICIDE RESISTANCE

Section 3.1 Herbicide resistance

339. The potential risk of herbicide-resistant weeds developing if the Roundup Ready® MON 88913 crop-herbicide combination is used inappropriately will be managed by the APVMA, under conditions of permits or registration for the use of agricultural chemicals in Australia. Therefore, the Regulator has not imposed specific conditions in relation to management of herbicide resistance, however the requirement to comply with conditions imposed by the APVMA is noted in the licence.

Section 3.2 Insecticide resistance

340. The potential risk of insect resistance to the insecticidal proteins expressed by Roundup Ready® MON 88913/Bollgard® II cotton is being managed by APVMA, under conditions of permits or registration for the use of insecticidal genes as agricultural chemicals in Australia. Therefore, the Regulator has not imposed specific conditions in relation to management of insecticide resistance, however the requirement to comply with conditions imposed by the APVMA is noted in the licence.

APPENDIX 7 LICENCE CONDITIONS

Note in relation to approval of genetically modified foods for human consumption

Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority), is responsible for human food safety assessment. Currently, Monsanto has not applied to FSANZ for evaluation of material from the GM cottons for use in human food. FSANZ approval would need to be obtained before any parts of the GM cottons such as oil and linters derived from GM cotton seed could be used as human food.

Note in relation to Herbicide Tolerance and Insecticide Resistance Management

The *Gene Technology (Consequential Amendments) Act (2000)* requires the Australian Pesticides and Veterinary Medicines Authority (APVMA, formerly the National Registration Authority for Agricultural and Veterinary Chemicals, NRA) to consult the Gene Technology Regulator for the purposes of making certain decisions regarding registration or issuing a permit for a chemical product that is or contains a genetically modified product.

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

One of the genetically modified organisms referred to in this licence also 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 one of the genetically modified organisms (Bollgard® II), including specifying maximum areas for release, for the purpose of managing the development of insecticide resistance in the target pest species. Conditions of this licence do not relate to management of insecticide resistance, and do not replace any conditions set by the APVMA. The licence holder must comply with any conditions imposed by the APVMA in relation to dealings with this GMO.

SECTION 1 GENERAL CONDITIONS

Duration of Licence

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

Holder of Licence

2. The holder of this licence ('the licence holder') is Monsanto Australia Ltd.

Project Supervisor

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

No dealings with GMOs except as authorised by this licence

4. Persons covered by this licence must not deal with the GMOs except as expressly permitted by this licence.

Permitted dealings

5. The permitted dealings with the GMOs are to plant, grow and conduct experiments with the GMOs, and the possession, supply, use, transport and disposal of the GMOs for the purpose of any of the permitted dealings with the GMOs, or in the course of any of these dealings.

Persons covered by this GMO licence

6. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged to undertake any activity in connection with GMOs grown in a Location pursuant to this Licence.

Informing people of their obligations

7. The licence holder must inform any person covered by this licence, to whom a particular condition of this licence applies, of the following:
 - (a) the particular condition (including any variations of it);
 - (b) the cancellation or suspension of the licence;
 - (c) the surrender of the licence.
8. The licence holder must provide the Regulator, on the Regulator's written request, signed statements from persons covered by this licence that the licence holder has informed those people of the conditions of this licence that apply to them.

Applicant to notify of circumstances that might affect suitability

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

Additional information to be given to the Regulator

10. It is a condition of a licence that the licence holder inform the Regulator if the licence holder:
- (a) becomes aware of additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or
 - (b) becomes aware of any contraventions of the licence by a person covered by the licence; or
 - (c) becomes aware of any unintended effects of the dealings authorised by the licence.

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

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

Remaining an Accredited organisation

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

SECTION 2 INTERPRETATIONS AND DEFINITIONS

This licence does not authorise dealings with GMOs that are otherwise prohibited as a result of the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

In this licence:

Words and phrases used in this licence have the same meaning as they do in the Act and the Regulations;

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) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

Where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning;

Specific conditions prevail over standard conditions to the extent of any inconsistency.

