Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 124

Commercial release of cotton genetically modified for insect resistance and herbicide tolerance (Bollgard®III and Bollgard®III x Roundup Ready Flex®)

Applicant: Monsanto Australia Ltd

June 2014

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 124

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the intentional, commercial scale release of a genetically modified organism (GMO) into the environment. A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that this commercial release poses negligible risks to human health and safety and the environment and no specific risk treatment measures are required. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the licence.

The application

Application number	DIR 124
Applicant:	Monsanto Australia Ltd (Monsanto)
Project Title:	Commercial release of cotton genetically modified for insect resistance and herbicide tolerance (Bollgard® III and Bollgard® III x Roundup Ready Flex®)[1]
Parent organism:	Cotton (*Gossypium hirsutum* L.)
Introduced genes and modified traits:	*vip3A* (vegetative insecticidal protein 3A) synthetic gene derived from a gene from the bacterium *Bacillus thuringiensis* (insect resistance) *cry1Ac* (*crystal protein 1Ac*) gene from *B. thuringiensis* (insect resistance) *cry2Ab* (*crystal protein 2Ab*) gene from *B. thuringiensis* (insect resistance) *cp4 epsps* (*5-enolpyruvylshikimate-3-phosphate synthase*) gene from the bacterium *Agrobacterium* sp. strain CP4 (herbicide tolerance) *nptII* (*neomycin phosphotransferase type II*) gene from the bacterium *Escherichia coli* (antibiotic resistance) *aph4* (*hygromycin B phosphotransferase*) gene from *E. coli* (antibiotic resistance) *uidA* (*β-glucuronidase*) gene from *E. coli* (reporter) *aad* (*3"(9)-O-aminoglycoside adenyltransferase*) gene from *E. coli* (antibiotic resistance)

[1] The title of the licence application submitted by Monsanto is "General release of cotton genetically modified for insect resistance and herbicide tolerance".

Proposed locations:	Current and potential cotton growing areas of Australia
Primary purpose	Commercial release of the GM cotton

This commercial release follows field trial work conducted under licence DIR 101.

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed release are negligible, either in the short or long term. No controls are required to treat these negligible risks.

The risk assessment process considers how the genetic modification and activities conducted with the GMO might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short and long term are considered.

Credible pathways to potential harm that were considered included: toxic and allergenic properties of the GM cotton; increased spread and persistence leading to increased weediness of the GM cotton relative to unmodified plants; and vertical transfer of the introduced genetic material to other sexually compatible plants.

The principal reasons for the conclusion of negligible risks are: the GM cottons have been produced by conventional breeding from GM parental cotton lines that have previously been assessed and authorised for field trial and/or commercial release in Australia; two of the parental cottons have been grown on a commercial scale in Australia since 2006 without adverse effects on human health or environment; the limited capacity of the GM cotton to spread and persist in undisturbed natural habitats; the widespread presence in the environment of proteins the same as or similar to the those encoded by the introduced genes; and the lack of toxicity of the introduced proteins to vertebrates and most invertebrates. Toxicity of the introduced insect-resistance proteins is limited to certain insects, including major pests of cotton.

Risk management

The risk management plan concludes that the risks from the proposed dealings, either in the short or long term, to the health and safety of people, or the environment, are negligible. No specific risk treatment measures are imposed.

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks and considers general risk management measures. The risk management plan is given effect through licence conditions.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions under post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

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Abbreviations

AGSWG	Australian Glyphosate Sustainability Working Group
aad	3"(9)-O-aminoglycoside adenyltransferase gene
Act2	Actin2
aph4	hygromycin B phosphotransferase gene
APVMA	Australian Pesticides and Veterinary Medicines Authority
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
CFIA	Canadian Food Inspection Agency
cp4 epsps	*epsps* gene from *Agrobacterium* sp. strain CP4
Cry	Crystal protein
CSD	Cotton Seed Distributors
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CP4 EPSPS	EPSPS protein from *Agrobacterium* sp. strain CP4
ctp	Chloroplast transit peptide
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FDA	United States Food and Drug Administration
FMV	Figwort mosaic virus
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
GUS	β-glucuronidase protein
ha	Hectare
HGT	Horizontal gene transfer
LGA	Local government area
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
nptII	Neomycin phosphotransferase II
OGTR	Office of the Gene Technology Regulator
PRR	Post release review

RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
TGA	Therapeutic Goods Administration
Ubi3	Ubiquitin3
USDA-APHIS	Animal and Plant Health Inspection Service of the United States Department of Agriculture
Vip	Vegetative insecticidal protein

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

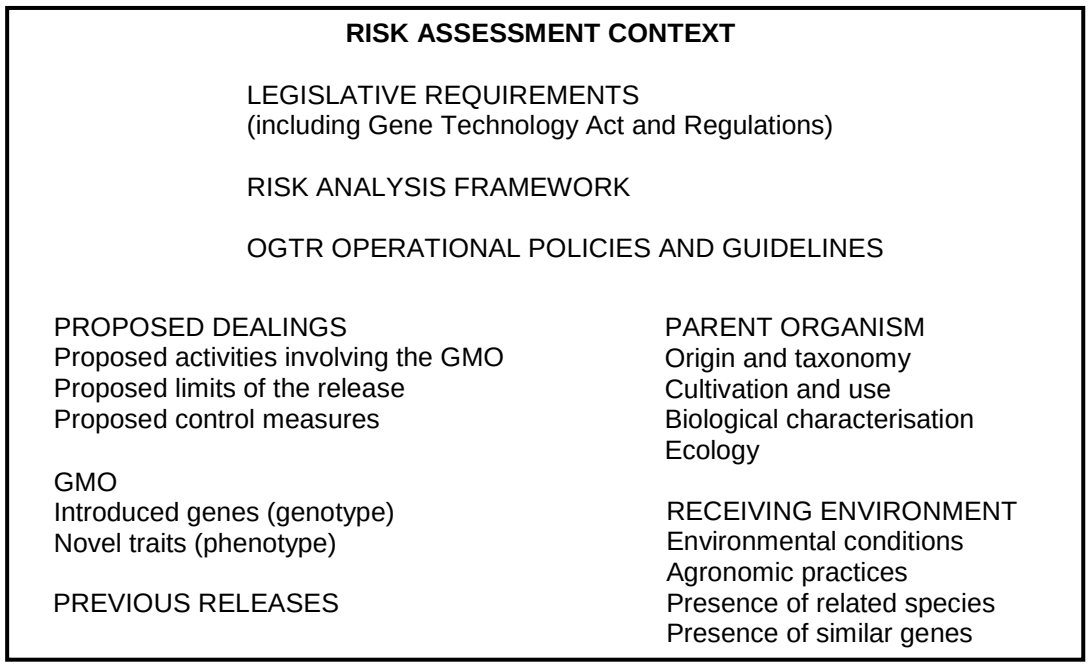

Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom he must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform his decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. This means that, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, local councils that the Regulator considered appropriate (being those in which

commercial cotton crops may be grown) and the Minister for the Environment. A summary of issues contained in submissions received is given in Appendix A.

6. Section 52 of the Act requires the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public. Advice from the prescribed experts, agencies and authorities for the second round of consultation, and how it was taken into account, is summarised in Appendix B. Four public submissions were received and their consideration is summarised in Appendix C.

7. The Risk Analysis Framework (OGTR 2013a) explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

8. Any dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration, National Industrial Chemicals Notification and Assessment Scheme and Department of Agriculture Biosecurity (formerly Australian Quarantine Inspection Service). These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 3 Proposed dealings involving intentional release of GM cotton plants

3.1 The proposed dealings

9. Monsanto Australia Ltd (Monsanto) proposes to release into the environment two types of GM cotton. The first type, Bollgard® III cotton, contains three introduced genes that confer insect resistance. The second type, Bollgard® III x Roundup Ready Flex® cotton, additionally contains a gene that confers tolerance to the herbicide glyphosate.

10. Bollgard® III cotton was produced by conventional breeding between Bollgard® II cotton, a commercially released insect resistant GM cotton, and VIP3A cotton (also known as COT102 cotton), a different insect resistant GM cotton that has been previously approved for limited and controlled release but not yet approved for commercial release. Bollgard® III x Roundup Ready Flex® cotton was produced by conventional breeding between Bollgard® II cotton, VIP3A cotton, and Roundup Ready Flex® cotton, a commercially released herbicide resistant GM cotton.

11. The applicant proposes release of the GM cottons in the current and potential cotton growing areas of Australia. No controls are proposed to restrict the release. The main cotton growing areas of Australia are in central to northern New South Wales and southern to central Queensland. Cotton is also grown on a trial basis in north western Victoria, northern Queensland and northern regions of Western Australia.

12. The GM cottons and GM cotton-derived products would enter general commerce, including use in human food and animal feed. FSANZ has assessed and approved food made from the parent GM cottons (Bollgard® II cotton, VIP3A cotton, and Roundup Ready Flex® cotton). These approvals include food made from any offspring produced through conventional breeding, and therefore cover Bollgard® III cotton and Bollgard® III x Roundup Ready Flex® cotton.

13. The dealings involved in the proposed intentional release are:

(a) conducting experiments with the GMO

(b) making, developing, producing or manufacturing the GMO

(c) breeding the GMO

(d) propagating the GMO

(e) using the GMO in the course of manufacture of a thing that is not the GMO

(f) growing, raising or culturing the GMO

(g) transporting the GMO

(h) disposing of the GMO

(i) importing the GMO

and the possession, supply or use of the GMO for the purposes of, or in the course of, any of the above.

Section 4 Comparator plants (baseline)

14. In establishing the risk context, details of the parent organism form part of the baseline for a comparative risk assessment (Figure 1 and OGTR 2013a). For the current application, two of the parent plants (Bollgard® II and Roundup Ready Flex®) are GM cottons that, alone and in combination, constitute over 95% of the existing Australian cotton crop.

15. The relevant comparator plants for the GM plants proposed for release are:

- Non-GM *Gossypium hirsutum* cotton (the 'grandparent') and
- The GM parental *Gossypium hirsutum* cottons Bollgard® II, Roundup Ready Flex® and VIP3A.

4.1 Non-GM cotton

16. The parent organism is the cultivated cotton species *Gossypium hirsutum* L. This cotton species is exotic to Australia and is grown as an agricultural crop, mainly in NSW and in southern and central Queensland.

17. The most relevant risk assessment and risk management information on non-GM cotton is included here. More detailed information on all those aspects of non-GM cotton can be found in the document, *The Biology of* Gossypium hirsutum L. *and* Gossypium barbadense L. *(cotton)* (OGTR 2013b), which was produced to inform the risk assessment process for licence applications involving GM cotton plants. This document is available from the Risk Assessment References page of the OGTR website.

4.1.1 Uses of non-GM cotton and its products

18. Cotton is grown commercially for a variety of uses:

- Use in the textile industry: Cotton is primarily grown as a fibre crop. It is harvested as 'seed cotton' which is packed into large bales or modules and transported to ginning facilities, where the seed cotton is 'ginned' to separate the seed and lint. The long lint fibres are further processed by spinning to produce yarn that is knitted or woven into fabrics.
- Use of products in human food: Ginned *G. hirsutum* seed is covered in short, fuzzy fibres, known as 'linters', which must be removed before the seed can be used for planting or crushed for oil. The linters are used as a cellulose base in high fibre dietary products as well as a viscosity enhancer (thickener) in ice cream, salad dressings and toothpaste. De-linted cotton seed, i.e. seed with no lint or linters, is crushed for oil for human consumption.

- Use in animal feed: Cotton seed meal is the product remaining once the oil has been removed by crushing and can contain up to contain 41% protein. Cotton seed, or meal, flour or hulls derived from it, are used for animal feed, but this is limited by the presence of natural toxins (see below).

4.1.2 Non-GM cotton as a crop

19. Cotton is a domesticated crop that grows best under agricultural conditions. It prefers soils with high fertility and responds well to irrigation. Cotton has been commercially cultivated in Australia since the 1860s (OGTR 2013b). It is a perennial plant that is cultivated as an annual.

20. A summary of climatic data and production systems for current and potential cotton growing areas can be found in the RARMP for DIR 066/2006. This provides a general overview of abiotic factors relevant to release in commercial cotton growing areas, including consideration of potential areas of development north of latitude 22°South (OGTR 2006b).

21. Areas where cotton can be grown in Australia are mainly limited by water availability, the suitability of the soil, temperature and the length of the growing season.

22. Temperature is the dominant environmental factor affecting cotton development and yield. Cotton is planted when the minimum soil temperature at 10 cm depth is 14°C for at least three successive days. Seedlings may be killed by frost and a minimum of 180–200 frost-free days of uniformly high temperatures (averaging 21–22°C) is required after planting *G. hirsutum*. Cold temperatures also have a significant effect on cotton germination and can lead to decreased yield, shorter plants and delayed flowering (OGTR, 2013b). Growth and development of cotton plants below 12°C is minimal and a long, hot growing season is crucial for achieving good yields. However, *G. hirsutum* has also been shown to be sensitive to high temperatures at all stages of growth, but in particular during reproductive development (Reddy et al. 1992).

23. The majority of Australia's cotton crop is grown in the Murray-Darling Basin under irrigation. Typically in Australia, more than 80% of the cotton is grown as a furrow irrigated crop and fields are commonly irrigated five or six times during the growing season between flowering and peak boll development. The remaining cotton production occurs on dryland farms.

24. In the major commercial cotton growing regions of Australia, the timing of cultivation varies slightly depending on climate. In northern New South Wales (NSW), sowing typically occurs in late September or early October, whereas in central Queensland (Qld), it is likely to occur four weeks earlier. Cotton farming activities include soil preparation during August–September, planting in September–October, managing weeds, pests and watering during the growing season in November–February. Defoliation, harvesting and transportation for processing are done during March–May.

25. Only a small percentage of cotton is currently grown in northern Australia. All cotton cultivation (GM and non-GM) in the Northern Territory (NT) was banned in 2002. However, Western Australia (WA) lifted its moratorium on the commercial production of GM cotton in the Ord irrigation area in November 2008.

26. In northern Australia, early attempts at cotton production as a summer crop were largely unsuccessful and cropping practices have now been tailored specifically to those regions. One key difference anticipated with cotton cultivation in northern Australia is winter (dry season) cropping, which may be necessary in certain areas to avoid periods of highest insect abundance (Australian Cotton Cooperative Research Centre 2004). Additionally, the wet season would impact adversely on cotton plant growth, cotton fibre quality, and the ability to access and operate machinery in the cotton fields.

27. In the Ord River Irrigation Area, for example, insect resistant GM cotton can be grown successfully if sown as a winter crop (March to April) and picked before significant rainfall commences (September to October); management guidelines specific to this region have been developed (Yeates et al. 2007).

4.1.3 Non-GM cotton and herbicide resistance

28. A number of agricultural practices are used to control weeds in fields prepared for the planting of cotton and also to manage cotton volunteers. These control practices include the application of herbicide treatments (OGTR 2013b). In addition, integrated weed management practices are used to avoid selection of resistant weed biotypes. The Australian cotton industry uses such weed management practices to decrease the possibility that herbicide tolerant weeds will become a problem (Cotton Australia website).

29. With respect to the control of cotton itself, glyphosate is generally not used against adult plants, as it usually fails to kill them. Adult cotton plants can be controlled by other herbicides and mechanical means.

30. Issues regarding herbicide use and resistance most appropriately fall under the Agricultural and Veterinary Chemicals Code Act 1994, and as such are the responsibility of the APVMA. The APVMA assesses all herbicides used in Australia and sets their conditions of use, including for resistance management.

4.1.4 Management of pests in non-GM cotton crops

31. In conventional cotton crops, two insect species, cotton bollworm (*Helicoverpa armigera*) and native budworm (*Helicoverpa punctigera*), are season-long pests requiring repeat insecticide applications during the growing season (Fitt 1994). On average, 8-12 sprays per season are applied against *Helicoverpa* spp. These sprays also control other pests such as plant bugs and stink bugs, but secondary pests such as two-spotted mite and cotton aphid may increase in number, since their natural enemies have been removed by broad spectrum insecticides.

32. Shaw (1992) listed six major chemical groups for use in conventional cotton: synthetic pyrethroids, organophosphates, cyclodienes, carbamates, biologicals, and chitin inhibitors. The timing of pesticide applications is determined by regular scouting of crops (2-3 times/week) and the use of pest thresholds (Fitt, 1994).

33. Historically, reliance on insecticides led to increasing problems with insecticide resistance in key pest species, and an Insecticide Resistance Management Strategy (IRMS) was implemented in 1983 in an effort to prolong the useful life of synthetic pyrethroids, and ultimately other insecticide groups (Forrester NW et al. 1993).

4.2 Non-GM cotton outside cultivation - weediness

34. In the context of this RARMP, characteristics of cotton when present as a volunteer in the relevant agricultural land uses, in intensive use areas such as roadsides and in nature conservation areas are examined.

35. The Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol rates the weed risk potential of plants according to properties that strongly correlate with weediness for each relevant land use (Standards Australia New Zealand & CRC for Australian Weed Management 2006). These properties relate to possible harms of the plant, its potential to spread and persist (its invasiveness) and its potential distribution in Australia.

4.2.1 Potential to cause harm

36. In summary, as a volunteer (rather than as a crop), non-GM cotton is considered to exhibit the following potential to cause harm:

- low potential to negatively affect the health of animals and/or people
- low potential to reduce the establishment or yield of desired plants
- low potential to reduce the quality of products or services obtained from all relevant land use areas
- low potential to restrict the physical movement of people, animals, vehicles, machinery and/or water
- some potential to act as a reservoir for a range of pests and pathogens
- low potential to adversely affect soil salinity and the water table.

37. With respect to the potential to negatively affect the health of people, it should be noted that workers in gins may develop byssinosis, an allergy to cotton (OGTR 2013b).

38. Mammals, including people, can be fatally poisoned when ingesting cotton plant parts, due to the presence of natural toxins in cotton. These are gossypol and the cyclopropenoid fatty acids (malvalic acid, sterculic acid and dihydrosterculic acid), all of which are found in seeds and certain other plant tissues (Bell 1986). Gossypol is an antioxidant and polymerisation inhibitor, the general symptoms of its toxicity being constipation and depressed appetite, death occurring from circulatory failure (Makkar et al. 2007), while the fatty acids reduce the activity of fatty acid desaturases (Raju & Reiser 1967; Yang et al. 1999). These compounds limit the use of cotton seed meal in human food and animal feed. Inactivation or removal of these components during processing enables the use of some cotton seed meal for farmed fish, poultry and swine. Ruminants such as cattle and sheep can tolerate some amounts of these toxins if they are slowly introduced into the diet. However, if inappropriate quantities are fed to ruminants then these animals may die.

4.2.2 Invasiveness

39. With regard to invasiveness, non-GM cotton has:

- low ability to establish amongst existing plants
- low tolerance to average weed management practices in cropping and intensive land uses, but a high tolerance in nature conservation areas (as they are not specifically targeted for weed management or because weed management is not applied in the area where cotton is present)
- a short time to seeding (less than one year)
- low annual seed production
- the ability to reproduce sexually, but not by vegetative means
- some ability for long distance spread by natural means (wind dispersal)
- high ability for spread long distance by people from dryland and irrigated cropping areas, as well as from intensive land uses such as road sides, but low ability to be spread by people from or to nature conservation areas.