In this licence:

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

‘Clean’ (or **‘Cleaned’**), as the case requires, means:

- (a) in relation to a Location or other area, the Destruction of the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants in that Location or area, to the reasonable satisfaction of the Regulator; or
- (b) in relation to Equipment, the removal and Destruction of the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants from the Equipment, to the reasonable satisfaction of the Regulator.

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

‘Destroy’, (or **‘Destroyed’** or **‘Destruction’**) means, as the case requires, killed by one or more of the following methods:

- (a) stalk pulling; or
- (b) uprooting by ploughing; or
- (c) burning; or

- (d) treatment with herbicide; or
- (e) hand weeding.

Note: ‘As the case requires’ has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate. For example, in the case of killing the remains of harvest of the GMOs, treatment of post harvest remains by herbicide would not be a sufficient mechanism.

‘Equipment’ includes harvesters, seeders, storage equipment, transport equipment (eg bags, containers, trucks), clothing and tools.

‘GM’ means genetically modified.

‘GMOs’ means the genetically modified organism or organisms authorised for release by this licence.

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

‘Material from Pollen Trap plants’ means seed, stubble, pollen or any GM material (including parts of a plant) that is derived from or produced by cotton from a Pollen Trap.

‘Material from the GMOs’ means genetically modified material, including parts of GMOs that are derived from or produced by the GMOs.

‘Natural Waterways’ means waterways other than irrigation channels, holding dams or storage ponds used to collect water runoff from irrigated areas.

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

‘Pollen Trap’ means an area of land, extending at least 20 metres in all directions from the outside edge of a Location.

‘Pollen Trap plant’ means cotton from a Pollen Trap.

‘Regulator’ means the Gene Technology Regulator.

‘Research Zone’ means an area of land, extending at least 400 metres in all directions from the outside edge of a Location.

‘Volunteer plants’ means progeny of the GMOs or Pollen Trap plants, or regrowth of previous GM or non-GM cotton plants.

SECTION 3 SPECIFIC CONDITIONS

Locations and size of trial

- The permitted dealings with the GMOs may be undertaken during the Summer 2003/2004 cotton growing season in the Shires and valleys and sizes and quantities as indicated in below in Table 1:

Table 1: Shires in which the GMOs may be grown and maximum Location sizes

Shire	Valley	State	Maximum area of GMOs Location	Maximum area of GMOs and conventional cotton on Location minus Pollen Trap	Maximum Total Area
Narrabri Shire	Upper Namoi	NSW	0.1608 ha	0.25 ha	1.32 ha
Narrabri Shire	Lower Namoi	NSW	0.1068 ha	0.535 ha	2.16 ha
Narromine Shire	Macquarie	NSW	0.0744 ha	0.596 ha	1.591 ha
Moree Plains Shire	Gwydir	NSW	0.0384 ha	0.48 ha	1.353 ha
Millmeran Shire	Darling Downs	QLD	0.0744 ha	0.596 ha	1.591 ha
Narrabri Shire	Namoi	NSW	0.2 ha	0.2 ha	

- The permitted dealings with the GMOs may be undertaken during the cotton growing seasons in Winter 2004, Summer 2004/2005 and Winter 2005 within the Shires set out in following table:

Table 2: Shires where permitted dealings with GMOs may be conducted

NSW	QLD	NT	WA
Balranald	Aramac	Katherine	Broome
Bingara	Balonne		Wyndham – East Kimberley
Bland	Banana		
Bogan	Cambooya		
Bourke	Chinchilla		
Brewarrina	Clifton		
Broken Hill	Dalby		
Carrathool	Emerald		
Central Darling	Fitzroy		
Coonamble	Flinders		
Deniliquin	Gatton		
Dubbo	Goodiwindi		
Forbes	Inglewood		
Griffith	Jondaryan		
Gunnedah	Kingaroy		
Hay	Milmerran		
Jerilderie	Monto		
Lachlan	Murilla		
Moree Plains	Murweh		
Murrumbidgee	Peak Downs		
Narrabri	Pittsworth		
Narromine	Richmond		
Parry	Rosalie		

Quirindi	Toowoomba
Tamworth	Waggamba
Walgett	Wambo
Warren	Warroo
Yallaroi	Wondai

3. For the cotton growing seasons in Winter 2004, Summer 2004/2005 and Winter 2005, the maximum number of Locations for those growing seasons (where permitted dealings may be conducted) are set out in Table 3 at Column 2. The maximum combined area of all Locations where permitted dealings may occur in those growing seasons is limited to the size set out in Table 3 at Column 3:

Table 3: Maximum numbers of Locations and combined areas

Growing season	Maximum number of Locations	Maximum combined area of all Locations
Winter 2004	4	30
Summer 2004/5	32	885.8
Winter 2005	4	30

4. In the Summer 2004/2005 growing season, the maximum size of any individual Location is 50 hectares. (No individual field trial site can be more than 50 hectares.)
5. The licence holder must be able to access and control a Location to the extent necessary to comply with this licence, for the duration of the life of the licence.

Notification of planting of the GMOs

6. The licence holder must provide notices in writing to the Regulator of the actual date or dates of commencement of planting of the GMOs at each Location (and Pollen Trap in respect of each Location) ('the actual planting date notice'), together with the Location's GPS coordinates and either a street address, or other directions to the Location. This notice must be provided within 7 days of commencement of planting of the GMOs at the Location.

Notification of commencement of flowering of the GMOs

7. The licence holder must provide notices in writing to the Regulator in respect of each of the following:
- (a) the short term forecasted date or dates of commencement of flowering of the GMOs at each Location (and Pollen Trap in respect of each Location) ('the short term forecast flowering date notice'). This notice must be provided at least 7 days, and not more than 20 days, prior to the forecasted date or dates of commencement of flowering set out in the notice; and
 - (b) the actual date or dates of commencement of flowering of the GMOs at each Location (and Pollen Trap in respect of each Location) ('the actual flowering date notice'). This

notice must be provided within 7 days of commencement of flowering of the GMOs at a Location.

Notification of commencement of seed set of the GMOs

8. The licence holder must provide notices in writing to the Regulator in respect of each of the following:
 - (a) the short term forecasted date or dates of commencement of seed set of the GMOs at each Location (and Pollen Trap in respect of each Location, if any) ('the short term forecast seed set date notice'). This notice must be provided at least 7 days, and not more than 20 days, prior to the forecasted date or dates of commencement of seed set, as set out in the notice;
 - (b) the actual date or dates of commencement of seed set of the GMOs at each Location (and Pollen Trap in respect of each Location, if any) ('the actual seed set date notice'). This notice must be provided within 7 days of commencement of seed set of the GMOs at the Location.

Notification of commencement of harvest of GMOs

9. The licence holder must provide notices in writing to the Regulator in respect of each of the following:
 - (a) the short term forecasted date or dates of commencement of harvesting of the GMOs at each Location (and Pollen Trap in respect of each Location) ('the short term forecast harvest date notice'). This notice must be provided at least 7 days, and not more than 20 days, prior to the forecasted date or dates of commencement of harvesting set out in the notice; and
 - (b) the actual date or dates of commencement of harvesting of the GMOs at each Location (and Pollen Trap in respect of each Location) ('the actual harvest date notice'). This notice must be provided within 7 days of commencement of harvesting of the GMOs at a Location.

Pollen Traps

10. A Pollen Trap must surround every Location.
11. Each Pollen Trap must contain non-genetically modified cotton that is grown in such a way as to reasonably promote a dense and vigorous growth and flowering of the non-genetically modified cotton at the same time as the GMOs.
12. The edge of every Pollen Trap that is farthest from the GMOs (the 'outer edge of the Pollen Trap') must not be within 50 metres of a Natural Waterway.
13. Pollen Trap plants must be handled and controlled as if they are the GMOs (ie subject to other applicable conditions elsewhere in this licence), and Material from Pollen Trap plants must be handled and controlled as if it is Material from the GMOs (ie subject to other applicable conditions elsewhere in this licence).

14. A Pollen Trap must be able to be accessed and controlled by the licence holder to an extent that is commensurate with the licence holder's rights to access and control the Location within it.

Research Zones

15. Each Location of 1 hectare or more in size that is planted to the GMOs in the Winter 2004, Summer 2004/2005 and Winter 2005 growing seasons must be surrounded by a Research Zone unless the licence holder has a notice in writing from the Regulator that a Research Zone in connection with the Location is not required.
16. Each Research Zone must be able to be accessed and controlled by the licence holder to the extent necessary to enable the licence holder to meet its obligations under this licence to conduct research in the Research Zone.