4.2.3 Management of volunteer cotton

40. Seedlings are easier to control than older plants and volunteer seedlings which emerge over winter (in the south) are likely to be killed by frosts. Seedlings that emerge later in the year are likely to establish and grow, whether in a channel, a rotation crop or elsewhere on the farm. In wet winters, much of the seed dies before spring and relatively few volunteer seedlings are likely. The control of cotton volunteers is usually achieved by mechanical means or use of a range of herbicides, including bromoxynil, carfentrazone and a combination of paraquat and diquat (Roberts et al. 2002). Glyphosate is not generally used to control established cotton volunteers, as it usually fails to kill the plants beyond 6-leaf stage.

4.2.4 Spread

41. Seed may be spread off-farm, primarily through overland flows associated with irrigation runoff into common drainage lines and via module road freight to gins. A survey begun in 2012 in Qld and northern NSW recorded volunteer cotton plants as either recent recruits or longer term perennially growing plants (DAFF QLD); a second phase of the survey revisited sites where the longer term perennial plants had been recorded. In summary, the survey showed that plants were generally localised just beyond the farm gate and very little cotton had moved into the broader agricultural landscape. Densities were highest adjacent to cotton farms, within a 5 km radius, and in close proximity to ginning facilities (Spotlight on Cotton, Spring 2013).

4.2.5 Potential distribution

42. Climex® modelling has been employed to predict the areas that are climatically suitable for long-term survival of *G. hirsutum* cotton in Australia (Rogers et al. 2007). Results indicate that dry stress is the major limiting factor for potential distribution of cotton in northern Australia. The modelling program predicted that the naturalisation potential for *G. hirsutum* in Australia is confined to the coastal regions of north east Australia. This outcome is consistent with the majority, but not all, of the reports of naturalised populations in Australia (Australia's Virtual Herbarium). The modelling program also predicted that the winter temperatures in all of the current (southern) cotton growing areas of Australia were too cold to support the establishment of permanent populations of *G. hirsutum*. There is potential for commercial cotton production to expand into northern Australia, where winter temperatures would not be limiting, but as noted above dry stress would be a limiting factor.

43. When overall soil fertility was considered in addition to climatic data, the area suitable for cotton is further restricted primarily to coastal areas. However, the majority of these most favourable areas for cotton either carry forests (with >50% canopy closure) or are already used for some form of managed agricultural system and it is therefore not expected that cotton plants would be able to establish in these areas. Weed competition and fire were also identified to further reduce the probability of permanent cotton populations establishing in the identified areas (Rogers et al. 2007).

44. Naturalised populations of *G. hirsutum* have been found in a few relatively natural areas in the north of Australia, indicating that it is possible for this species to establish outside agricultural cultivation, but cotton seems to have a limited ability to spread and persist in undisturbed nature conservation areas.

45. It has been noted by scientists over many years that the morphology of many of these naturalised cotton populations is distinct from that of the cultivated cotton varieties. When grown in a glasshouse, they tend to have poor architecture and produce small bolls and seed with sparse, grey lint. They also produce mainly tufted rather than fuzzy seeds, which is a strong indication that they are not derived from modern cultivars which are all fuzzy seeded cotton plants (Curt Brubaker and Lyn Craven, CSIRO, pers. comm., 2002). It seems likely that many naturalised cotton populations result from attempts in the early 19th century to establish cotton industries in northern Qld and the NT (Curt Brubaker and Lyn Craven, CSIRO, pers. comm., 2002).

46. Some naturalised cotton populations have been observed which appear to be from a more recent origin, but none seem to have originated from the current commercial types of *G. hirsutum* that have been cultivated since the 1970's (Eastick 2002).

4.3 Sexually compatible plants

47. Cotton is largely self-pollinating and no self-incompatibility mechanisms exist. Where cross-pollination does occur it is likely facilitated by honeybees. *G. barbadense* is sexually compatible with *G. hirsutum,* but the likelihood that *G. hirsutum* could hybridise successfully

with any of the native Australian cottons is extremely low, due to genetic incompatibility (see discussion below).

48. There are 17 native species of *Gossypium* in Australia, most of which are found in the NT and the north of WA (OGTR 2013b). Only three of these species are likely to occur in the regions of Australia where cotton is cultivated: *G. sturtianum, G. nandewarense*, and *G. australe*. However, native *Gossypium* species prefer well-drained sandy loams and are rarely found on heavy clay soils favoured by cultivated cotton.

49. In the natural environment, for successful hybridisation to occur, the parent plants would have to occur in close proximity, flower at the same time, have pollen from one plant deposited on the stigma of the other, fertilisation occur and progeny survive to sexual maturity. Any progeny seed would have to be viable.

50. Genetic differences between the cultivated cottons, *G. barbadense* and *G. hirsutum*, and native Australian species make the possibility of hybridisation extremely low. Cultivated cottons are tetraploids of the A and D genomes (AADD, 2n=4x=52), whereas the Australian *Gossypium* species are diploids of the C, G or K genomes. Hybrids between *G. hirsutum* and *G. sturtianum* have been produced under field conditions between plants grown in close proximity but the hybrids were sterile, eliminating the possibility of introgression of genes from *G. hirsutum* into *G. sturtianum* populations (OGTR 2013b). Attempts to hybridise cultivated cottons and other native species under optimal artificial conditions, including use of plant hormones, have produced some hybrid seed, but in nearly all cases this seed has not been viable.

Section 5 The GM Parental Cottons

5.1 GM Bollgard® II cotton

51. Bollgard® II cotton is phenotypically very similar to non-GM cotton. For example, it is limited by the same abiotic factors as its non-GM parent, sexually compatible with the same plants and its products are used identically to non-GM cotton. Apart from the expression of selectable marker genes (which do not influence its behaviour outside the laboratory), the only phenotypic difference between Bollgard® II and non-GM cotton is expression of proteins toxic to certain insects in the order Lepidoptera, including the most important insect pests of cotton crops in Australia. Accordingly, agricultural management of Bollgard® II cotton differs from non-GM cotton in the application of insecticides and also reduced irrigation, as less insect damage of early bolls leads to the retention of bolls on the plant early in the growing season and to earlier maturity than in non-GM cotton. Refuge crops are also grown in combination with Bollgard® II cotton as part of insect resistance management plans.

5.1.1 Genetic modification

52. A detailed description of the genetic modification is provided in the RARMP for DIR 012/2002 (OGTR 2002).

5.1.2 Introduced genes

53. Bollgard® II cotton contains the *cry1Ac* and *cry2Ab* genes derived from *Bacillus thuringiensis* (Bt) subsp. *kurstaki*. The *cry1Ac* and *cry2Ab* genes encode insecticidal proteins which are specifically toxic to caterpillar larvae of certain species of lepidopteran insects including significant pests of cotton. The genes and their encoded proteins have been described in detail in the RARMPs for the commercial release applications DIR 059/2005 and DIR 066/2006, and will not be discussed in detail here (OGTR 2006a; OGTR 2006b).

54. *B. thuringiensis*, produces a range of insecticidal proteins, including the crystal (Cry) proteins (also known as delta-endotoxins) and vegetative insecticidal proteins (Vips). Vips are secreted by various *Bacillus* species during vegetative growth stages and sporulation, whereas

the Cry proteins are expressed by Bt only during sporulation and form crystalline inclusions in spores (reviewed by Estruch et al. 1997).

55. Bollgard® II contains antibiotic resistance selectable marker genes (Table 2): neomycin phosphotransferase II (*nptII*), 3"(9)-O-aminoglycoside adenyltransferase (*aad*) and hygromycin B phosphotransferase (*aph4*). These genes were originally derived from the common gut bacterium *Escherichia coli*. The *nptII* gene confers resistance to antibiotics such as kanamycin and geneticin, and the *aph4* gene confers resistance to the antibiotic hygromycin. These genes were used as selective markers during early stages of development of the GM plants in the laboratory. The *aad* gene, which confers resistance to the antibiotics spectinomycin and streptomycin, is linked to a bacterial promoter that does not function in plants so the gene is not expected to be expressed in the GM cotton plants. Bollgard® II also contains the beta-glucuronidase (*uidA*) gene from *E. coli*, which encodes an enzyme enabling visual identification of plant tissues in which this gene is being expressed. More detail on marker genes can be found in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website.

Table 1. Introduced genetic elements in GM Bollgard® II cotton

Plasmid name	Promoter	Gene	Terminator	Additional genetic elements	Function
PV GHBK042	35S	*cry1Ac*	7S 3'		insect resistance
	35S	*nptII*	nos		antibiotic resistance
	Tn7	*aad*	none		antibiotic resistance
PF-GHBK11	35S	*cry2Ab*	nos	PetHSP70, Ctp2	insect resistance
	35S	*uidA*	nos		reporter

Table 2. Details of the introduced genetic elements

Genetic element	Full name	Source organism / further description
Gene sequences		
cry1Ac	crystal protein 1Ac	Modified synthetic fusion protein with amino acids 1-466 from cry1Ab and amino acids 467-1178 from cry1Ac, both from *Bacillus thuringiensis* subsp. *kurstaki*
nptII	neomycin phosphotransferase type II	*Escherichia coli* Tn5 transposon
aad	3"(9)-O-aminoglycoside adenyltransferase	E. coli. This gene is under the control of its native (bacterial) promoter. Therefore, the gene will not be expressed in the GM cottons.
cry2Ab	crystal protein 2Ab2	Modified synthetic version of cry2Ab2 gene from *B. thuringiensis* subsp. *kurstaki*
uidA	beta-glucuronidase	E. coli
Promoters		
35S	CaMV 35S promoter	Cauliflower mosaic virus (CaMV), a pararetrovirus that infects a wide range of cruciferous plant species.
Tn7	Transposon 7 promoter	*E. coli*
Terminators		
nos	3' non-translated sequence of the nopaline synthase gene	*Agrobacterium tumefaciens*
7S 3'	3' non-translated sequence of the beta-conglycinin alpha-subunit gene	*Glycine max* (soybean)
Other elements		
PetHSP70	5' untranslated leader of the heat shock protein 70 gene	*Petunia x hybrida* (petunia)
Ctp2	Chloroplast targeting peptide from the epsps gene	*Arabidopsis. thaliana* (thale cress)

5.1.3 Introduced regulatory elements

56. In addition to the introduced genes, Bollgard® II cotton contains short regulatory elements which control expression of the genes (Table 1 and Table 2). These sequences are derived from plants (including thale cress, pea, petunia and soybean), a soil bacterium (*A. tumefaciens*) and plant viruses (CaMV and FMV).

57. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Also required for gene expression in plants are mRNA terminators, including a poly-adenylation signal. Other regulatory sequences, such as enhancers, may contribute to the expression pattern of a given gene. Further details of the regulatory sequences used in Bollgard® II cotton can be found in the RARMPs for DIR 059/2005 and DIR 066/2006. They are summarised in Table 2. Although some of these regulatory sequences are derived from organisms that are plant pathogens, by themselves they do not cause disease. The regulatory elements present in the parental GM cotton have been previously assessed by Australian and international regulators without identifying an increase in risk compared to endogenous regulatory elements of cotton.

58. Recently, there has been public commentary suggesting that protein P6, encoded by gene VI of the Caulimovirus and Soymovirus families, could result in harm to humans if expressed in GM plants (Latham & Wilson 2013). The cauliflower mosaic, figwort mosaic and peanut chlorotic streak viruses belong to the Caulimovirus family, and the CaMV 35S, FMV 35S and PC1SV promoters overlap sequences of gene VI (Podevin & du Jardin 2012). However, bioinformatics searches, experience from the wide consumption of non-GM food naturally infected with these viruses, and the safe release of other GM plants with these promoters, indicate that the P6 protein does not possess any allergenic or toxic properties. Likewise, there is no evidence of any environmental harms associated with use of these promoters in GM plants on a commercial scale.

5.1.4 Introduced proteins

Cry proteins

59. Recent reviews of *B. thuringiensis* crystal toxins (Pardo-Lopez et al. 2013) provide updated information on their mode of action and broadly support the model whereby Bt toxins exert their effect primarily through their ability to form pores in the plasma membrane of the midgut epithelial cells of susceptible insects.

Toxicity/allergenicity to humans and toxicity to animals, including livestock

60. Previous assessments of Ingard® and Bollgard® II cotton have involved the evaluation of the insecticidal proteins Cry1Ac and Cry2Ab. These proteins have been shown to have a safe history of use, low or no mammalian toxicity or allergenicity, and rapid digestion in the gut, and so are not considered to be harmful to human or animal health. This finding has been borne out by the expression of these same genes in a number of GM corn and soybean varieties that have been cultivated over a wide area (over 160 million hectares worldwide in 2011 (James 2011)) over the last 15 years.

61. Bollgard® II cotton has been approved for use in stockfeed since 2002, and the use of cotton seed products derived from the GM cotton has not shown any adverse impacts for livestock. In feeding studies where dairy cows were fed diets containing raw cottonseed meal at 10% of dry matter intake, Bollgard® II cotton performed similarly to the control cottonseed and did not affect dry matter intake, milk yield, milk composition, and body condition (Castillo et al. 2001). Studies on catfish, quail or broiler chickens showed no significant differences in weight gain and feed conversion between animals fed Bollgard® II cotton seed meal and animals fed non-GM cotton seed meal (Gallagher et al. 2000; Li & Robinson 2000; Mandal et al. 2004).

62. Most recently, CERA reviewed information and data relating to the environmental risk assessment of Cry1Ac and Cry 2Ab proteins (CERA 2010). Toxicity testing of these proteins with a range of representative non-target organisms (including honeybee, lacewing, ladybird, springtail and mouse) produced No Observed Effect Level (NOEL) values at concentrations representing levels at least ten-fold higher than the expected environmental concentrations of Cry1Ac or Cry2Ab.

Toxicity to invertebrates

63. Cry2Ab may be active against invertebrates from both Lepidopteran and Dipteran families (McNeil & Dean 2011), while Cry1Ac is usually characterised as having specific activity against a narrow range of Lepidopteran pests, including significant pests of cotton such as *Helicoverpa armigera* and *H. punctigera*. However, in a recent review of the current literature on Bt protein toxicities, Frankenhuyzen et al. (2013) examined cross-order, cross-class and cross-phylum activity of a range of Bt proteins and noted that Cry1Ac may also affect species in two other insect orders: it is highly toxic to tsetse flies (*Glossina morsitans* - Diptera) and has some toxicity to pea aphid (*Acyrthosiphon pisum* - Hemiptera). The hemipteran toxicity of Cry1Ac was reportedly low, but dipteran toxicity was within the reference range for diptera-active proteins.

64. There are a number of recent reviews of the potential impact of Bt crops on non-target invertebrates (Duan et al. 2009; Kaur 2012; Yu et al. 2011). Kaur (2012) reported that some studies found a degree of adverse effect to specific predator species, but results were sometimes conflicting or criticised for poor methodology. Yu et al. (2011) reviewed studies from 2005 to 2010 on effects of Bt cotton and maize on predators and parasitoids and concluded that adverse effects on predators (larval survival, consumption rate, and body mass) were only reported in the studies where Bt susceptible insects were used as prey. No negative effects were found when Bt-resistant, or even sublethally-damaged herbivores, were used as prey. Similarly, deleterious effects observed on parasitoids were due to the lower quality of hosts caused by Bt toxin ingestion, but not the direct toxicity of Bt toxins (Yu et al., 2011 and references therein).

65. In contrast, a large number of laboratory and field studies reported impacts ranging from no detrimental effect to increased abundance of beneficial insects. A meta-analysis of data collected from 42 field studies indicated that non-target invertebrates are generally more abundant in Bt cotton and Bt maize fields than in non-transgenic fields managed with insecticides (Marvier et al. 2007). In addition, a comprehensive review of short and long-term field studies on the effects of invertebrate populations in Bt corn and cotton fields indicated that no significant adverse effects are taking place as a result of wide scale Bt crop cultivation (Sanvido et al. 2006). Another review of field tests published to date concluded that the large-scale studies in commercial Bt cotton have not revealed any unexpected non-target effects other than subtle shifts in the arthropod community caused by the effective control of the target pests (Romeis et al. 2006). Slight reductions in some invertebrate predator populations will result from all pest management practices, which result in reductions in the abundance of the pests as prey.

66. The potential for indirect secondary effects of Bt cottons on non-target herbivores was explored by Hagenbucher et al (2013). Under controlled greenhouse conditions, reduced feeding by lepidopterans in Bollgard® II cotton was found to be associated with a decrease in levels of induced terpenoids. This was thought to be associated with resulting increase in numbers of *A. gossypii*, an herbivore not targeted by the Bt trait. However, the effect was less visible in the field, and aphid populations were not correlated with measured terpenoids in the cotton plants, indicating that one factor alone is not sufficient to explain aphid population dynamics.

Toxicity to honeybees

67. Cotton is primarily self-pollinating, but cross pollination does occur and is most likely facilitated by honeybees.

68. A list of regulatory assessments of GM plants that express the insecticidal proteins Cry1Ac or Cry2Ab proteins can be found in recent environmental reviews of these proteins (CERA 2010; CERA 2013). In summary, it was concluded from consideration of exposure of a range of organisms (including bees, springtails, greenbugs, aphids) that plants containing these proteins would not harm non-target arthropods. Table 3 presents data on ecotoxicological testing on honeybees, which supports a conclusion of no harm to honey bees from exposure to the purified proteins. Average expression levels of each protein in pollen from Bollgard® II GM parental cotton are included for comparison.

Table 3. Ecotoxicological testing on honey bees (*Apis mellifera*) using purified proteins

Protein	Life stage	Method of exposure	Duration	Result†	Expression level in pollen*
Cry1Ac	Adult Larvae	Protein in honey/water Single injection into cells with developing larvae	NA Single dose	NOEL 20 ppm NOEL 20 ppm	0.2 – 0.4 µg/g fwt in Bollgard II
Cry2Ab	Adult Larvae	68µg/g in diet 100 µg/ml in diet	Single dose Single dose	NOEC 68µg/g NOEC 100 µg/ml	1.9 – 9.3 µg/g fwt in Bollgard II

NOEC: No observed effect concentrations; NOEL: No observed effect level; NA: not applicable

† data reported in CERA 2010, 2013; *data provided by applicant: See Table 5.

Toxicity to soil microbes

69. In reviewing the literature relating to effects of GM plants on soil microorganisms, a number of authors have commented on the technical difficulties in measuring, assessing and interpreting such effects (Bruinsma et al. 2003; O'Callaghan et al. 2005a; Weinert et al. 2010). In general, however, no evidence has been found to suggest that the Cry1Ac, or Cry2Ab proteins or similar proteins are toxic to microorganisms including various species of protozoa, bacteria, fungi, algae and diatoms (OGTR 2006a and b) and it appears that bacterial soil communities are probably less affected by GM plants expressing Bt proteins than by variables such as field site, plant growth stage or field heterogeneities (Baumgarte & Tebbe 2005).