Harvest and post-harvest procedures

17. If the GMOs or Pollen Trap plants are harvested, they must be harvested separately from any other Cotton.
18. If seed cotton harvested from the GMOs or from Pollen Trap plants is ginned, it must be ginned separately from any other Cotton.
19. The GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants, must not be used for stockfeed and must not enter the human food supply.
20. Following ginning, seed from the GMOs and Pollen Trap plants and must be:
 - (a) stored in a sealed container, within a locked facility that is signed so as to indicate that GM cotton seed is stored within the facility;
 - (b) exported; or
 - (b) Destroyed by burning.
21. Any GM cotton seed obtained from ginning may only be transported to the extent necessary to store them, export them, Destroy them by burning or take it to a facility certified by the Regulator to physical containment level PC2.
22. Cotton lint obtained from ginning of seed cotton harvested from the GMOs or Pollen Trap plants may be sold.

Cleaning – post harvest and generally

23. Equipment, a Location or other area used pursuant to this licence in respect of GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants, must be Cleaned.
24. For each Location, either within 14 days of harvest of the GMOs or by 31 May 2004, whichever occurs first, the Location must be Cleaned.
25. If Equipment is Cleaned, the area in which the Equipment is Cleaned must also be Cleaned. (For the sake of clarity, it is not necessary for Equipment to be Cleaned only at a Location.)

26. Cleaning must occur immediately or as soon as practicable after the use and before it is used for any other purpose. (For example, if GM seed is ginned, the gin must be Cleaned immediately following its use and before any other Cotton is ginned.)
27. On the request of the Regulator, the Regulator must be provided with written documentation of the procedures in place to ensure continuing compliance with the Cleaning conditions in this licence.

Inspection

28. Following Cleaning of the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants at a Location or other area, the following places must be inspected for the existence of Volunteer plants:
 - (a) the Location;
 - (b) the Pollen Trap in respect of the Location;
 - (c) irrigation channels and drains through which water flows to and from the Location and the Pollen Trap; and
 - (d) any areas used to Clean Equipment used in connection with the GMOs or to Destroy the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants.
29. Inspection must be performed by a person who is able to recognise Volunteer plants.
30. All the places required to be inspected must be inspected at least once every 2 months for a period of at least 12 months that commences the last day of Cleaning of the Location or until the GMOs are once again grown at the Location in a new growing season, pursuant to this Licence.
31. The results of inspection activities must be recorded in a logbook. The logbook must be available on request for examination or photocopying by the OGTR. The findings of the inspections as recorded in the logbook must be included in the licence holder's annual report to the Regulator. The logbook must contain at least the following:
 - (a) details of the areas inspected;
 - (b) details of the date of inspection;
 - (c) the names of the person or persons who undertook the monitoring and details of the experience, training or qualification that enabled them to recognise Volunteer plants;
 - (d) the number of Volunteer plants observed, if any;
 - (e) details of the development stages reached by the Volunteer plants, if any; and
 - (f) details of methods used to Destroy Volunteer plants, if any.
32. Any Volunteer plant identified must be Destroyed prior to the plant flowering.

General conditions on use of Locations post-harvest

33. If the GMOs are grown at a Location, no other Cotton plant of any kind may be grown at the Location or Pollen Trap in respect of the Location, after harvest of the GMOs or Pollen Trap plants, except in accordance with this licence, until inspection obligations are completed.

34. If the GMOs are grown at a Location, no plants may be planted at the Location or Pollen Trap in respect of the Location, except in accordance with this licence, until inspection obligations are completed unless:
- (a) the plants are grasses (grass pastures), cereals (cereal crops); or
 - (b) the plants are plants agreed to in writing by the Regulator; and
 - (c) the Regulator is satisfied that monitoring and Destruction of Volunteer plants prior to setting seed will not be adversely affected by the planting.