Presence of synergistic, antagonistic or combination effects

70. Synergistic, additive or antagonistic effects can occur when different Cry proteins, or other insecticidal proteins, are ingested by an insect at the same time (del Rincon-Castro et al. 1999; Schnepf et al. 1998). The scientific literature contains publications that report greater than additive interactions between Cry proteins. The potential for such effects between Cry1Ac and Cry2Ab has been discussed in the RARMPs for Bollgard II (DIR 059/2005 and DIR 066/2006) and is briefly updated here.

71. In general, it appears that proteins common to one family (eg Cry1) compete for similar binding sites, while proteins from different families (eg a Cry1 and a Cry2) do not share binding sites. For example, Hernandez et al. (2008) used competitive binding assays with *H. zea* and *H. armigera* brush boder membrane vesicles (BBMVs) to show that Cry2Aa, Cry2Ab, and Cry2Ae shared common binding sites, but did not compete for binding sites with the Cry1Ac protein in these species.

72. In addition, Luo et al. (2007) performed binding tests between the Cry toxins Cry1Aa, Cry1Ab, Cry1Ac and Cry2Ab with *Helicoverpa armigera* BBMVs. All of the toxins could bind these BBMVs. Cry2Ab could not displace labelled Cry1Ac and Cry1Ab. The same results were observed in reciprocal binding tests, demonstrating that Cry1A and Cry2Ab had different binding sites in *H. armigera.*

73. Similarly, Greenplate et al. (2003) performed experiments to examine the interaction of Cry1Ac and Cry2Ab in Bollgard® II cotton using *Heliothis virescens*, *Helicoverpa zea* and *Spodoptera frugiperda*. In this study the results for individual single-gene near isolines were used to calculate a predicted value for the response to the two-gene near-isoline, under the assumption that the interaction of the two toxins was additive. The resulting expected value was compared with the observed response to the two-gene near-isoline. The authors reported that for every tissue-type, the expected value was not significantly different from the observed mean response, indicating purely additive activity (i.e., no interaction) of the two toxins against these three insect species.

74. In contrast, Ibargutxi et al. (2008) reported low levels of synergy between Cry1Ac and Cry2Ab for growth and mortality responses of *H. armigera* and *Earias insulana* neonate larvae. A synergistic factor of 0.9 up to 3 for mortality was reported and 0.67 up to 1.40 for growth for Cry1Ac and Cry2Ab mixtures.

75. Overall, studies generally support the conclusion that, at the organism level (toxicity) and cellular level (BBMV binding) the Cry1Ac and Cry2Ab act independently as insecticidal proteins, without synergistic, additive or antagonistic effects.

5.1.5 Presence of identical or similar genes and proteins in the environment

76. All the source organisms for the introduced genetic elements in Bollgard® II are widespread and prevalent in the Australian environment and thus humans and other organisms would commonly encounter their genes and encoded proteins.

77. The introduced genes for all the parental GM lines are derived from common soil-borne microorganisms. The regulatory sequences (promoters, terminators, leader sequences) are derived from plants (cotton, soybean, pea, thale cress, petunia), plant viruses (peanut chlorotic streak caulimovirus, tobacco etch virus, cauliflower mosaic virus, figwort mosaic virus) and a common soil bacterium (*Agrobacterium tumefaciens*).

78. The insecticidal genes are derived from the common soil organism *Bacillus thuringiensis*, so other soil organisms would commonly be exposed to the expressed proteins. In the case of GM plants expressing these genes, the proteins can enter the soil from roots or from plant residues remaining on the field after harvest (Saxena et al. 1999; Zwahlen et al. 2003), resulting in continuous exposure of soil organisms to the Bt proteins. Measuring persistence and testing the effects of GM plants on soil organisms has presented experimental difficulties (O'Callaghan et al. 2005b; Weinert et al. 2010) and estimates of the persistence of Bt toxins in soil vary considerably.

79. Research on the biodegradation of the Cry1Ac and Cry2Ab proteins in soil has been discussed in significant detail in previous RARMPs prepared for the commercial release of Bollgard® II and Roundup Ready® Flex cottons (DIR 012/2002, DIR 023/2002, DIR 059/2005 and DIR 066/2006). Results indicate that plant-encoded Cry1Ac degrade with a half-life of up to 46 days (Palm et al. 1996). Additionally, soil samples collected three months after tillage showed no effects on susceptible insect larvae in bioassays (Head et al. 2002). A study by Dubelman et al. (2001) using an insect bioassay indicated that insecticidal activity of soil containing ground MON 15985 leaf tissue dissipated rapidly within the first week, more slowly over the next week, and was undetectable after 6 weeks.

5.1.6 Molecular stability

80. Molecular characterisation of the insertion of *cry1Ac* and *cry2Ab* genes in Bollgard® II is discussed in the RARMPs prepared for DIR 012/2002 and DIR 022/2002.

81. The data, including phenotypic and Southern blot analysis, demonstrate that the genes have been stably maintained as single dominant Mendelian traits over many generations of crossing and backcrossing. DNA sequencing was used to verify the inserted genes and to determine the regions flanking all of the insertion sites.

5.1.7 Method of genetic modification

82. The methods by which Bollgard® II cotton was produced have been described in detail in the RARMP for DIR 012/2002 and are briefly summarised here.

83. Bollgard® II (MON15985) was originally derived from MON531 (INGARD® cotton) and is the product of two transformation events: MON531 cotton contains the *cry1Ac*, *nptII* and *aad* genes, which were inserted using *Agrobacterium* mediated transformation; the *cry2Ab* and *uidA* genes were then added into the genomic DNA of MON531 cotton using projectile bombardment. Both these methods have been widely used in Australia and overseas for introducing new genes into plants and further information can be found in the document *Methods of plant genetic modification* available from the Risk Assessment References page of the OGTR website.

84. The parental *G. hirsutum* variety for transformation of MON 15985 was the cultivar Coker 312, which was released in 1974 by the Coker Pedigree Seed Company.

5.1.8 Experience with Bollgard® II cotton and its products

85. Commercial DIRs with Bollgard® II cotton, with and without some restrictions, have been approved under licences DIR 012/2002, DIR 059/2005 and DIR 066/2006. Activities with Bollgard II are permitted either individually or when combined with glyphosate herbicide tolerance (Roundup Ready Flex cotton, see below).

86. Release of GMOs may be subject to other regulatory requirements, including those of the Department of Agriculture – Biosecurity (formerly the Australian Quarantine and Inspection Service), Food Standards Australia New Zealand (FSANZ), and Australian Pesticides and Veterinary Medicines Authority (APVMA).

Australian experience from cultivation of Bollgard® II cotton

87. To date, the Regulator has not received any reports of adverse effects caused by Bollgard® II as a crop. There are no scientific studies showing adverse effects of Bollgard® II cultivation on human health or the environment in Australia.

88. APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. Bollgard® II cotton meets the definition of an agricultural chemical product under the Agricultural and Veterinary Chemicals Code Act 1994, due to its production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA.

89. Cultivation of GM insect resistant cotton varieties needs to comply with an approved insect resistance management plan and any other relevant conditions that may be imposed by the APVMA. The limitations and requirements under a resistance management plan include mandatory growing of refuges to produce susceptible insects, defined planting windows, restrictions on the use of foliar Bt sprays, mandatory cultivation of crop residues and the control of volunteer plants.

90. A resistance management plan for Bollgard® II cotton varieties grown south of latitude 22ºS has been developed by the Transgenic and Insect Management Strategy committee of the

Australian Cotton Growers' Research Association in consultation with the APVMA (Farrell & Johnson 2005; Monsanto Australia Limited 2004). The APVMA requires implementation of this plan as a condition of registration. A similar resistance management plan for areas north of latitude 22ºS has also been developed.

91. The aim of refuge crops is to generate significant numbers of susceptible moths that have not been exposed to selection pressure from the Bt proteins. Bollgard® II volunteers in cotton refuges can diminish the value of the refuge, as some of the emergent moths have had exposure to the insecticidal proteins. A key part of the Resistance Management Plan for growers of Bollgard® II cotton is the control of volunteer and ratoon cotton.

92. The Regulator has not received any reports of adverse effects caused by Bollgard® II cotton as a weed in nature conservation areas or in intensive land use areas such as roadsides since its release in Australia in 2002.

Australian experience from use of products of Bollgard® II cotton

93. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has previously given approval for the use in food of cotton seed oil and linters derived from INGARD® and Bollgard® II GM cottons (under applications A341 and A436, respectively; assessments are available from the FSANZ website).

94. People have been consuming these products in Australia since 2002 without any reported adverse health effects.

95. Cotton seed and meal from Bollgard® II has been fed to domestic animals since its commercial release. The Regulator has not received adverse reports from these activities.

International experience with Bollgard® II cotton

96. Bollgard® II cotton has been approved for environmental release as well as food and feed use in a number of other countries (Table 4). The Regulator has not received adverse reports from any of these countries. There are also no reports of adverse health or environmental effects in the scientific literature.

Table 4. International approvals of Bollgard II cotton

Country	Environment	Food and/or Feed	Food	Feed
Brazil	2009	2009		
Burkina Faso	2008			
Canada			2003	2003
China		2006		
Colombia			2009	
European Union		2005		
India	2006			
Japan			2002	2003
Korea			2003	2004
Mexico		2003		
Philippines			2003	2003
South Africa	2003	2003		
United States (US)	2002	2002		

5.2 GM Roundup Ready Flex® cotton

97. Roundup Ready Flex® cotton is phenotypically very similar to non-GM cotton. For example, it is limited by the same abiotic factors as its non-GM parent, sexually compatible with the same plants and its products are used identically to non-GM cotton. The only differences between Roundup Ready Flex® cotton and non-GM cotton are that the former is tolerant to glyphosate-based herbicides (see below).

98. Accordingly, agricultural management of Roundup Ready Flex® cotton differs from non-GM cotton in the application of herbicides. GM Roundup Ready Flex® cotton volunteers in subsequent crops are targeted for management and must be controlled with non-glyphosate herbicides or by mechanical means.

5.2.1 Genetic modification

99. A detailed description of the genetic modification is available in the RARMP for DIR 035/2003.

5.2.2 Introduced genes

100. Roundup Ready Flex® cotton contains two copies of the 5-enolpyruvylshikimate-3-phosphate synthase (*cp4 epsps*) gene from the soil bacterium *Agrobacterium* sp. strain CP4 (Barry et al. 1992). Unlike plant EPSPS enzymes, the CP4 EPSPS enzyme can function in the presence of glyphosate, the active constituent of a number of herbicides including Roundup Ready® herbicide. Expression of *cp4 epsps* in Roundup Ready Flex® GM cotton confers tolerance to glyphosate (Barry et al. 1992).

101. The *epsps* gene encodes 5-enol-pyruvylshikimate-3-phosphate synthase, EPSPS, which catalyses the conversion of phosphoenol pyruvate (PEP) and shikimate 3-phosphate (SHKP) to 5-enol-pyruvylshikimate-3-phosphate (EPSP). This reaction, part of the shikimic acid pathway, is essential for the biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Herrmann & Weaver 1999).

5.2.3 Introduced regulatory elements

102. In addition to the introduced genes, Roundup Ready Flex® cotton contains short regulatory elements used to control expression of the genes (Table 5 and Table 6). These sequences are derived from plants (thale cress and pea), a soil bacterium (*A. tumefaciens*) and plant viruses (CaMV and FMV).

Table 5. Genetic modifications of Roundup Ready Flex cotton

Plasmid name	Promoter	Gene	Terminator	Additional genetic elements	Function
PV GHGT35	P-FMV/ TSF2	*cp4 epsps*	rbcS-E9	Ctp2	herbicide tolerance
	P-35S/ ACT8	*cp4 epsps*	rbcS-E9	Ctp2	herbicide tolerance

Table 6. Details of the introduced genetic elements

Genetic element	Full name/description	Source organism
cp4 epsps	5-enolpyruvylshikimate-3-phosphate synthase	*Agrobacterium* sp. strain CP4
P-FMV/TSF2	EF1alpha promoter (including non-translated leader with intron sequences) with FMV 35S enhancer sequence	*A. thaliana*, Figwort mosaic virus (FMV)

Genetic element	Full name/description	Source organism
P-35S/ACT8	actin8 promoter (including non-translated leader with intron sequences) with CaMV 35S enhancer sequence	*A. thaliana*, CaMV
rbcS-E9	3' non-translated sequence of the ribulose 1,5 bisphosphate carboxylase small subunit E9 gene	*Pisum sativum* (pea)
Ctp2	Chloroplast targeting peptide from the *epsps* gene	*A. thaliana* (thale cress)

103. A more detailed description of the regulatory sequences used in Roundup Ready Flex cotton can be found in the RARMP for DIR 059/2005. Although some of these regulatory sequences are derived from organisms (a bacterium and viruses) that are plant pathogens, by themselves they do not cause disease. The regulatory elements present in the parental GM cotton have been previously assessed by Australian and international regulators without identifying an increase in risk compared to endogenous regulatory elements of cotton.

5.2.4 Introduced CP4 EPSPS protein

104. EPSPS catalyses the conversion of phosphoenol pyruvate (PEP) and shikimate 3-phosphate (SHKP) to 5-enol-pyruvylshikimate-3-phosphate (EPSP). This reaction, part of the shikimic acid pathway, is essential for the biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Herrmann & Weaver 1999).

105. The *epsps* gene and its encoded protein have been described in detail in the RARMPs for commercial release applications DIR 059/2005 and DIR 066/2006, and the description was recently updated in the RARMP for commercial release application DIR 118.

Toxicity/allergenicity to humans and animals

106. From an extensive body of experimental work, there is no evidence that the EPSPS protein is toxic or allergenic. Toxicity experiments with animals (mainly mice and rats), often involving the feeding of exaggerated doses of the protein, have failed to establish any deleterious effects upon the subjects (Hammond et al. 2004; Harrison et al. 1996; Teshima et al. 2000; Zhu et al. 2004). Current literature on the toxicity of the CP4 EPSPS protein has recently been discussed in the RARMP for DIR 118 and further information can also be found in the RARMPS for DIR 055/2004, DIR 059/2005 and DIR 066/2006.

107. Analysis of the amino acid sequence of the CP4 EPSPS protein has failed to demonstrate any significant homology with any known toxin or allergen. Further, the protein is rapidly denatured by heat, enzymatic digestion and acid in simulated mammalian digestive fluid, indicating it is unlikely to have any toxic or allergenic effects (Harrison et al. 1996). In assessments for GM lucerne and GM soybean) expressing the CP4 EPSPS protein, FSANZ note that there is no evidence of toxic and allergenic properties associated with these proteins (FSANZ 2006; FSANZ 2007).

Effects on soil microorganisms

108. In reviewing the literature relating to effects of GM plants on soil microorganisms, a number of authors have commented on the technical difficulties in measuring, assessing and interpreting such effects (Bruinsma et al. 2003; O'Callaghan et al. 2005b; Weinert et al. 2010). As the habitat of *Agrobacterium tumefaciens* is the soil and roots of plants, it is expected that soil microorganisms are regularly exposed to EPSPS proteins or their degradative peptide products; in this context, one study has found that 90% of the CP4 EPSPS protein is degraded in the soil within 9 days (Dubelman et al. 2005).

109. In general, no evidence has been found to suggest that the CP4 EPSPS protein or similar proteins are toxic to microorganisms including various species of protozoa, bacteria, fungi, algae and diatoms (OGTR 2006a and b). For example, no permanent effects on soil biota were

observed in a series of experiments designed to estimate the effect of glyphosate tolerant soybean and maize, and their management, on the abundance of detritivorous soil biota and crop litter decomposition (Powell et al. 2009). While statistically significant effects were observed in a few of the measured groups, in most cases the effects were only observed in the first year of the study and were not consistent across sample dates or across the four study years. The most frequent effect of the glyphosate tolerant herbicide system was a transient shift toward more fungal biomass relative to bacterial. The genetic modification in the soybean and maize had little effect on litter decomposition, however the use of glyphosate did reduce decomposition of surface (but not buried) litter.

110. In a field experiment conducted at six sites in Canada, repeated plantings of glyphosate tolerant wheat and glyphosate tolerant canola grown in rotation had only minor and inconsistent effects on soil microorganisms over a wide range of growing conditions and crop management regimes (Lupwayi et al. 2007). As is the case for many studies that show an effect of herbicide resistant cropping systems on microbial communities, the effects of the glyphosate tolerance trait and the herbicide applications were not separated in this study. Application of herbicides can affect proportions of soil microbes (for example, see Becker et al. 2001; Gyamfi et al. 2002; Kremer & Means 2009; Mijangos et al. 2009).

111. Crop type (GM or non-GM) made no difference to the abundance or structure of microbial communities in a study designed to separate the effects of GM glyphosate tolerant maize from the use of glyphosate on denitrifying bacteria and fungi (Hart et al. 2009). The GM maize in this study expressed the *cp4 epsps* gene, and the authors note that the use of a protein derived from a common soil bacterium may affect soil microbial communities less than modifications that introduce novel proteins into the soil.

112. No novel metabolic products are formed in Roundup Ready Flex cotton as the only difference between the introduced CP4 EPSPS protein and the native enzyme is the reduced affinity of the former for glyphosate (OECD 1999).

113. Glyphosate is the active ingredient in a number of broad-spectrum systemic herbicides that have been approved for use in Australia, and was first marketed as the proprietary herbicide Roundup®. The action of glyphosate is due to its structural resemblance to PEP. In plants, glyphosate binds preferentially to the active site of endogenous (plant) EPSPS proteins (Steinrucken & Amrhein 1980). However, the CP4 EPSPS enzyme has a greater affinity for PEP than glyphosate, this difference in substrate affinity being sufficient for GM plants carrying the gene coding for this bacterial enzyme to be tolerant to the herbicide.

114. The potential toxicity of the herbicide metabolites is considered by the APVMA in its assessment of a new use pattern for particular herbicides. The APVMA found that there is no difference in the metabolic fate of glyphosate in non-GM canola and in GM canola expressing *goxv247* and *cp4 epsps*.

5.2.5 Presence of identical or similar genes and proteins in the environment

115. All source organisms for the introduced genetic elements are widespread and prevalent in the Australian environment and thus humans and other organisms commonly encounter their genes and encoded proteins.

116. Homologues of the *cp4 epsps* gene are widespread in plants and microorganisms, implying that both vertebrates and invertebrates are regularly exposed to EPSPS protein homologues in their diet and these are unlikely to have any adverse toxic or allergenic effects. Further, as the habitat of *Agrobacterium tumefaciens* is the soil and roots of plants, it is expected that soil microorganisms are regularly exposed to EPSPS proteins or their degradative peptide products. In this context, one study has found that 90% of the CP4 EPSPS protein is degraded in the soil within 9 days (Dubelman et al. 2005).

5.2.6 Molecular stability

117. Molecular characterisation of Roundup Ready Flex cotton is described in the RARMPs prepared for DIR 035/2003 and DIR 066/2006 and includes Southern blot and PCR analyses, as well as molecular cloning and sequencing of the site of insertion. Stable integration and inheritance of the inserted DNA was demonstrated in all of the lines. DNA sequencing was used to verify the inserted genes and to determine the regions flanking all of the insertion sites.

118. Data pertaining to the characterisation of the insertion of the *cp4 epsps* genes in the parent Roundup Ready Flex® *G. hirsutum* line in the United States is discussed in the RARMPs prepared for DIR 035/2003 and DIR 066/2006. Southern blot analysis has demonstrated the stability of the insert over five generations, as assayed by the number and size of plant DNA fragments hybridising to selected DNA probes (Groat et al. 2004). The insert in Roundup Ready Flex® *G. hirsutum* contains two complete *cp4 epsps* genes at a single locus (Cerny et al. 2010).

5.2.7 Method of genetic modification

119. The methods by which Roundup Ready cotton was produced have been described in detail in the RARMP for DIR 035/2003 and are summarised here.

120. Roundup Ready Flex® cotton (MON 88913) was developed using *Agrobacterium tumefacien*s mediated transformation with the disarmed binary vector plasmid described in Table 4. Transformed cotton cells were selected through their ability to grow in the presence of glyphosate as the selective agent (the *cp4 epsps* gene encodes tolerance to this herbicide, for in-vitro cell cultures as well as whole plants). GM cotton plants were regenerated from the selected cells. *Agrobacterium tumefacien*s mediated transformation is widely used in Australia and overseas for introducing new genes into plants and further information can be found in the document *Methods of plant genetic modification* available from the Risk Assessment References page of the OGTR website.

121. The parental *G. hirsutum* germplasm for transformation of Roundup Ready Flex cotton was the cultivar Coker 312, which was released in 1974 by the Coker Pedigree Seed Company.

5.2.8 Experience with Roundup Ready Flex® cotton

Australian experience from cultivation of Roundup Ready Flex cotton

122. Roundup Ready Flex® GM cotton has previously been described and assessed for commercial release (refer to RARMPs for DIR 059/2005 and DIR 066/2006), in addition to other applications for limited and controlled release. Assessments of Roundup Ready Flex cotton, individually or in combination with Bollgard® II, in the context of commercial release throughout Australia concluded that it poses negligible risks to human health and safety and the environment.

123. To date, the Regulator has not received reports of adverse effects caused by Roundup Ready Flex® cotton as a crop. There are no scientific studies showing adverse effects of Roundup Ready cotton grown as a crop on human health or the environment in Australia.

124. As a volunteer, in subsequent crops and around farms, Roundup Ready Flex® cotton has been described by farmers as a weed. Crop resistance to glyphosate, along with a general move to reduced tillage, has reportedly led to increased survival of volunteer cotton plants on farms as a consequence of heavy reliance on glyphosate for weed control. On this basis, glyphosate tolerant cotton has been termed an agricultural weed by Thornby et al (2012).

125. This is reflected in responses to the Crop Consultants Association (CCA) surveys of Australian cotton growers (2010-2013), which report a yearly increase in prevalence of cotton volunteers and ratoons: in 2013, 48% of consultants identified ratoon and volunteer cotton as

more prevalent compared with the previous season and in many cases volunteer cotton was the dominant weed in the farming system (Spotlight on Cotton, Spring 2013).

126. In addition, an increase in prevalence of volunteer cotton in irrigated cotton systems was identified by Werth et al. (2013), who undertook surveys in southern Qld and northern NSW of irrigated and non-irrigated cotton systems over the 2008/09 and 2010/11 seasons. The incidence of volunteer cotton was found to have increased from 5% of fields in 2008 to 31% in 2010, largely due to more cotton being grown in the survey region over that time (26 600 ha in 2007/08 compared with 146 000 ha in 2009/10). The volunteers were predominately glyphosate-resistant cotton plants that had survived fallow glyphosate sprays.

127. Management of cotton volunteers between growing seasons is important for cotton pest and disease control. For example, aphids, mealybug and cotton bunchy top can survive on volunteer cotton between growing seasons (Wilson et al. 2013). Removal of weed hosts in general over the winter period has been identified by the cotton industry as a major shared principle of integrated pest and disease management strategies and stewardship plans (Spotlight on Cotton, Winter 2013). As outlined in section 4.2.3, management practices for volunteer cotton include selection of appropriate herbicides for four to six leaf plants and manual chipping for more established plants.

Australian experience from the use of products of Roundup Ready Flex cotton

128. The applicant has received approval from FSANZ for the use of oil and linters derived from Roundup Ready Flex® *G. hirsutum* and Roundup Ready Flex® *G. barbadense* (pima cotton) in food (FSANZ 2005). These approvals also cover material derived from cotton containing both Roundup Ready Flex and Bollgard II.

129. Cotton seed and meal from Roundup Ready Flex cotton has been fed to domesticated animals such as cattle since its approval for commercial release and no adverse effects have been reported to the Regulator.

130. Roundup Ready® herbicide has been registered by the APVMA for use on Roundup Ready® cotton since 2000 and on Roundup Ready Flex® Flex cotton since 2006.

International experience with Roundup Ready® Flex cotton

131. Roundup Ready Flex cotton has been approved for environmental release as well as food and feed use in a number of countries (Table 7).

Table 7. Approvals of Roundup Ready Flex cotton in other countries

Country	Environment	Food and/or Feed	Food	Feed
Canada			2005	2005
China		2007		
Colombia			2010	
Japan			2005	2006
Korea			2006	2006
Mexico		2006		
Philippines		2005		
South Africa	2007	2007		
United States	2004	2005		

132. Additionally, cottons containing the combined Roundup Ready Flex® and Bollgard® II events have been approved in a number of other countries.

Table 8. Approvals of Bollgard II/Roundup Ready Flex cotton in other countries

Country	Environment	Food and/or Feed	Food	Feed
Colombia				2010
Japan			2005	2006
Korea			2006	2008
Mexico		2006		
Philippines			2006	2006
South Africa	2007	2007		

133. In the United States, releases of GM cotton in which the Roundup Ready Flex® and Bollgard® II events have been combined by conventional breeding are approved automatically as the individual GM parents are approved.

134. To date, there have not been any reports of adverse effects on human health or the environment caused by these authorised releases. There have been reports of glyphosate resistant weeds developing in the United States as a result of glyphosate overuse. However, glyphosate resistant weeds were not caused specifically by cropping with GM Roundup Ready Flex cotton but rather through heavy over-reliance on glyphosate herbicides over a number of years in both GM and non-GM cropping systems.

5.3 GM VIP3A cotton

135. VIP3A cotton is also known as COT102 cotton. It is phenotypically very similar to non-GM cotton. For example, it is limited by the same abiotic factors as its non-GM parent, sexually compatible with the same plants and its products are used identically to non-GM cotton. The only phenotypic difference between VIP3A and non-GM cotton are that it expresses a protein toxic to certain insects in the order Lepidoptera, including the most important insect pest of cotton crops in Australia. Accordingly, agricultural management of VIP3A cotton differs from non GM cotton in the application of insecticides.

5.3.1 Genetic modification

136. Detailed information on the genetic modification of VIP3A cotton (either individually or in combination with the Bollgard® II and Roundup Ready Flex® modifications) is available in the RARMPs for DIR 017/2002, DIR 025/2002, DIR 034/2003, DIR 036/2003, DIR 058/2005, DIR 065/2006 and DIR 073/2007.

5.3.2 Introduced genes

137. VIP3A cotton contains a synthetic copy of the *vip3Aa1* gene, which encodes a vegetative insecticidal protein (VIP)3Aa1 that confers toxicity to various lepidopteran insects including significant pests of cotton. The *vip3a19* gene is a modified synthetic copy of a *vip3Aa1* gene from *Bacillus thuringiensis* (Bt) strain AB88, which was isolated from sour milk (Estruch et al. 1996). VIPs isolated from Bt and *Bacillus cereus* have shown activity against a range of lepidopteran species and several coleopteran species (Warren 1997), and the activity of VIP3A has been shown to contribute significantly to the toxicity of Bt spores to insects (Donovan et al. 2001). To date, 53 *vip3Aa*-class genes have been cloned (Crickmore et al. 2014), and closely related genes have been detected in approximately 50% of the Bt strains surveyed (Hernandez-Rodriguez et al. 2009; Liu et al. 2007). Hernandez-Rodriguez et al. (2009) surveyed 507 strains of Bt and found that 91.5% of those with *vip3* genes also contained *cry1A* and *cry2* genes, and speculated that these genes are encoded by the same plasmids.

138. In addition, VIP3A cotton contains the *aph4* gene from *E. coli* which confers resistance to the antibiotic hygromycin B.

5.3.3 Introduced regulatory elements

139. In addition to the introduced genes, VIP3A cotton contains short regulatory elements used to control expression of the genes (Table 9 and Table 10). These sequences are derived from thale cress and a common soil-borne bacterium (*A. tumefaciens*).

Table 9. Genetic modifications present in VIP3A cotton

Plasmid name	Promoter	Gene	Terminator	Additional genetic elements	Function
pCOT1	actin2 promoter	*vip3A*	nos	First exon and intron of actin-2	insect resistance
	ubiquitin3 promoter	*aph4*	nos	ubi3 intron	antibiotic resistance

Table 10. Details of the introduced genetic elements

Genetic element	Full name	Source organism / further description
vip3A	vegetative insecticidal protein 3A gene	Modified synthetic version of *vip3Aa1* gene from *Bacillus thuringiensis* strain AB88
aph4	hygromycin B phosphotransferase gene	*Escherichia coli* strain K-12
Act2	actin2 gene promoter, including untranslated leader sequence with the first exon and intron of the actin2 gene.	*Arabidopsis thaliana* (thale cress)
Ubi3	ubiquitin3 gene promoter	*A. thaliana*
nos	3' non-translated sequence of the nopaline synthase gene	*Agrobacterium tumefaciens*

140. Although one of these regulatory sequences is derived from a plant pathogen, by itself it does not cause disease. The regulatory elements present in VIP3A cotton have been previously assessed by Australian and international regulators without identifying an increase in risk compared to endogenous regulatory elements of cotton.

5.3.4 The Introduced Vip3A protein

141. Vegetative insecticidal proteins (Vips) are soluble proteins secreted by various *Bacillus* species during vegetative growth stages and sporulation. In this they differ from the Cry proteins, which are expressed by Bt only during sporulation and form crystalline inclusions in spores (reviewed by Estruch et al. 1997). Vips do not exhibit any structural similarity with the Cry toxins and bind to different receptors in the insect midgut (Lee et al. 2006; Sena et al. 2009). Currently, all Vip-related sequences that have been described fall into three different families, Vip1, Vip2, and Vip3 (Crickmore et al. 2014), each having a different insect host range. As will be discussed further below, Vip3 proteins are mainly active against a range of lepidopteran species. Of these, the Vip3Aa proteins in particular, for which over 50 variants have been identified, have been developed to confer an insect control trait in GM plants.

142. The *vip3Aa19* gene (Entrez Accession number DQ539887) is a variant of the native *vip3Aa1* gene found in *Bacillus thuringiensis* strain AB88. It has been modified to accommodate the preferred codon usage in plants (Murray et al. 1989). In the expressed Vip3Aa19 protein, glutamine is present at position 284, whereas Vip3Aa1 has lysine at the same position. The substitution is conservative in that lysine and glutamine are polar amino acids having a molecular weight of 146 kDa. The other amino acid residues are identical in both proteins. However, comparison of the reported activity of Vip3A with VIP, a closely related Vip described by Selvapandiyan et al. (2001), raised the possibility that the amino acid substitution in Vip3A could contribute to altered specificity (Hill et al. 2003). The related Vip varies by two amino acids from Vip3A (one of which is the lysine to glutamine substitution at residue 284) and has been shown to be toxic to *Plutella xylostella* (diamondback moth), whereas Vip3Aa1 is not. Nonetheless, like Vip3Aa1, Vip3Aa19 is reportedly not toxic to

diamondback moth (USDA-APHIS 2005) and the amino acid substitution does not appear to have otherwise changed the insecticidal activity of the protein.

143. For the purposes of discussion in ensuing sections of the RARMP, variants of Vip3Aa (including Vip3Aa19) will be referred to as Vip3A or Vip3Aa, except where a distinction is necessary in the context of the topic.

Toxicity/allergenicity of Vip3A to humans

144. Vip3A proteins are not expected to be toxic to organisms that lack the receptors to which Vip3A binds, such as those found on the brush border membrane vesicles in the midguts of some lepidopteran larvae (Lee et al. 2003). Therefore, humans and livestock are highly unlikely to be susceptible to Vip proteins. In addition, searches of the FARRP Allergen Database, performed according to CODEX guidelines (Codex Alimentarius Commission 2003b) have recovered no matches of the Vip3A protein to known allergens (information supplied by applicant). Similarly, the US EPA uses a weight-of-evidence approach for allergenicity consistent with the CODEX guidelines and has concluded that the potential for Vip3Aa to be a food allergen is minimal (US EPA 2008).

145. Syngenta has conducted studies to test for toxicity of Vip3A to a range of non-target organisms, which are summarised in Hill (2003), a document submitted to regulatory authorities in the USA (USDA-APHIS 2005). These studies were considered in detail in the RARMP prepared in respect of application DIR 058/2005. More recent non-target organism toxicity data for Vip3A can be found in an ecological risk assessment prepared by Raybould and Vlachos (Raybould & Vlachos 2011) for MIR162 maize, which expresses Vip3Aa20, and in the US EPA Biopesticide registration action document for COT 102 x COT 67B cotton (US EPA 2008). The latter includes characterisation of representative Vip3A proteins, acute oral toxicity studies, amino acid sequence comparisons to known allergens and toxins and *in vitro* digestibility of the protein. No treatment-related adverse effects were observed in any of these studies and this was used as further indication that Vip3Aa is non-toxic to mammals, including humans.

146. To summarise the above studies, laboratory experiments indicate that Vip3A protein expressed by *E. coli* or GM maize is not toxic to mice, Bobwhite quail and channel catfish, in addition to a range of non-lepidopteran insect species (including representative Coleoptera, Hymenoptera, Isotomidae and Neuroptera) and several other invertebrates (including water fleas and earthworms). The exposure levels reached in these studies were estimated to be substantially greater than levels expected in the field, based upon expression levels of Vip3A in VIP3A GM cotton or maize. The studies also indicate no adverse effects to predators, parasitoids (e.g., parasitic Hymenoptera), decomposers and herbivores exposed to or feeding on cotton or maize expressing the VIP3A protein.

147. Further, the most recent review of the environmental safety of Vip3Aa by CERA (2012) concluded that these proteins are active specifically against the subset of lepidopteran pests which consume the crop and are harmless to vertebrate species and other non-target organisms.

Toxicity to invertebrates

148. Following ingestion by insects, Vip3Aa1 protein is activated by proteolytic processing in the midgut (Lee et al. 2003; Yu et al. 1997). In susceptible insect species, activated Vip3Aa1 binds to midgut epithelium cells and the insects undergo gut paralysis, lysis of midgut epithelium cells and death (Yu et al. 1997). Vip3Aa1 forms channels in midgut epithelium cell membranes (Lee et al. 2003) which are thought to mediate its effects on the midgut. Binding of Vip3 proteins to the midgut requires the presence of specific receptor proteins on the midgut epithelium surface, and is thought that this is the mechanism by which Vip3 proteins have a high degree of target insect specificity.

149. The mode of action of Vip3A proteins has some similarities to that of Cry proteins, however Vip3A proteins bind to different insect midgut receptor proteins than do Cry proteins (Lee et al. 2006; Sena et al. 2009), and form membrane channels that have different biophysical properties (Lee et al. 2003). The different biochemistries underlying the activity of these toxins correlates with reports that insects resistant to the Cry1Ac protein remain susceptible to Vip3A (Jackson et al. 2007). There is no sequence similarity between Vip3A proteins and Cry proteins (Estruch et al. 1996).

150. As outlined in the previous section, the potential for harm to non-target insects by Vip3Aa has been considered as part of regulatory risk assessments for GM crops expressing the *vip3Aa* gene, which determined that adverse effects are unlikely (CERA 2012). This is based on the narrow spectrum of insecticidal activity displayed by Vip3Aa, and on the results of tier 1 testing of a range of invertebrate species (or their surrogates) that are present in maize and cotton agricultural communities. Tier 1 testing is part of a tiered risk assessment approach for pesticides that relies on a worst-case exposure regimen targeted at indicator species. Laboratory toxicity assays are conducted that expose selected non-target species to a maximum-hazard dose, usually1–20 times the expected environmental exposure concentration (USEPA 2003). The tests are often focused on six to eight indicator species (such as honeybees, springtails, earthworms, daphnia, predatory beetles or pirate bugs, and parasitoid wasps), which represent different functional guilds (e.g., pollinators, detritivores, predators, parasitoids).

151. The predictive validity of laboratory studies for non-target effects of Bt Cry proteins has been explored by Duan et al. (2010). The authors used meta-analyses to test whether laboratory studies were consistent with results from field studies that compared the abundance of non-target organisms in Bt crops versus non-Bt counterparts. In summary, the meta-analysis supported the assumption that tier 1 laboratory studies show effects that are either consistent with, or more conservative than, those found in field studies of GM insect resistant crops.

152. In field trials, the VIP3A GM cotton has been shown to have activity against a range of lepidopteran insects: *Helicoverpa armigera* (cotton bollworm), *H. punctigera* (native budworm), *H. zea* (corn earworm), *Heliothis virescens* (tobacco budworm), *Spodoptera exigua* (beet armyworm), *Pectinophora gossyipiella* (pink bollworm), *Pseudoplusia includens* (soybean looper), *Trichoplusia ni* (cabbage looper) and *Bucculatrix thurberiela* (cotton leaf perforator) (unpublished studies summarised by Hill et al. 2003; Llewellyn et al. 2007; Whitehouse et al. 2007). Laboratory assays using various preparations of Vip3Aa1 protein have also shown activity against some of these species (Estruch et al. 1996; Liao et al. 2002) and also *S. frugiperda* (fall armyworm), *Manduca sexta* (tobacco hornworm) and *Agrotis ipsilon* (black cutworm) (Cotton Catchment communities CRC 2007; Lee et al. 2003).

153. *H. armigera, H. punctigera, S. exigua* and *P. gossypiella* are cotton pests in Australia, as are some species related to *Agrotis ipsilon, B. thurberiela, S. exigua* and *S. frugiperda*.

154. Whitehouse et al. (2007) studied the effects of VIP3A cotton on the invertebrate community at two Australian field sites, in comparison to non-GM cotton. The sites were chosen to represent a major commercial cotton growing region and a tropical cotton region north of 22ºS. The authors found that there were no major differences in species richness or diversity of beneficial and non-target communities. Several indirect effects were detected, including higher numbers of predatory beetles and mirids in the GM cotton plots; these effects were thought to result from factors such as the increased numbers of bolls on VIP3A plants and the reduced abundance of the target *Helicoverpa* spp. larvae (which are prey for several species).

Toxicity to honeybees

155. A recent environmental review of Vip3Aa (CERA 2013 and references therein) concluded that plants containing these proteins would not harm non-target arthropods. In particular, ecotoxicological testing on honeybees found no harm to honey bees from exposure to the purified proteins. There was no observed adverse effect when honeybee larvae were exposed to the purified Vip3A protein at 500µg/g diet for 24 days. As a comparison, measured expression levels in VIP3A in cotton pollen range from 0.8–3.2 µg/g fwt.