Transportation of the GMOs, Material from GMOs, Pollen Trap plants and Material from Pollen Trap plants

35. Subject to the conditions immediately below in respect of transportation, the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants must be transported in accordance with the OGTR Guidelines for the Transport of GMOs (June 2001) issued by the Regulator.
36. Harvested GMOs, Pollen Trap plants, Material from the GMOs or Material from Pollen Trap plants may be transported to a ginning facility in a cotton module that is:
- (a) completely enclosed within 2 layers of tarpaulin ('double wrapped in tarpaulin'); or
 - (b) completely enclosed within a layer of tarpaulin inside a layer of shade cloth ('double wrapped in tarpaulin and shade cloth'); or
 - (c) contained within an enclosed chain-bed truck specifically designed for the purpose of transporting cotton modules.

(Explanatory note: double wrapping is intended to prevent dissemination of the enclosed material during transportation.)

37. Cotton lint derived from GMOs and Pollen Trap plants from ginning is not subject to transportation conditions.
38. Every container used to transport the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants must be labelled:
- (a) to indicate that it contains genetically modified Cotton; and
 - (b) with telephone contact numbers for the licence holder and instructions to contact the licence holder in the event that the container is broken or misdirected.
39. The licence holder must have in place accounting procedures to verify whether the same quantity of GMOs, Material from the GMOs, Pollen Trap Plant or Material from Pollen Trap plants sent is delivered and must document routes, methods and procedures used for transportation of GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants.

Contingency Plans

40. Within 30 days of the date of the commencement of this licence, a written Contingency Plan must be submitted to the Regulator detailing measures to be taken in the event of the unintended presence of the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants, outside an area that must be inspected.

41. The Contingency Plan must include details of procedures to:
 - (a) ensure the Regulator is notified immediately if the licence holder becomes aware of the event;
 - (b) destroy any of the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants; and
 - (c) inspect and Destroy any Volunteer plants that may exist as a result of the event.
42. The Contingency Plan must be implemented in the event that the unintended presence of the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants is discovered outside an area that must be inspected.

Compliance Management Plan

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

Reporting

44. 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 information on any adverse impacts on human health and safety or the environment, caused as a result of the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants.

Research requirements

45. The licence holder must, in consultation with the OGTR, develop an agreed research program to collect information regarding:
 - (a) genetic segregation and molecular characterisation of the introduced genetic material;
 - (b) the levels of expression of proteins by the introduced genes;
 - (c) the stability and persistence of introduced proteins in the soil under Australian conditions;
 - (d) the expression of the glyphosate tolerant phenotype during development of the GM plant;
 - (e) the agronomic characteristics indicative of potential weediness of the GM cottons under Australian conditions;
 - (f) validation of previous research on the efficacy of the Pollen Trap; and
 - (g) validation of previous research on gene transfer from GM Cotton to other Cotton using the Research Zone.

46. In accordance with any Guidelines issued by the Regulator in relation to annual reporting, the licence holder must provide the Regulator with a written report of the progress and results of the research program. This report must accompany the annual report to be sent to the Regulator.

Testing methodology

47. The licence holder must provide a written instrument to the Regulator describing an experimental method that is capable of reliably detecting the presence of the GMOs and the presence of the genetic modifications described in this licence in a recipient organism. The instrument must be provided within 12 months of the issuing of this licence.

Use of GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants

48. The licence holder must ensure that the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants are not consumed by humans or used in manufacture of animal feed or therapeutics.

APPENDIX 8 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES

SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

341. 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).

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

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

344. 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).

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

346. 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

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

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

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

348. The application must also contain:

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

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

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

SECTION 3 THE INITIAL CONSULTATION PROCESSES

351. In accordance with Section 50 of the Act, the Regulator must seek advice in preparing a RARMP from prescribed agencies:

- State and Territory Governments;
- the Gene Technology Technical Advisory Committee (GTTAC);

- prescribed Australian Government agencies (Regulation 9 of the *Gene Technology Regulations 2001* refers);
- the Environment Minister; and
- relevant local council(s) where the release is proposed.

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

353. 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

354. 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, Australian 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.

355. 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, Australian Government 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.

356. In considering this information and preparing the RARMP, 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.

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

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

SECTION 5 FURTHER CONSULTATION

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

359. All issues relating to the protection of human health and safety and the environment raised in written submissions on an application or a risk assessment and a risk management plan are considered carefully, and weighed against the body of current scientific information, in reaching the conclusions set out in a final RARMP. 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.