Fate of Vip3A in soil

156. The US EPA (2008) reviewed studies on the fate of the Vip3A protein in soil. Based on bioactivity studies of Vip3A protein incorporated into various types of soil, they found no indication that the proteins expressed in COT102 were likely to persist or accumulate in soil after continuous cultivation.

5.3.5 Presence of identical or similar genes and proteins in the environment

157. The introduced genes for VIP3A cotton are derived from common bacteria. The regulatory sequences (promoters, terminators, leader sequences) are derived from plants (thale cress) and a common soil bacterium (*Agrobacterium tumefaciens*). The coding sequences are derived from common bacteria (*B. thuringiensis* and *E. coli*). All the source organisms for the introduced genetic elements are widespread and prevalent in the Australian environment and thus humans and other organisms would commonly encounter their genes and encoded proteins.

5.3.6 Molecular stability

158. Molecular characterisation of VIP3A cotton included Southern blot and PCR analyses, as well as molecular cloning and sequencing of the site of insertion. Stable integration and inheritance of the inserted DNA was demonstrated in all of the lines. DNA sequencing was used to verify the inserted genes and to determine the regions flanking all of the insertion sites.

159. Data showing Mendelian inheritance of the *vip3A* gene through five generations of selfing and crossing, as assayed by the number and size of plant DNA fragments hybridising to selected DNA probes, has been previously assessed in RARMPs for DIR 034/2003, DIR 058/2005, DIR 065/2006 and DIR 073/2007.

160. Molecular analyses were provided by the applicant to confirm the genetic stability of the insert (Burgin 2013). Sequence analysis demonstrated that the insert (including coding sequences of *vip3Aa19* and *aph4*, the *Ubq3* and *Act2* promoters, and the *nos* terminators) is intact, as intended. Some truncation occurred at the right border (RB) and left border (LB) ends of the T-DNA during the transformation process. Such deletions have been previously observed in transformations with *A. tumefaciens* and are unlikely to affect the functionality of the DNA insert.

161. Additional information relevant to detail of the molecular characterisation of COT102 has been declared confidential commercial information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on this application and RARMP..

5.3.7 Method of genetic modification

162. VIP3A cotton was developed using *Agrobacterium tumefaciens* mediated transformation with the disarmed binary vector plasmids described in Table 9. *Agrobacterium tumefacien*s mediated transformation is widely used in Australia and overseas for introducing new genes into plants and further information can be found in the document *Methods of plant genetic modification* available from the Risk Assessment References page of the OGTR website

163. The VIP3A GM cotton was produced using protocols similar to those described by Murray et al (1999). Transformed cotton cells were selected through their ability to grow in the presence of the appropriate selective agent, hygromycin. GM cotton plants were regenerated from the selected cells.

164. The parental *G. hirsutum* germplasm for the transformation was the cultivar Coker 312, which was released in 1974 by the Coker Pedigree Seed Company.

5.3.8 Australian experience from cultivation of VIP3A cotton

165. To date, VIP3A cotton has only been released under limited and controlled conditions in Australia. A number of field trials have been authorised under DIR 017/2002, DIR 025/2002, DIR 034/2003, DIR 036/2003, DIR 058/2005, DIR 065/2006 and DIR 073/2007. The Regulator has not received any reports of adverse effects caused by VIP3A cotton in these trials.

5.3.9 Australian experience from the use of VIP3A cotton products

166. FSANZ has previously given approval for the use in food of cotton seed oil and linters derived from VIP3A GM cottons (under application A509; the assessment is available from the FSANZ website). FSANZ assessed human food derived from linters and cotton seed oil from VIP3A cotton, and concluded that studies to determine potential toxicity of Vip3A demonstrate that it is non-toxic to mammals (FSANZ 2004). Also, the Vip3A protein has been demonstrated to be heat labile (Estruch et al. 1996), which would lead to a decreased exposure in processed products. The studies assessed by FSANZ are summarised in Hill et al. (2003).

167. VIP3A cotton meets the definition of an agricultural chemical product under the Agricultural and Veterinary Chemicals Code Act 1994, due to their production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA. The APVMA has issued permits for the field trials of VIP3A cotton authorised by the Regulator.

5.3.10 International experience with VIP3A cotton

168. VIP3A cotton (COT102) has been released on a commercial scale and for food and/or feed use in the United States (US; in 2005) and in Mexico (in 2010).

169. Field trials of VIP3A cotton have been conducted in Argentina (2001-2002), Burkina Faso (2004-2006), China (2001-2003), Costa Rica (2002, 2007-2009), India (2002-2006), Republic of South Africa (2002-2005), the USA (2000-2009), Vietnam (2002-2003) and Zimbabwe (2003-2004).

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

Section 6 The GMOs

6.1 Introduction to the GMOs

171. The GM cottons proposed for release are:

- Bollgard® III cotton and
- Bollgard® III x Roundup Ready Flex® cotton.

172. These GM cottons have been generated by conventional crossing of:

- GM Bollgard® II cotton (insect resistant) with GM VIP3A cotton (insect resistant; produced by Syngenta and licenced to Monsanto) and of
- GM Bollgard® II x Roundup Ready Flex® (insect resistant and glyphosate herbicide tolerant) cotton with GM VIP3A cotton, respectively.

173. Bollgard® III and Bollgard ®III x Roundup Ready Flex® have been bred into cotton varieties suitable for Australian conditions.

174. Tables 1, 2, 5, 6, 9 and 10 list the genetic elements present in the GM parental cottons. All of the relevant genetic elements are present in Bollgard® III cotton and Bollgard® III/Roundup Ready Flex® cotton. Therefore, in addition to genes conferring insect resistance and herbicide tolerance, the GM cottons contain introduced antibiotic resistance genes, a reporter gene and regulatory elements.

175. Bollgard® III and Bollgard® III/Roundup Ready Flex® *G. hirsutum* have been approved by the Regulator for limited and controlled release (field trials) under licence DIR 101. Further, Bollgard® II and Bollgard® II/Roundup Ready Flex® *G. hirsutum* have been approved for commercial release in DIR 066/2006. The latter licence also authorises the release of several other GM cotton lines that possess the same introduced genes.

176. The RARMP for DIR 101 identified additional information that may be required for a large scale or commercial release of Bollgard® III and Bollgard® III/Roundup Ready Flex® cotton. The relevant information can be summarised as:

- additional data on the potential toxicity of Vip3A in combination with the proteins encoded by the other introduced insect resistance genes to non-target invertebrates
- phenotypic characterisation of the GM cottons, in particular of traits which may contribute to weediness, persistence, and ability to disperse in the environment
- data on the effects on non-target insects and weediness potential of Bollgard III or Bollgard® III/Roundup Ready Flex® combined with insect resistant WideStrike™ cotton.

177. Monsanto has provided information in relation to the first two of these points in application DIR 124, and this is discussed in the relevant sections below. The third point is considered in Chapter 2, risk scenario 8.

6.2 Characterisation of the GMOs

6.2.1 Molecular stability

178. The applicant has provided Southern blot data to demonstrate stability of the DNA inserts in Bollgard® III and Bollgard® III x Roundup Ready Flex® (Garnaat et al. 2013a; Garnaat et al. 2013b).

179. For both GM cottons, Southern blot analysis demonstrated that the fingerprints obtained by analysis of the combined-trait products are consistent with the corresponding fingerprints obtained with each of the individual GM parental lines. These results confirmed the presence of the individual inserts in the GM cottons.

6.2.2 Expression of the introduced proteins

180. The applicant measured protein expression levels in GM cotton during Australian field trials between 2010 and 2012 (Table 9). The levels of Vip3A, Cry1Ac, Cry2Ab and CP4 EPSPS proteins were determined by validated enzyme-linked immunosorbent assays in leaf, pollen and seed tissues from Bollgard® III cotton, Bollgard® III x Roundup Ready Flex® cotton, and the parental GM cottons.

181. Table 11 shows that the ranges of expression levels of introduced proteins in Bollgard® III cotton and Bollgard® III x Roundup Ready Flex® cotton are comparable to the expression levels in the parental cotton lines.

Table 11. Expression levels of introduced proteins in 2010-12 Australian field trials of GM cotton

Protein	Cotton line	Leaf protein (µg/g fresh weight)	Seed protein (µg/g fresh weight)	Pollen protein (µg/g fresh weight)
Vip3A	Parental COT102	11 - 67	1.2 – 1.8	0.8 – 3.2
	Bollgard® III	20 - 56	1.0 – 1.7	0.5 – 2.7
	Bollgard® III x Roundup Ready Flex®	12 - 68	1.0 – 3.7	0.3 – 3.3
Cry1Ac	Parental Bollgard® II	2.1 – 9.8	4.9 – 6.5	0.2 – 0.4
	Bollgard® III	2.2 – 9.4	4.4 – 6.1	0.2 – 0.4
	Bollgard® III x Roundup Ready Flex®	1.5 - 12	3.4 – 9.8	0.1 – 0.7
Cry2Ab	Parental Bollgard® II	25 - 100	240 - 320	1.9 – 9.3
	Bollgard® III	27 -110	270 - 310	1.4 – 1.9
	Bollgard® III x Roundup Ready Flex®	15 - 170	180 - 340	0.7 - 19
CP4 EPSPS	Parental Roundup Ready Flex®	150 - 410	160 - 240	17 - 66
	Bollgard® III x Roundup Ready Flex®	100 - 460	89 - 280	3.8 - 99

6.2.3 Phenotypic characterisation of the GM cottons

Compositional analysis

182. Compositional analysis of seed from each of the GM parent cottons has been previously considered by the OGTR and FSANZ. The GM seed was assessed to be compositionally equivalent to conventional cotton (OGTR: DIR012/2002, DIR017/2002, DIR034/2003, DIR035/2003, DIR055/2004, DIR059/2005, DIR066/2006, DIR074/2007, DIR101; FSANZ: A338, A362, A363, A416, A525, A548, A553, A592, A1049, A1063).

183. The applicant has provided compositional data for Bollgard® III and Bollgard® III x Roundup Ready Flex® cotton (Venkatesh 2013a; 2013b), comparing the GM to a conventional cotton variety with a similar genetic background. Compositional analyses were conducted on acid-delinted cottonseed collected from these test and control substances as well as twelve unique conventional cotton varieties (reference substances). Cottonseed analysed was grown at eight sites across representative cotton growing regions in the US during the 2011 growing season.

184. Analyses of the cottonseed samples were conducted for nutrients including calories, carbohydrates, moisture, protein, total fat, acid detergent fibre, neutral detergent fibre, crude fibre, total dietary fibre, amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc) and vitamin E; and anti-nutrients including gossypol (free and total) and cyclopropenoid fatty acids (dihydrosterculic, malvalic and sterculic acids). In all, 65 different analytical components were measured. Of

these, 13 had more than 50% of the observations below the assay limit of quantitation and were excluded from statistical analysis. Therefore, 52 components were statistically assessed using a mixed-model analysis of variance method.

185. For both GM cottons, statistical comparisons to the conventional control were based on compositional data combined across all eight individual field sites (the combined-site analysis). Statistical differences were identified at a 5% level of significance ($p<0.05$). Compositional data from the reference substances, grown concurrently in the same trial as test substances and the conventional control, were combined across all sites and used to calculate a 99% tolerance interval for each component to estimate the natural variability in cottonseed varieties with a history of safe consumption.

186. For Bollgard® III compared to the conventional control, the combined-site analysis of cotton seed showed statistically significant differences for 32 of the 52 mean value comparisons. Amino acids represented 18 of the 32 observed differences and the applicant suggests this is likely a reflection of changes in protein. Protein and all amino acid mean values from the combined-site analysis of Bollgard® III were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same field production (Venkatesh et al. 2013a). With the exception of tryptophan, mean values for protein and all other amino acids, were also within the limits of natural variability published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI-CCDB). Tryptophan levels in both Bollgard® III and conventional control were higher than the maximum value found in ILSI-CCDB, but were within the tolerance interval calculated from values from references grown in the same field trial.

187. For Bollgard® III x Roundup Ready Flex® compared to the conventional control, the combined-site analysis showed statistically significant differences for 36 of the 52 mean value comparisons. Amino acids represented 17 of the 36 observed differences and are likely a reflection of changes in protein (Venkatesh et al. 2013b).

188. From this analysis, mean values for all significantly different nutrient and anti-nutrient components from the combined-site analyses of both GM cottons were within the 99% tolerance interval established from the conventional commercial reference substances grown concurrently in the same field production. The mean component values also fall within the range of the natural variability of commercial cotton composition as published in the scientific literature and/or available in the ILSI-CCDB.

189. In summary, the compositional data analysis supports the compositional parity of the GM cottons proposed for release and conventional cotton. Component values that were statistically significantly different between the test substance and the conventional control represented differences that are not considered meaningful from a food or feed safety or nutritional perspective.

Effects of the GMOs on desirable invertebrates in Australia

190. A study was conducted by CSIRO between 2010 and 2012 to establish whether growing of Bollgard® III and Bollgard® III x Roundup Ready Flex® cottons would alter the structure of associated invertebrate communities in comparison to Bollgard® II alone or unsprayed non-Bt cotton (Whitehouse et al. 2014). Using beatsheet and suction sampling methods, invertebrate communities were compared in 5 experiments across 3 sites in eastern Australian cotton regions and north western Australia. The authors found significant differences between invertebrate communities of non-Bt and Bt (Bollgard® II and Bollgard® III) cotton only in experiments where lepidopteran larval abundance was high; when lepidopteran abundance was low, no differences between Bt and non-Bt cotton were found. In beatsheet samples, changes in the community reflected a higher abundance of flowers and bolls in Bt cotton due to less

feeding damage by lepidopterans. For example, there was a greater abundance of grasshoppers in Bt cottons relative to non-Bt cottons.

191. In suction sampling data, insects usually associated with plant damage and lepidopteran frass were more common in non-Bt crops (Whitehouse et al 2014). When comparing individual taxa responses, differences between Bt and non-Bt cotton invertebrate communities were largely driven by changes in the abundance of lepidopteran larvae. Taxa showing significant differences between crop types were several generalist predators and some pests including several spider families; many of these are more common in non-Bt cotton, most probably due to prey preferences. In suction samples, there were differences observed in small dipterans between Bt and non-Bt plants. It was not clear why these insects were shown to be more common on non-Bt plants although it is possible that these populations may have been influenced by prolonged vegetative growth caused by insect damage in non-Bt cotton (Whitehouse 2014). Hence, most differences between Bt and non-Bt communities reflected altered food availability for different functional groups. Overall, the data supports the authors' conclusion that there was no significant difference between Bollgard® II and Bollgard® III communities, despite the addition of the Vip3A gene in Bollgard® III.

192. The Australian studies are largely consistent with results provided by the applicant from US field studies (Galadima & Bommireddy 2013). For example, in individual qualitative site assessments of arthropod damage, no differences were observed between Bollgard® III and the conventional control for any of 91 comparisons for the assessed arthropod stressors, including aphids, boll weevils, fleahoppers, grasshoppers, plant bugs, spider mites, stink bugs, thrips, and white flies.

6.2.4 Experience from cultivation of the GM cottons

193. To evaluate basic phenotypic characteristics, the applicant has collected data from field trials of Bollgard® III and Bollgard® III x Roundup Ready Flex® conducted in the US in 2011 (Galadima & Bommireddy 2013) and in Australia in 2011 and 2012 (Conaty 2013). The GM cottons at each site were assessed in comparison to the parental GM cottons Bollgard® II and Roundup Ready Flex®, as well as non-GM cotton varieties and, in the US, four conventional reference upland varieties.

194. The agronomic and phenotypic performance and environmental interactions of Bollgard® III was compared with the non-GM cotton control. In addition, Bollgard® III x Roundup Ready Flex® was treated (T) with glyphosate and compared to the parental conventional control in terms of agronomic and fibre quality characteristics.

195. Comparisons were made for a range of agronomic and phenotypic characteristics, including: stand count at 14 and 30 days after planting (DAP); final stand count; plant vigour at 14 and 30 DAP; plant height at 30 DAP and at harvest; nodes above white flower; seed cotton yield; mainstem nodes; nodes to first fruiting branch; total boll; total first position bolls; vegetative boll count; percent retention first position bolls; percent first position bolls of total bolls; seed index; total seed per boll; mature seed per boll; immature seed per boll; boll weight; and fibre quality data (micronaire, elongation, strength, uniformity, and length).

196. In the combined-site analysis of the plant growth and development data, no statistically significant differences were detected between Bollgard® III and the conventional control for all growth and development characteristics.

197. No statistically significant differences were detected between Bollgard® III x Roundup Ready Flex® (T) cotton and the non-GM cotton control for the assessed characteristics except one. A statistically significant difference for height at 30 DAP was detected (13.9 vs. 15.4 cm). However, the mean plant height value of Bollgard® III x Roundup Ready Flex® (T) was within the reference range and the difference was relatively small in magnitude; such variant data

would be expected in any program of conventional plant breeding (Acquaah 2007; Bradford et al. 2005).

198. Within sites, measures of environmental interactions included plant response to abiotic stressors, disease damage, and arthropod damage (qualitative assessments). These observations were performed four times during the growing season at ten sites. In the assessment of plant response to abiotic stressors, disease, and arthropod damage, no differences were observed between Bollgard® III and the conventional control.

199. Based on the assessed characteristics, this study indicates a similar weed risk potential in Bollgard® III, Bollgard® III x Roundup Ready Flex® and the non-GM cotton control.

6.2.5 Phenotypic and agronomic characterisation of the GM cottons in Australia

200. Six trial sites were planted in the 2011-12 cotton season throughout the Australian cotton growing region in Eastern Australia and one trial site in winter 2012 at Kununurra in northern Western Australia (Conaty 2013).

201. The agronomic and phenotypic performance of Bollgard® III and Bollgard® III x Roundup Ready Flex® were compared with the following controls:

- Bollgard II® × Roundup Ready Flex®
- Roundup Ready Flex®
- Bollgard II® and
- Non-GM cotton.

202. A range of characteristics were measured as indicators of growth, development, agronomic performance and fibre quality to assess the potential effect of the added *vip3A* gene. These included early stand count at 21 DAP, final stand count, plant vigour at 21 DAP, plant height and nodes every 21 days until harvest, nodes above white flower, seed cotton weight, seed index, total seed per boll and fibre quality data (including lint percent, length, strength and micronaire).

203. Based on the assessed characteristics, no significant difference was observed in the phenotype or the agronomic performance of Bollgard® III x Roundup Ready Flex® over the 2011/12 growing season, when compared to its GM parental plant, Bollgard® II x Roundup Ready Flex®. Similarly, no differences were observed between Bollgard III compared to Bollgard® II. The plant height, number of nodes, nodes above white flower and yield were the same across the season, and across six sites representing different climatic conditions, and representative of the Australian cotton growing region.

204. There were consistent differences in yield between the GM cottons proposed for release and the non-Bt controls, ie Roundup Ready Flex® cotton and non-GM cotton. These differences were ascribed to the insect protection afforded to the cotton by the addition of the *cry1Ac*, *cry2Ab* and *vip3A* genes, the efficacy of which was not assessed in this study. The lack of insecticide spraying resulted in severe damage to the non-Bt plots, and is consistent with their low yield.