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

361. 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

362. 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.

363. The Regulator must also have regard to any policy guidelines issued by the Ministerial Council that relate to risks to human health and safety and the environment, or the management of such risks. At this time no policy guidelines have been issued.

364. The Regulator also must not issue a licence if this would be inconsistent with a policy principle issued by the Ministerial Council. The Gene Technology Ministerial Council recently issued a policy principle "Gene Technology (Recognition of Designated Areas) Principle 2003" (the Principle), which allows for recognition of GM or non-GM designated areas for marketing purposes. The Principle is designed to ensure the valid operation of State and Territory laws declaring areas to be GM, non-GM or both for marketing purposes.

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

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

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

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

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

- limit the scope of the dealings;

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

369. 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.

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

APPENDIX 9 SUMMARY OF PUBLIC SUBMISSIONS ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

Submission from: **A:** agricultural organisation; **I:** individual, **C:** conservation organisation, **F:** food interest organisation.

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

Sub. No:	Type	Summary of issues raised	Issue	Consideration of issue
1	A	Demand for clean and natural produce is reflected in the rapid growth of the global organic market, currently valued at \$40 billion.	FC, MA	FSANZ, OSA
		Uptake of GMOs and their use in the food chain must be accountable, with appropriate labelling of all GMO products and accurate identification to ensure product integrity and consumer confidence.	FC, L	FSANZ, OSA
		Cottonseed meal comprises up to 5% of pig's diet in Australia, and could safely be raised to 20% during feed shortage. Thus, the introduction of GM cotton into the food chain holds strong implications for the pork industry.	FC, MA	FSANZ, OSA
		There are risks through partial or complete rejection of animals fed GM products. Australian pork exporters are now required to provide written assurance to Japanese customers that the diets of their pigs are GMO free.	FC, MA	FSANZ, OSA
		Introduction of GM cotton is likely to increase the amount of cotton seed meal for use in pig diets provided any changes result in cheaper prices. This will offer improvements in ingredient supply and could enhance competitiveness in markets where GMOs are not an issue. However in our major Asian markets ... unlikely to provide a competitive advantage.	FC, MA	FSANZ, OSA
		Imperative that tracking, tracing 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.	FC, L, MA, SEG	FSANZ, OSA
		While it is beyond the scope of the current RARMP, further research needs to be conducted aimed at the prospects of improving and expanding on-farm food safety systems and to develop effective tracking and tracing and identity preservation systems. If the benefits, if any, are to be realised, ... produce must be traced from its point of origin through to its final destination ... through to the end of the production process.	D, FC, L, SEG, MA	FSANZ, OSA

	Need for the cotton growers and policy makers to consider identifying appropriate risk management, tracking and tracing, communication strategies and appropriate labelling, for not just the cotton fibre end product but also for the GM inputs to other industries.	FC, L, SEG, MA	FSANZ, OSA
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Sub. No:	Type	Summary of issues raised	Issue	Consideration of issue
2	I	These GMOs pose unknown and unknowable risks to the environment and so to human health. However slight these risks are ... no right to take them.	D	App 2 -7
3	C	Does not support development of cotton industry in northern Australia	MA	OSA
		Insufficient evidence to conclusively demonstrate no risk to environment or human health.	RA	App2-7
		Protect environment in northern Australia from impacts of proposed release and future large scale and commercial releases.	RA	App3-7
		Location of sites not disclosed	RA	App7
		Concerned about management of potential weediness of GM cottons in northern Australia.	W, RM	App 4 and 7
		No scientific basis for conclusions relating to gene transfer.	G	App 5
4	F	Benefits of insecticidal and herbicide tolerant transgenic cotton on industry and environment.	EN	OSA
		Reduction in pesticide use due to GM cottons.	EN	OSA
		The GM cottons will allow successful control of weeds in cotton.	W, RM	App 7, OSA
		A risk of weeds developing resistance.	HT, RM	App 5, 6
		Lack of cotton plants establishing themselves as weeds.	W	App 4
		Likelihood of the GM cottons becoming weeds is low.	W	App 4
		Supports the proposed field trials.		

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