205. There were some local differences in early plant vigour, plant stand and yield, which were either compensated for after the point of measurement (plant vigour and stand count) resulting in no difference later in the season, or were ascribed to external factors that were not to do with plant performance. The season in which these experiments were carried out was characterised by significant differences in rainfall and environment between the sites, and some local differences were expected. Nonetheless, these differences did not result in changes in yield, or consistent changes in plant height, growth rate or number of nodes.

206. In summary, the study showed no consistent differences from comparisons between the test GM cotton plots and the control Bt cottons. These similarities indicate that the phenotype

of the GM cottons proposed for release are comparable with the insect resistant GM cottons currently commercially produced in the Australian cotton industry.

Evaluation of germination and dormancy characteristics

207. Characteristics that might impart increased survival in the environment have been considered for each of the parental GM cottons: Vip3A cotton, Bollgard® II and Roundup Ready Flex® displayed the same dormancy and germination characteristics, ie percent seed germinated, percent viable hard seed, percent viable firm swollen seed, and percent degenerated seed, as non-GM cotton (information provided by applicant).

208. Dormancy and germination characteristics are not expected to be altered in Bollgard® III or Bollgard® III x Roundup Ready Flex® when these traits are crossed by conventional breeding.

Agricultural management

209. The applicant has stated that stacking of Vip3A with the two Cry proteins is likely to provide Bollgard® III with more sustainable long-term protection against emergence of resistant insect pests than previous GM cottons. This is based on the assumption that, since the Bt toxins have different receptors (Section 5.3.4), selection for resistance to one toxin will not cause cross-resistance to the other toxins, and insects resistant to all three toxins are likely to be rare (Tabashnik et al. 2013). As noted by Brevault (2013) this assumption may not always hold true, but is more likely if other resistance management measures such as refuges are in place.

210. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. Bollgard® III cotton meets the definition of an agricultural chemical product under the Agricultural and Veterinary Chemicals Code Act 1994, due to its production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA. Resistance management is an issue considered by the APVMA in registration of herbicides and insecticidal products. The applicant has applied to the APVMA for registration of Bollgard® III as an insecticidal product and will need to comply with an approved insect resistance management plan and any other relevant conditions that may be imposed.

211. It is intended that Roundup Ready® herbicide be applied to Bollgard® III x Roundup Ready Flex® cotton and this would also be subject to regulation by the APVMA. Roundup Ready® herbicide has been registered for use on Roundup Ready cotton since 2000 and on Roundup Ready Flex® Flex cotton since 2006.

6.2.6 Previous releases of the GM cottons proposed for release

212. To date, there have been no international approvals for the commercial release of Bollgard® III and Bollgard® III x Roundup Ready Flex® cottons. However, regulatory regimes in some jurisdictions do not require separate authorisation for environmental release of GMOs produced by conventional crossing between other already authorised GMOs, such as Bollgard® III and Bollgard® III x Roundup Ready Flex® cottons. This is also the case for FSANZ, which does not need to assess these GM cottons for food use as products of the GM parental cottons are authorised for food use in Australia.

Section 7 Other relevant considerations for the Australian environment

7.1 Other relevant plants

213. The Regulator or his predecessor has issued licences for the commercial release of other herbicide tolerant and/or insect resistant cottons (Table 11). These also form part of the risk context for this DIR licence application.

Table 12. Other relevant GM cottons in Australia

GM cotton	DIR licence number and approval type	Comment
Other herbicide tolerant GM cottons (containing the *bar* gene for glufosinate ammonium tolerance)	DIR 062/2005 is for commercial release. It superseded DIR 038/2003 which was for limited and controlled release.	Liberty Link® cotton
Other insect resistant cottons	DIR 091 is for commercial release. It superseded DIR 044/2003 and DIR 040/2003 which were for limited and controlled release.	WideStrike™ cotton

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

Chapter 2 Risk assessment

Section 1 Introduction

215. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 2). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

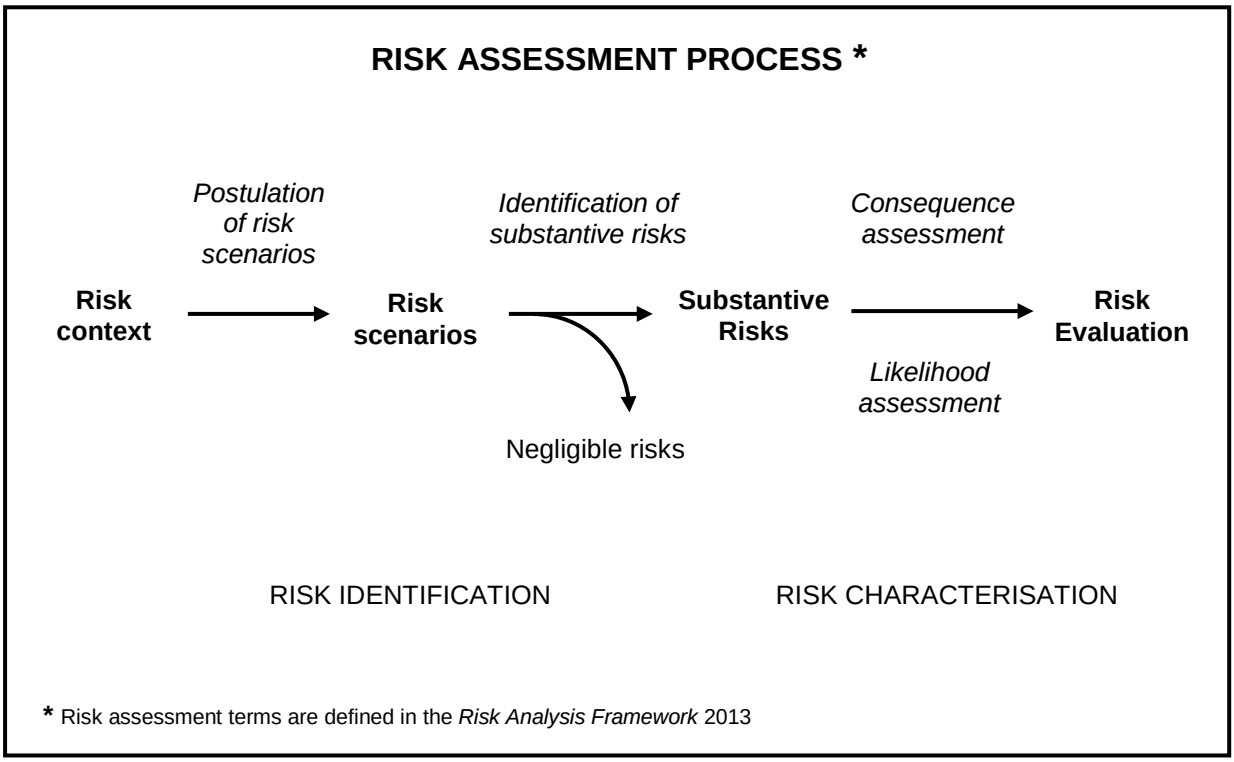

Figure 2. The risk assessment process

216. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios) in the short and long term.

217. Postulated risk scenarios are screened to identify substantive risks that warrant detailed characterisation. A substantive risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

218. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013a). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are considered in postulating risk scenarios (Keese et al. 2013). In addition, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

219. Substantive risks (*i.e.* those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments. The level of risk, together with analysis of interactions between potential risks, is used to evaluate these risks to determine if risk treatment measures are required.

Section 2 Risk Identification

220. Postulated risk scenarios are comprised of three components (Figure 3):

i. The source of potential harm (risk source).

ii. A plausible causal linkage to potential harm (causal pathway).

iii. Potential harm to an object of value (people or the environment).

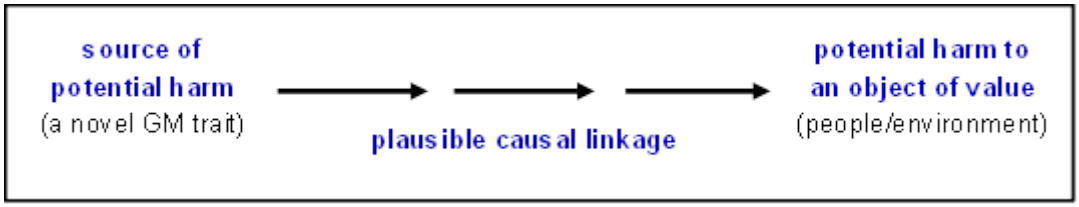

Figure 3. Risk scenario

221. In addition, the following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- any proposed limits including the extent and scale of the proposed dealings
- any proposed controls to restrict the spread and persistence of the GMO
- characteristics of the parent organism(s).

2.1 Risk source

222. The source of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

223. As discussed in Chapter 1, the GM cottons have been modified by the introduction of three insect resistance genes. Bollgard® III x Roundup Ready® Flex cotton also has a glyphosate herbicide tolerance gene. These introduced genes are considered further as potential sources of risk.

224. In addition, the GM cottons contain three selectable marker genes that confer antibiotic resistance (*npt II*, *aph4* and *aad*) and a reporter gene (*uidA*) (see Chapter 1). However, these genes and their products have already been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator as well as other regulatory agencies in Australia and overseas. As these genes have not been found to pose substantive risks to either people or the environment, their potential effects will not be further assessed for this application.

225. All of the introduced genes include regulatory sequences derived from other organisms, including pathogens. As described in Chapter 1, these sequences have been widely used in other GMOs, including the parental GM lines that are grown commercially, without reports of adverse effects. Hence, risks from these regulatory sequences will not be further assessed for this application.

226. The genetic modifications also have the potential to cause unintended effects in several ways including altered expression of endogenous cotton genes by random insertion of introduced DNA in the genome, increased metabolic burden due to expression of the introduced proteins, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. Unintended effects might result in adverse outcomes such as toxicity or allergenicity.

227. However, the range of possible unintended effects produced by genetic modification is not likely to be greater than that from accepted traditional breeding techniques (Bradford et al. 2005; Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health 2004; The GM Science Review Panel 2003). Conventional methods of plant breeding may also induce unanticipated changes in plants (Haslberger 2003), but new varieties produced by such techniques have rarely had traits that are undesirable for human health, safety or the environment (Hajjar & Hodgkin 2007)[2]. Therefore, unintended effects resulting from the process of genetic modification will not be considered further.

228. Some details of the molecular characterisation of COT102 have been declared confidential commercial information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on this application and RARMP..

2.2 Causal pathway

229. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) on the properties of the organism
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- agronomic management practices for the GMOs
- spread and persistence (invasiveness) of the GM plant, including
    - establishment
    - reproduction
    - dispersal by natural means and by people
- tolerance to abiotic conditions (eg climate, soil and rainfall patterns)
- tolerance to biotic stressors (eg pest, pathogens and weeds)
- tolerance to cultivation management practices

[2] More detail on potential for unintended effects as a result of the process of genetic modification can be found in the document *Methods of plant genetic modification* available from the Risk Assessment References page on the OGTR website.

- gene transfer to sexually compatible organism
- gene transfer by horizontal gene transfer (HGT)
- unauthorised activities.

230. Although all of these factors are taken into account, some have been considered in previous RARMPs or are not expected to give rise to substantive risks.

2.2.1 Tolerance to abiotic factors

231. The geographic range of non-GM cotton in Australia is limited by a number of abiotic factors; including climate and soil compatibility, as well as water and nutrient availability (see *The Biology of* Gossypium hirsutum L. *and* Gossypium barbadense L. *(cotton)* (OGTR 2013b). The introduced genes are unlikely to make the GM cotton plants more tolerant to abiotic stresses that are naturally encountered in the environment, and are therefore unlikely to alter the potential distribution of the GM cotton plants.

2.2.2 Weed management measures

232. Extensive practices (including use of herbicides) are used in agriculture to control cotton volunteer plants (see Chapter 1, section 4.2.3). As discussed there, glyphosate is not generally used to control adult cotton plants.

233. Some feral cotton does occur outside of cultivation in northern Australia, including in nature reserves. However, these plants are not routinely subjected to control measures such as the use of herbicide. Information provided by the Department of Environment indicates that the Kakadu National Parks Service has undertaken intermittent attempts to remove some feral populations by herbicide spraying and mechanical removal; this reportedly occurred up until 2007 in some areas, but more recent information is not available. The presence of herbicide tolerance genes in these feral cottons would not be expected to provide a selective advantage in the absence of herbicide application. The weediness potential of the parent GM cotton Roundup Ready Flex® was considered in the RARMPs for DIRs 059/2005 and 066/2006 and no risk greater than negligible was identified; there have been no adverse reports since commercial release in 2005.

2.2.3 Gene transfer to sexually compatible relatives

234. Vertical gene flow is the transfer of genetic information from an individual organism to its progeny by conventional heredity mechanisms, both asexual and sexual. In flowering plants, pollen dispersal is the main mode of gene flow (Waines & Hegde 2003). For GM crops, vertical gene flow could therefore occur via successful cross-pollination between the crop and neighbouring crops or plants of the same species, related weeds or related native plants (Glover 2002).

235. Baseline information on vertical gene transfer associated with non-GM cotton plants can be found in *The Biology of* Gossypium hirsutum L. *and* Gossypium barbadense L. *(cotton)* (OGTR 2013b). In summary, cotton is predominantly self-pollinating with no self-incompatibility mechanisms present. The average inter-plant outcrossing rates in Australia are less than 2 % between adjacent cotton rows and not significant beyond 20m. As pollen is large, sticky and heavy it is not easily dispersed by wind, any cross-pollination is likely to be facilitated by insects, including honeybees. Expression of the introduced genes is not expected to change the pollination characteristics of the GM cottons compared to non-GM cotton.

236. As discussed in Chapter 1, Section 5.6, *G. hirsutum* is sexually compatible with all GM and non-GM *G. hirsutum* varieties, as well as *G. barbadense*. Therefore some cross-hybridisation with these plants is inevitable. However, gene transfer to Australian native

cotton species is not expected due to genetic incompatibility. Therefore, only gene transfer to *G. hirsutum* and *G. barbadense* will be considered further.

2.2.4 Gene transfer by HGT

237. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in the literature (Keese 2008) as well as assessed in many previous RARMPs. HGT was most recently considered in detail in the RARMP for DIR 108. This and other RARMPs are available from the GMO Record on the OGTR website **Error! Hyperlink reference not valid.**or by contacting the OGTR. No risk greater than negligible was identified due to the rarity of these events and because the gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, HGT will not be assessed further.

2.2.5 Unauthorised activities

238. The potential for unauthorised activities to lead to an adverse outcome has been considered in previous RARMPs. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires the Regulator to have regard to the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities, and no risk greater than negligible was identified in previous RARMPs. Therefore unauthorised activities will not be considered further.

2.3 Potential harm

239. Potential harms from GM plants include:

- reduced biodiversity through harm to other organisms or ecosystems
- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced establishment of desirable plants, including having an advantage in comparison to related plants
- reduced yield of desirable vegetation
- reduced products or services from the land use
- restricted movement of people, animals, vehicles, machinery and/or water
- reduced quality of the biotic environment (eg providing food or shelter for pests or pathogens) or abiotic environment (eg negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table).

240. These harms are based on those used to assess risk from weeds (Standards Australia 2006). Judgements of what is considered harm depend on the management objectives of the land where the GM plant is expected to spread to and persist. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.3.1 Production of a substance toxic or allergenic to people or toxic to other organisms

241. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000).

242. Allergenicity is the potential of a substance to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

243. Expression of the introduced genes involved in insect resistance or herbicide tolerance could result in production of novel toxic or allergenic compounds, or alter the production of endogenous compounds of cotton that are toxic or allergenic.

The CP4 EPSPS protein and associated metabolites

244. The introduced herbicide tolerance gene encodes the CP4 EPSPS protein, which has been rigorously assessed for toxicity and allergenicity in humans and for toxicity in a range of other organisms. As discussed in Chapter 1 Section 5.2.4, this extensive body of experimental work has produced no evidence that the EPSPS protein is toxic or allergenic. In Australia, the applicant has received approval from FSANZ for the use of oil and linters derived from Roundup Ready Flex® *G. hirsutum* (FSANZ 2005). FSANZ has also approved material derived from other GM plants expressing the CP4 EPSPS protein (lucerne and soybean) for consumption (FSANZ 2006; FSANZ 2007). The assessments by FSANZ note that there is no evidence of toxic and allergenic properties associated with these proteins.

245. In addition, no new herbicide metabolic products have been identified in GM plants expressing CP4 EPSPS (Chapter 1, Section 5.2.4). Therefore, on the basis of the substantial knowledge base relating to the CP4 EPSPS protein, the toxicity and allergenicity of the EPSPS protein will not be considered further.

Endogenous cotton toxins

246. Cotton (*G. hirsutum* and *G. barbadense*) tissue, particularly the seeds, can be toxic if ingested in excessive quantities because of the presence of endogenous anti-nutritional and toxic factors including gossypol and cyclopropenoid fatty acids (including dihydrosterculic, sterculic and malvalic acids).

247. The presence of gossypol and cyclopropenoid fatty acids in cotton seed limits its use as a protein supplement in animal feed. Ruminants are less affected by these components because they are detoxified by digestion in the rumen (Kandylis et al. 1998). However, its use as stockfeed is limited to a relatively small proportion of the diet and it must be introduced gradually to avoid potential toxic effects (Blasi & Drouillard 2002).

248. The presence of the introduced genes is not expected to directly affect the levels of endogenous toxins. This is supported by data provided by the applicant (Chapter 1, section 6.2.3) showing that gossypol levels in seed from the GM cottons lie within the recorded range for the parental cottons. Furthermore, there are established management practices to control the preparation and use of cottonseed products as feed for livestock, including poultry. Therefore, endogenous cotton toxins will not be considered further.

2.4 Postulated risk scenarios

249. Ten risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 13 and more detail of these scenarios is provided later in this Section. Postulation of risk scenarios considers impacts of the GM cotton or its products on people undertaking the dealings, as well as impacts on people and the environment exposed to the GM cotton or its products as the result of the commercial use or the spread and persistence of plant material, including pollen.

250. In the context of the activities proposed by the applicant and considering both the short and long term, none of the ten risk scenarios gave rise to any substantive risks that could be greater than negligible.

Table 13. Summary of risk scenarios from dealings with the GM cottons

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced insect resistance genes	Expression of insect resistance genes in GM plants ⬇ Exposure of people undertaking the dealings to the GM plants or products thereof, or exposure of the public by consumption of GM cotton products, contact with GM cotton products, or inhalation of GM cotton pollen	Increased toxicity or allergenicity for people	No	• There is limited exposure of humans to the expressed proteins. • The Cry 1Ac, Cry2Ab and Vip3A proteins have no demonstrated toxicity or allergenicity to humans.
2	Introduced insect resistance genes	Expression of insect resistance genes in GM plants ⬇ Feeding of livestock with GM cotton plant material or meal	Increased toxicity for livestock	No	• Consumption of cotton by livestock is limited, largely due to the presence of natural toxins (eg gossypol). • Low toxicity of Cry 1Ac, Cry2Ab and Vip3A proteins to organisms other than certain insects.
3	Introduced insect resistance genes	Expression of insect resistance genes in cultivated or volunteer GM plants ⬇ Exposure of non-target insects to GM plant material through contact or ingestion	Increased toxicity for non-target insects	No	• There is no demonstrated ill-health of non-target insects resulting from Vip3A, as compared with the parental GM cottons. • There is no demonstrated increase in ill-health of desirable insects in comparison to effects of standard insect control measures applied to conventional cotton varieties.
4	Introduced insect resistance genes	Expression of insect resistance genes in cultivated or volunteer GM plants ⬇ Exposure of desirable organisms other than humans, livestock or non-target insects to GM plant material through contact or ingestion	Increased toxicity for desirable organisms other than humans, livestock or non-target insects	No	• Low toxicity of Cry 1Ac, Cry2Ab and Vip3A proteins to organisms other than certain insects.
5	Introduced insect resistance genes	Dispersal of GM cottonseed to nature reserves ⬇ Establishment of GM plants in nature reserves ⬇ Expression of insect resistance genes in GM plants ⬇ Reduced insect herbivory of GM plants, leading to increased spread and persistence	Reduced establishment of desirable native vegetation	No	• Cotton has limited ability to establish outside of cultivation. • Abiotic factors, rather than lepidopteran herbivory, are the major factors restricting the establishment of cotton populations outside of cultivation areas. • Cotton has limited ability to reduce establishment of desirable vegetation.
6	Introduced herbicide tolerance gene	Establishment of volunteer GM cotton plants in agricultural areas ⬇ Expression of the herbicide tolerance gene in GM plants ⬇ Reduced effectiveness of weed management measures to control the volunteer GM cotton plants	Reduced establishment or yield of desirable agricultural crops	No	• Standard agronomic practice for cotton cultivation includes integrated weed management practices that will effectively reduce volunteer populations.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
7	Introduced insect resistance genes	Expression of insect resistance genes in the GM plants ⬇ Reduced populations of target pest insects ⬇ Reduced use of chemical pesticides ⬇ Increased populations of other agricultural pests	Reduced establishment or yield of desirable agricultural crops	No	• Standard agronomic practice for cotton cultivation includes practices for effective management of secondary pests.
8	Introduced insect resistance genes	Transfer of insect resistance genes to other cultivated insect resistant GM cottons by pollen flow ⬇ Expression of insect resistance genes in the stacked GM cottons ⬇ Exposure of people or animals by contact or ingestion, or inhalation of cotton pollen	Increased toxicity or allergenicity for people or desirable organisms	No	• Transfer of the introduced genes to other cultivated cottons by pollen flow is likely to be limited. • The presence of the hybrids is expected to be transient. • There is limited exposure of humans to the expressed proteins. • The Cry 1Ac, Cry2Ab and Vip3A proteins have no demonstrated toxicity to humans or other desirable organisms or allergenicity to humans. • The Bt genes for insect resistance present in currently approved GM cottons are targeted to a limited range of lepidopteran pests. Stacking of these genes is not expected to increase toxicity for non-target invertebrates.
9	Introduced insect resistance genes	Transfer of insect resistance genes to feral cotton plants in nature reserves by pollen flow ⬇ Reduced insect herbivory of GM feral cotton, leading to increased establishment and reproduction of GM feral cotton in nature reserves	Reduced establishment of desirable native vegetation	No	• There are spatial limitations on the potential for movement of the insect resistance genes into feral cotton plants by pollen flow. • Abiotic factors restrict the establishment of cotton populations outside of cultivation areas. • There is limited potential to reduce establishment of desirable vegetation.
10	Introduced herbicide tolerance gene	Transfer of herbicide tolerance gene to other herbicide tolerant GM cotton plants by pollen flow ⬇ Establishment of volunteer GM cotton plants in agricultural areas ⬇ Reduced effectiveness of weed management measures to control volunteers	Reduced establishment of desirable agricultural crops	No	• Transfer of the introduced genes to other herbicide tolerant GM cottons by pollen flow is expected to be limited. • The presence of resulting hybrid volunteers is expected to be transient. • Tolerance to both glyphosate and glufosinate ammonium is not likely to impact on the control of cotton volunteers as these herbicides are of limited usefulness in controlling cotton volunteers. Other methods are available. • Standard measures for controlling cotton volunteers will limit volunteer numbers, further limiting their potential to reduce establishment of desirable crops.

2.4.1 Risk scenario 1

Risk source	*Causal pathway*	*Potential harm*
Introduced insect resistance genes	Expression of insect resistance genes in GM plants ⬇ Exposure of people undertaking the dealings to the GM plants or products thereof, or exposure of the public by consumption of GM cotton products, contact with GM cotton products, or inhalation of GM cotton pollen	Increased toxicity or allergenicity for people

Risk source

251. The source of potential harm for this postulated risk scenario is the introduced insect resistance genes.

Causal pathway

252. The insect resistance genes *vip3A*, *cry1Ac* and *cry2Ab* are expressed in the vegetative parts, pollen and seed of the GM cotton plants. Therefore, people may be exposed to the GM cotton or its products through contact, consumption or inhalation of pollen. However, the introduced genes and expressed proteins are not present in cotton products such as cottonseed oil, fibres and linters. Therefore, the main people that will be exposed to the introduced genes and their products will be workers involved in breeding, cultivating, harvesting, transporting and processing the GM cotton. The public, who consume cottonseed oil and cottonseed linters, or have contact with cotton fabrics, are not exposed to the introduced genes and their products.

Potential harm

253. People exposed to the proteins expressed from the introduced genes may show increased toxic reactions or increased allergenicity. From consideration of the causal pathway, these are primarily people undertaking the dealings.

254. The World Health Organisation's International Programme on Chemical Safety evaluated the environmental safety of use of Bt as a pest control agent and concluded that, because of the specificity of the mode of action of Bt toxins, Bt products are unlikely to pose any hazard to humans, other vertebrates, or the great majority of non-target invertebrates (International Programme on Chemical Safety 1999). In this report it was noted that Bt has not been reported to cause adverse effects on human health when present in drinking water or food. Two human studies found no observable health effect of an oral dose of 1000 mg of Bt spores per day for 3 or 5 days (reviewed by Betz et al. 2000; McClintock et al. 1995).

255. Inhalation and ingestion of Bt is not known to cause allergic reactions (International Programme on Chemical Safety 1999). There have been rare reports of occupational allergies associated with the use of Bt insecticidal products.

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

257. The US EPA determined that the dermal allergic reactions reported by Bernstein et al. (1999) were due to non-Cry proteins produced during fermentation or to added formulation ingredients, not to Bt itself or any of the Cry toxins (EPA 2001).

258. Searches of the FARRP Allergen Database, performed according to CODEX guidelines (Codex Alimentarius Commission 2003a) have shown no matches of the Vip3A protein to known allergens (information supplied by applicant). Similarly, the US EPA uses a weight-of-evidence approach for allergenicity consistent with the CODEX guidelines and has concluded that the potential for Vip3Aa to be a food allergen is minimal (USEPA 2008).

259. Food Standards Australia New Zealand (FSANZ) assessed the safety of human food derived from linters and cotton seed oil from VIP3A cotton, and concluded that studies to determine potential toxicity of Vip3A demonstrate that it is non-toxic to mammals (FSANZ 2004). Also, the Vip3A protein has been demonstrated to be heat labile (Estruch et al. 1996), which would lead to a decreased exposure in processed products. The studies assessed by FSANZ are summarised in Hill et al. (2003).

260. More recently, Syngenta provided four acute oral toxicity studies conducted on mice as part of the biopesticide registration document for COT 102 x COT67B (containing Vip3Aa and Cry1Ab, respectively) in the US (US EPA 2008). No treatment-related adverse effects were observed in any of the studies, providing further indication that Vip3Aa is non-toxic to mammals, including humans.

261. Therefore, the insect resistance gene products are not considered significantly toxic or allergenic to workers involved in breeding, cultivating, harvesting, transporting and processing the GM cotton.

Conclusion

262. Risk scenario 1 is not identified as a substantive risk, due to limited exposure of humans to the expressed proteins, and the lack of toxicity or allergenicity of the Cry 1Ac, Cry2Ab and Vip3A proteins to humans. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	*Causal pathway*	*Potential harm*
Introduced insect resistance genes	Expression of insect resistance genes in GM plants ⬇ Feeding of livestock with GM cotton plant material or meal	Increased toxicity for livestock

Risk source

263. The source of potential harm for this postulated risk scenario is the introduced insect resistance genes.

Causal pathway

264. The insect resistance genes *vip3A, cry1Ac* and *cry2Ab* are expressed in all parts of the GM cotton plants, including cottonseed and leaves. Therefore, livestock that are fed cottonseed meal and leaves will be exposed to the introduced gene products. However, the amount of cotton plant material (both GM and non-GM) that is consumed by livestock is, by necessity, limited due to presence of endogenous toxins such as gossypol.

Potential harm

265. As discussed in risk scenario 1 (and Chapter 1, Sections 5.1.4 and 5.3.4), the introduced gene products are not expected to be toxic to livestock.

Conclusion

266. Risk scenario 2 is not identified as a substantive risk due to limited consumption of cotton by livestock and low toxicity of the Cry 1Ac, Cry2Ab and Vip3A proteins to

organisms other than certain insects. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk source	*Causal pathway*	*Potential harm*
Introduced insect resistance genes	Expression of insect resistance genes in cultivated or volunteer GM plants ⬇ Exposure of non-target insects to GM plant material through contact or ingestion	Increased toxicity for non-target insects

Risk source

267. The source of potential harm for this postulated risk scenario is the introduced insect resistance genes.

Causal pathway

268. Expression of the insect resistance genes in pollen, seed and vegetative material of cultivated or volunteer GM plants will directly expose non-target insects through contact and ingestion, or indirectly via feeding on herbivores that feed on the GM material. Non-target insects may include: non-pest insect species that consume the GM crop, butterflies and desirable insects such as natural insect predators of the pest organisms, parasitoids, or pollinators such as bees.

Potential harm

269. Exposure of non-target insects to the Cry1Ac, Cry2Ab or Vip3A proteins expressed by the introduced insect resistance genes may result in adverse effects such as death, slowed growth rate or reduced fecundity if these proteins are toxic to exposed organisms.

270. Bollgard® III contains three insect resistance genes, each of which has a relatively narrow specificity for a limited range of insect species, including target insect pests. As discussed in Chapter 1, Sections 5.1.4 and 5.3.4, Cry 1Ac, Cry 2Ab and Vip3A proteins have been assessed for potential toxicity to non-target invertebrates through tiered testing of a range of representative arthropods (including bees, springtails, greenbugs, aphids); from such testing it was concluded that plants containing these proteins have only a narrow range of target specificity within Lepidopteran species and would not harm non-lepidopteran arthropods.

271. Addition of the *vip3A* gene to the *cry* genes already present in the Bollgard II® or Bollgard II®/Roundup Ready Flex® cotton could result in an interaction between the Cry and Vip3A proteins. If an interaction occurs between these proteins the combined effect could either be greater than (synergistic effect), equal to (additive effect), or less than (antagonistic effect) the sum of the effects of the individual proteins.

272. Synergistic or additive effects could be expected to occur between toxins isolated from the same or different strains of bacteria, particularly where different receptor molecules are involved (Schnepf et al. 1998). Evidence suggests that the Vip3A and Cry proteins bind to different receptors in the insect midgut epithelium (see Chapter 1, Section 5.3.4), so it is possible that an additive or synergistic effect between the three insecticidal proteins in combination in the GM cottons may occur. This could increase the toxic effect on insects sensitive to either the Vip3A or Cry1Ac and Cry2Ab proteins alone.

273. A search of the literature reveals limited evidence relating to synergistic effects between Cry and Vip proteins. Nonetheless, the specificities of the Vip3A, Cry1Ac and Cry2Ab proteins appear to be restricted to overlapping subsets of lepidopteran insects. Therefore, any

increase in the range of sensitive insects as a result of the expression of three insecticidal proteins is expected to be confined to lepidopteran species.

274. It is noteworthy that the same or similar proteins are present in the microbial formulations in commercial Bt insecticide preparations (Hill et al. 2003) and it is not expected that the range of sensitive insects would increase beyond those sensitive to the Bt insecticides.

275. The primary effect is toxicity to lepidopterans that feed on cotton. However, most of these organisms, including cotton bollworm (*H. armigera*), native budworm (*H. punctigera*), cluster caterpillar (*Spodoptera litura*) and pink bollworm (*Pectinophora gossypiella*) (Cotton Catchment communities CRC 2006; Strickland et al. 2003; Strickland et al. 2000) are considered pests of cotton that warrant control by farmers. These control measures include spraying with broad spectrum pesticides.

276. The potential impact of a range of Bt crops on non-target insects has been studied in both laboratory and field studies and, as summarised by Kaur (2012), the impact ranges from no detrimental effect, to minimal adverse effects (eg on beneficial predator insects), to increase in abundance of beneficial insects (also see Section 5.1.4).

277. A meta-analysis of data collected from 42 field studies indicated that non-target invertebrates are generally more abundant in Bt cotton and Bt maize fields than in non-transgenic fields managed with insecticides (Marvier et al, 2007). In addition, a comprehensive review of short and long-term field studies on the effects of invertebrate populations in Bt corn and cotton fields indicated that no significant adverse effects are taking place as a result of wide scale Bt crop cultivation (Sanvido, et al. 2007). Another review of published field tests concluded that the large-scale studies in commercial Bt cotton have not revealed any unexpected non-target effects other than subtle shifts in the arthropod community caused by the effective control of the target pests (Romeis et al., 2006). Slight reductions in some invertebrate predator populations will result from all pest management practices which result in reductions in the abundance of the pests as prey.

278. Field studies in Australia comparing Bollgard® II, Bollgard® III and sprayed conventional cotton drew similar conclusions (Chapter 1, Section 6.2.3 and Whitehouse et al. 2007; Whitehouse et al. 2014). In studies of arthropod abundance at field sites in south-eastern and northern Australia, most differences between Bt and non-Bt communities reflected altered food availability for different functional groups and there was no overall significant difference between Bollgard® II and Bollgard® III arthropod communities, despite the addition of the *vip3A* gene in Bollgard® III.

Conclusion

279. Risk scenario 3 is not identified as a substantive risk as there is no increase in adverse effects on non-target insects compared with the parental GM cottons or standard control measures applied to non-GM cottons. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

Risk source	*Causal pathway*	*Potential harm*
Introduced insect resistance genes	Expression of insect resistance genes in cultivated or volunteer GM plants ⬇ Exposure of desirable organisms other than humans, livestock or non-target insects to GM plant material through contact or ingestion	Increased toxicity for desirable organisms other than humans, livestock or non-target insects

Risk source

280. The source of potential harm for this postulated risk scenario is the introduced insect resistance genes.

Causal pathway

281. In addition to humans (Risk scenario 1), livestock (Risk scenario 2) and insects (Risk scenario 3), expression of the insect resistance genes in cultivated GM plants, or in volunteer GM cottons, may expose other non-target organisms to the GM plant material through contact or ingestion. The introduced insect resistance genes are expressed primarily in the vegetative parts of the GM cotton, with some expression in the pollen and seed. The insecticidal proteins may also occur at low levels in the soil due to exudation from roots or decomposition of plant material left after harvesting.

282. The exposure of insects to GM plant material is addressed in risk scenario 3. Other organisms, including birds, mammals, soil microbes and non-insect invertebrates are also expected to be exposed to cotton material in agricultural areas under cotton cultivation. These organisms may be exposed to the introduced insecticidal proteins through contact, ingestion or indirectly by feeding on herbivores that have ingested the GM cotton.

283. Cotton volunteers are commonly found along roadsides neighbouring cultivation sites and some transport routes so may provide a pathway for exposure. However, there appears to be limited ability for cotton to establish persistent populations at these locations, so extended exposure to the GM cotton will occur mostly in the agricultural context.

Potential harm

284. There is potential for adverse impacts on the health of other exposed organisms if the Cry1Ac, Cry2Ab or Vip3A proteins are toxic to these organisms.

285. However, as discussed in risk scenarios 1-3 and Chapter 1, Sections 5.1.4 and 5.3.4, the introduced insecticidal gene products are not expected to be toxic to organisms other than certain insects. In addition, review of the current literature has found no evidence to suggest that the Cry1Ac or Cry2Ab proteins or similar proteins are toxic to microorganisms including various species of protozoa, bacteria, fungi, algae and diatoms (Chapter 1, Sections 5.1.4). All of insecticidal genes are derived from the common soil bacterium *B. thuringiensis*, so other soil organisms would commonly be exposed to the expressed proteins.

Conclusion

286. Risk scenario 4 is not identified as a substantive risk due to low toxicity of the Cry1Ac, Cry2Ab or Vip3A proteins to organisms other than certain insects. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.5 Risk Scenario 5

Risk source	*Causal pathway*	*Potential harm*
Introduced insect resistance genes	Dispersal of GM cottonseed to nature reserves ⬇ Establishment of GM plants in nature reserves ⬇ Expression of insect resistance genes in GM plants ⬇ Reduced insect herbivory of GM plants, leading to increased spread and persistence	Reduced establishment of desirable native vegetation

Risk source

287. The source of potential harm for this postulated risk scenario is the introduced insect resistance genes.

Causal pathway

288. If GM cotton seed were dispersed into nature reserves and GM plants became established, expression of the introduced genes for insect resistance could lead to reduced herbivory from certain lepidopteran insects. In areas where lepidopteran herbivory is a significant limitation on the spread and persistence of cotton plants, the GM cotton lines expressing three insect resistance genes could have improved survival and persistence in the environment.

289. The potential for GM insect resistant cotton to disperse and become established outside agricultural cropping areas has been discussed at length in the RARMP for DIR 066/2006 (Bollgard® II and Bollgard® II/Roundup Ready Flex®). In summary, GM cotton is expected to occur as volunteers in agricultural areas and along roadsides and other transport routes. There is also potential for a limited amount of seed to spread to nearby nature reserves by natural means, primarily by water and possibly wind (OGTR 2103). Although cotton has limited ability to establish amongst existing vegetation, there is the possibility of establishment after disturbances such as flooding.

290. Expression of the introduced insect resistance genes could reduce herbivory by certain lepidopteran species. This could in turn enhance the possibility of survival and establishment of these cottons, leading to increased spread and persistence of the GM cottons in nature reserves. However, modern commercial cotton cultivars such as those proposed for release lack invasiveness characteristics that would enable them to readily establish outside the agricultural environment. This is consistent with only limited evidence of persistence of naturalised cotton populations outside of cultivation in southern Australia.

291. In contrast, there are a number of isolated small populations of cotton growing in the northern half of the Northern Territory, indicating that naturalisation may be possible in northern Australia. However, these appear to be derived from pre-modern cotton cultivars (Chapter 1, Section 4.2.5). In addition, naturalised cotton populations in the NT grow in sites close to watercourses, indicating that their spread is restricted by water availability. Furthermore, these small populations suggest limited ability to establish dense populations, which is consistent with the lack of invasiveness potential of cotton and related species (Randall 2012).

292. Although lepidopteran pests (mainly *H. armigera* and *H. punctigera*) are the main insect pests in cultivated cotton, they do not seem to be a major limiting factor in naturalised cotton populations. The RARMP for DIR 066/2006 (Bollgard® II cotton) canvassed the potential for GM insect resistant cotton to become weedy, particularly in northern Australia, and concluded that insect pressure is not the critical factor limiting establishment and growth of cotton populations, and expression of the *cry* genes does not confer increased fitness. Rather, a range of other biotic and abiotic factors seem to be far more important in limiting the spread and persistence of cotton than lepidopteran herbivory.

293. In particular, monitoring of seven naturalised cotton populations in the Northern Territory revealed abundant seed production, suggesting that these cotton plants were not significantly affected by lepidopteran pests (Eastick 2002). The major insect herbivores observed, particularly over the wet season, were grasshoppers. Grasshoppers are considered to be the most important insect herbivores in tropical savanna ecosystems (Andersen & Lonsdale 1990) and are unaffected by the Cry or Vip proteins present in Bollgard® III cotton.

294. As discussed in Chapter 1, Section 6.2.3, the introduction of the *vip3A* gene in Bollgard® III is not expected to significantly change the range of target insect species as compared with Bollgard® II. In addition, the agronomic characteristics of the Bollgard® III cotton plants are within the range of current commercial non-GM cotton and GM varieties (Chapter 1, section 6.2.5). Therefore, expression of the *vip3A* gene in Bollgard® III is similarly unlikely to confer increased selective advantage on the plants in the event of seed dispersal into nature reserves.

295. Rather than insect pressure, naturalisation of cotton in Australia is limited by abiotic factors such as water and nutrient availability, temperature and soil type (Chapter 1, Section 4.2.2). Evaluation of a number of phenotypic and agronomic characteristics (Chapter 1, Section 6.2.1) for the parent GM cottons and the GMOs indicates that the GM cottons proposed for release are comparable with the GM cottons currently commercially produced in the Australian cotton industry, so the abiotic factors limiting non-GM cotton will also limit the ability of the GM cottons to spread and persist.

296. The importance of these factors may vary between northern or southern Australia: cold stress is the most significant factor affecting persistence of cotton plants in southern Australia and dry stress is most significant in northern Australia. The germination and survival of any GM cotton seedlings is therefore likely to remain limited by abiotic factors rather than lepidopteran herbivory (OGTR 2013b).

297. Therefore, any expression of the insect resistance genes in the GM cottons is unlikely to increase its invasiveness potential, assessed as low for cotton according to the National Post-Border Weed Risk Management Protocol (Keese et al. 2013).

Potential harm

298. Increased spread and persistence of the insect resistant GM cottons in nature reserves may give rise to an increase in adverse effects on desirable native vegetation, including reduced establishment of desirable native plants, thereby reducing native plant numbers and organisms reliant on those native plants. This may in turn reduce species richness, or cause undesirable changes in species biodiversity.

299. However, cotton has limited ability to reduce the establishment of other plants (OGTR 2013) due to the lack of properties such as rambling growth or production of allelopathic compounds. The introduced genes do not lead to phenotypic changes that indicate an increased potential to reduce establishment of desirable vegetation, except by displacement through greater numbers.

Conclusion

300. Risk scenario 5 is not identified as a substantive risk due to: the limited ability of cotton to establish outside of cultivation; the influence of abiotic factors rather than lepidopteran herbivory in restricting the establishment of cotton populations outside of cultivation areas; and the limited potential of cotton to reduce establishment of desirable vegetation. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.6 Risk Scenario 6

Risk source	*Causal pathway*	*Potential harm*
Introduced herbicide tolerance genes	Establishment of volunteer GM cotton plants in agricultural areas ⬇ Expression of the herbicide tolerance gene in GM plants ⬇ Reduced effectiveness of weed management measures to control the volunteer GM cotton plants	Reduced establishment or yield of desirable agricultural crops

Risk source

301. The source of potential harm for this postulated risk scenario is the introduced herbicide tolerance genes.

Causal pathway

302. If volunteer GM cotton plants were to establish in agricultural areas, expression of the herbicide tolerance gene could reduce effectiveness of weed management measures for control of volunteer GM cotton.

303. Volunteer plants are likely to occur in the field following a cotton crop, but will also occur wherever bales or modules are placed, along roads travelled by module trucks and in channels and drains where trash accumulates (Chapter 1, Section 4.2). In southern Australia, most volunteer seedlings that emerge over winter are likely to be killed by frosts. However, seedlings that emerge later can establish and grow at all these locations.

304. As discussed in previous RARMPs (DIR 059/2005 and 066/2006), glyphosate resistant cotton volunteers would have a fitness advantage in environments where glyphosate is used to control weeds, eg along roadsides. If glyphosate herbicide were the primary means of weed control, expression of the herbicide tolerance gene in volunteer cotton plants could reduce the effectiveness of weed management measures to control those volunteers and enhance the possibility of survival and establishment of these cottons.

305. However, as noted in Chapter 1, Section 4.2.3, glyphosate is not generally used to control established cotton as it usually fails to kill even non-GM cotton plants. There are a number of herbicides registered for controlling cotton seedlings that are effective in controlling four to six node seedlings, but there are no herbicides registered for cotton plants beyond nine nodes of growth. After growing glyphosate-tolerant GM cotton, GM volunteer cotton seedlings cannot be controlled with glyphosate-based herbicides in the subsequent crop. Mechanical removal is the preferred option for older plants.

306. Cotton volunteers in intensive use areas such as roadsides are not known to give rise to self-perpetuating feral populations. Such areas may be subject to weed management (eg appropriate herbicide treatment or slashing/mowing) and/or grazed by livestock, thereby limiting the reproduction or survival of volunteers.

Potential harm

307. If left uncontrolled, volunteer cotton plants could establish and compete with other crops (Spotlight on Cotton, Winter 2013) or become host for pests and diseases, reducing establishment/yield of crops. However, weed management is a farm stewardship issue that is not confined to herbicide tolerant cotton. Cropping areas are subject to standard weed management practices that would minimise the impact of volunteers on the establishment of desirable crop plants and reduce their potential to harbour pests and diseases. In addition, intensive use areas such as roadsides may be subject to management for aesthetic and practical purposes, removing large or invasive weeds.

Conclusion

308. Risk scenario 6 is not identified as a substantive risk, as integrated weed management practices will reduce the density of volunteer populations in cropping use areas. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.7 Risk Scenario 7

Risk source	*Causal pathway*	*Potential harm*
Introduced insect resistance genes	Expression of insect resistance genes in the GM plants ⬇ Reduced populations of target pest insects ⬇ Reduced use of chemical pesticides ⬇ Increased populations of other agricultural pests	Reduced establishment or yield of desirable agricultural crops

Risk source

309. The source of potential harm for this postulated risk scenario is the introduced insect resistance genes.

Causal pathway

310. Expression of the introduced insect resistance genes in the GM cotton is expected to reduce populations of the target pest insects. This would allow a reduction in use of chemical pesticides, which may lead to an increase in populations of other agricultural pests which are otherwise controlled by the same pesticides.

311. Bollgard® III expresses three insect resistance genes, each of which has a relatively narrow specificity for a limited number of target insect pests. Expression of the insect resistance genes in pollen, seed and vegetative material of cultivated or volunteer GM plants directly exposes target insect pests to the proteins through ingestion, leading to a reduction in the number of target insect pests. Natural insect predators and parasitoids of the pest organisms may be indirectly affected through a reduction in numbers and/or quality of the hosts.

312. The additional presence of Vip3A in Bollgard® III is largely designed as a measure to address resistance development; Vip3A demonstrates a similar toxicity to Cry1Ac and Cry2Ab against larvae of certain lepidopteran species, including key pests of cotton, and the efficacy of Bollgard® III against target pests is shown to be similar to Bollgard® II (Chapter 1, Section 6.2.3).

313. Therefore, if Bollgard® III were released for commercial production, it is expected that overall pesticide usage patterns will be similar to those developed since Bollgard® II was introduced into Australian cotton cropping in 2003/4. In particular, adoption of insect resistant GM cottons in the last 15 years has led to a reduction in pesticide use of approximately 85% (Cotton Round Table Report 2013). At the same time, there has been increased survival of populations of non-target arthropods, both beneficial and pest species. In particular, there has been an increase in a range of sucking pests (such as cotton aphid, green mirid and spider mites) that would formerly have been controlled coincidentally by insecticides applied to control *Helicoverpa* species. The most significant of these is the green mirid, which feeds on developing squares and bolls, causing younger bolls to shed and damaging the lint in maturing bolls, potentially reducing yield. In addition, there have also been substantial increases in beneficial arthropod populations in GM cotton crops which have helped to manage other insects (Mansfield 2006). These pests are being successfully managed in cotton crops and associated agricultural systems.

314. It has also been suggested that reduction in endogenous terpenoids such as gossypol in the GM cotton may contribute to observed increases in populations of non-target herbivores such as aphids. The presence of the introduced genes is not expected to directly affect the levels of endogenous toxins, but there may be some indirect effects under insect predation. Hagenbucher (2013) reported reduced levels of induced terpenoids in Bt cotton and suggested

that this may result from effective suppression of Bt-sensitive lepidopteran herbivores (Chapter 1, Section 5.1.4). In greenhouse studies, this was strongly associated with increased populations of aphids, but the effect was less visible in the field under natural infestation of lepidopteran pests.

315. In summary, adoption of Bollgard® III would maintain the reduction in pesticide usage that has been a feature of commercial production of GM cottons. It is therefore unlikely to lead to any further changes in populations of other agricultural pests such as aphids, thrips, mirids and spider mites.

Potential harm

316. The increased presence of secondary pests in the cropping environment could lead to a reduction in yield of desirable agricultural crops. However, pest management is part of standard agronomic practice for cotton cultivation and there are now well established sampling protocols, threshold and control options for managing pests that have been developed since the introduction of existing GM insect resistant cottons. These management practices would be the same for cultivation of Bollgard® III.

Conclusion

317. Risk scenario 7 is not identified as a substantive risk as secondary pest management is part of standard agronomic practice for cotton cultivation and is not expected to be substantially different for Bollgard® III compared to Bollgard® II. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.8 Risk Scenario 8

Risk source	*Causal pathway*	*Potential harm*
Introduced insect resistance genes	Transfer of insect resistance genes to other cultivated insect resistant GM cottons by pollen flow ⬇ Expression of insect resistance genes in the stacked GM cottons ⬇ Exposure of people or other organisms by contact or ingestion, or inhalation of cotton pollen	Increased toxicity or allergenicity for people or desirable organisms

Risk source

318. The source of potential harm for this postulated risk scenario is the introduced insect resistance genes.

Causal pathway

319. The GM cotton is sexually compatible with all *G. hirsutum* cultivars and *G. barbadense*, but not any native cotton species (Chapter 1, section 5.6.2). Therefore, the introduced genes have the potential to be transferred by pollen flow to cultivated cotton that is grown nearby.

320. Most of these cultivated cottons are likely to be the parental GM cottons Bollgard® II and Roundup Ready Flex®, which constitute the large majority of Australian commercial cotton production (Chapter 1, Section 4). A limited amount of Liberty Link (glufosinate herbicide tolerant) cotton is also grown. In addition, WideStrike™ insect resistant cotton is approved for commercial cultivation in areas south of latitude 22°S, but there have been no commercial plantings to date. Nonetheless, if WideStrike™ were adopted for commercial production in the future, the potential exists for hybridisation with Bollgard® III, resulting in hybrid progeny that express the synthetic *Bt* proteins Cry1Ac(synpro) and Cry1F(synpro) in addition to those already present in Bollgard® III.

321. Agronomic practices for GM cotton require crops to be planted from new seed each season and the introduced genes are not expected to increase the persistence of any hybrid plants. Therefore the presence of the hybrids is expected to be transient and represent a small proportion of volunteers compared with the parental cottons.

322. Nonetheless, people harvesting any of these cottons may come in contact with the hybrid seed, as could livestock fed cottonseed meal, leading to exposure to all of the proteins expressed from the introduced insect resistance genes in the hybrid GM cottons. In addition, desirable organisms such as native birds, butterflies, earthworms, natural insect predators of the pest organisms, parasitoids and pollinators such as bees may all be exposed to these hybrid plants.

Potential harm

323. Expression of the introduced insecticidal genes in other cultivated cottons could lead to toxicity or allergenicity for people or toxicity to other desirable organisms such as livestock or certain invertebrates. However, as discussed in risk scenarios 1-4, the Cry 1Ac, Cry2Ab and Vip3A proteins have no demonstrated toxicity or allergenicity to humans or toxicity to other desirable or non-target organisms.

324. The toxicity of Cry 1Ac, Cry2Ab and Vip3A is limited to certain insect species, primarily some of the major lepidopteran pests of cultivated cotton. This is also the case for the synthetic proteins expressed by WideStrike™ cotton, Cry1Ac (synpro) and Cry1F (synpro), which are toxic to a similar (but not identical) range of lepidopteran species, but have not been shown to be toxic or allergenic to humans or toxic to other animals (see RARMP for DIR 091). The literature on laboratory non-target toxicity studies of WideStrike™ (Cry1Ac and Cry1F) was reviewed in the RARMP for DIR 091 (OGTR, 2009) and indicated no adverse effects by the Cry1Ac or Cry1F protein on honey bees, adult ladybird beetles, green lacewing larvae, Daphnia magna, Collembola or adult earthworms. When monarch butterfly larvae were tested at two to ten-fold higher levels of Cry1Ac or Cry1F than levels found in genetically modified corn or cotton plants, some effects were observed (OGTR, 2009). WideStrike™ GM cotton has not yet been grown on a commercial scale, but in the event of future planting greater than 500 ha, Dow AgroSciences Australia Ltd (Dow AgroSciences; the holder of licence DIR 091) is required to provide further data on potential toxicity of the insecticidal proteins to key non-target invertebrates present in the Australian environment.

325. The expression of all these proteins in a stacked hybrid may lead to additive toxic effects against lepidopteran pest species. Evidence from competitive binding studies (Gouffon et al. 2011; Hernandez & Ferre 2005; Ibargutxi et al. 2008; Sena et al. 2009) suggests that, for Cry1, Cry2 and Vip3 families, proteins common to one family compete for similar binding sites, while proteins from different families do not share binding sites. Therefore, in the case of a Bollgard® III and WideStrike™ field cross, it would be predicted that the Cry1Ac and Cry1F proteins would compete for binding sites and show an antagonistic interaction.

326. No literature has been identified that shows combining Cry proteins results in an increase in the range of insects affected compared to the range of insects affected by the individual Cry proteins alone. No literature has been found to suggest that the specificity of individual Cry proteins change in the presence of another Cry protein. In addition, it should be noted that commercial Bt sprays contain whole bacteria, with their endogenous mixture of insecticidal proteins; there have been no reported adverse effects for humans or other desirable organisms resulting from exposure to these sprays.

327. Synergistic effects of Cry proteins have also been reported (Chakrabarti et al. 1998; Ibargutxi et al. 2008), with combined proteins showing a greater toxicity to the same insects targeted by the individual proteins. The potential for synergistic effects between the Cry proteins present in a stack between Bollgard® II and Widestrike™ cotton was discussed in the RARMP for DIR 091 (Widestrike™) and identified as an area of future research to be addressed by Dow AgroSciences in the event of an application for future release in northern Australia.

Conclusion

328. Risk scenario 8 is not identified as a substantive risk as transfer of the introduced genes to other cultivated insect-resistant GM cottons is expected to be limited, the resulting hybrids would be transient, and would not lead to increased toxicity for people or other desirable organisms. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.9 Risk Scenario 9

Risk source	*Causal pathway*	*Potential harm*
Introduced insect resistance genes	Transfer of insect resistance genes to feral cotton plants in nature reserves by pollen flow ⬇ Reduced insect herbivory of GM feral cotton, leading to increased establishment and reproduction of GM feral cotton in nature reserves	Reduced establishment of desirable native vegetation

Risk source

329. The source of potential harm for this postulated risk scenario is the introduced insect resistance genes.

Causal pathway

330. Transfer of insect resistance genes to feral cotton plants in nature reserves could result in reduced insect herbivory of these plants, leading to increased establishment and reproduction of GM feral cottons in nature reserves.

331. The GM cottons are sexually compatible with all *G. hirsutum* cultivars and *G. barbadense*, but not with native cotton species (Chapter 1, section 4.3 and Chapter 2, Section 2.2). Cotton is primarily self-pollinating, with pollen that is not easily dispersed by wind, and the main mechanism for gene transfer is via insect mediated pollen flow (Chapter 1, Section 4.3). The frequency of gene transfer to feral cotton would depend on a range of factors, including the occurrence of feral cotton, survival and reproduction rate of GM plants, and abundance and behaviour of insect pollen vectors. For transfer of the introduced genes to occur, the GM cotton (either planted or volunteers) would need to flower simultaneously with, and be within pollination distance of, the recipient *G. hirsutum* or *G. barbadense* plants. Therefore, pollen mediated gene flow is likely to occur at low frequency and almost solely to cultivated cotton varieties or feral cottons that occur close by.

332. In the short term, it is unlikely that commercial plantings of the GM cottons would be sufficiently close to feral cotton populations for pollen flow to occur. In southern areas of Australia, only transient volunteer populations of cotton occur, mainly due to the impact of frost. In the north, small, naturalised cotton (*G. hirsutum* and *G. barbadense*) populations have been recorded, particularly in areas associated with a prolonged supply of fresh water (Hnatiuk 1990). The majority of naturalised *G. hirsutum* populations occur in the Northern Territory, mostly in Kakadu National Park, while naturalised *G. barbadense* occurs mainly along the eastern regions of QLD (data from Australian Virtual Herbarium). No feral cotton

populations were reported in nature reserves in the Kimberley region of Western Australia (Eastick 2002).

333. While cotton has been evaluated previously as a crop in many regions of the Australian semi-arid tropics (Yeates 2001), other than some test-farming in the Ord River Irrigation Area (ORIA) and Burdekin Irrigation Area there is currently no commercial scale cotton production (Yeates et al. 2013). The growing of all cotton (GM and non-GM) is currently banned in the Northern Territory and this is unlikely to change in the near future; commercial scale cotton production would require land clearing permits as well as licences to access the limited volumes of irrigation water.

334. At present, then, the potential for pollen mediated gene flow between GM cotton in commercial cropping areas and feral cotton populations in nature reserves is very low, due to physical isolation between the populations. The feral cottons are remote from agricultural areas and transport routes, and so are unlikely to fulfil any potential for hybridisation. In addition, cotton growing is currently banned in the Northern Territory, where almost all feral cotton populations have been reported (Eastick 2002).

335. However, if the GM cottons were commercially approved and grown in Northern Australia, over time it is likely that roadside populations may occur as a result of cottonseed transport. These are unlikely to persist as they would be subject to the normal abiotic limitations such as water insufficiency.

336. All of the above limitations and conditions would need to be overcome for pollen mediated gene flow between the GM cottons and feral cottons to be possible. Nonetheless, there is a possibility that small amounts of GM cottonseed may be moved by water or animals into nature conservation areas, establish and hybridise with individuals from established feral populations of non-GM cotton.

337. Expression of the introduced insect resistance genes in these feral cotton varieties could reduce herbivory from lepidopteran insect species. If lepidopteran herbivory were normally a limiting factor, this could enhance the survival, establishment and reproduction of these cottons and lead to their increased spread and persistence in nature reserves.

338. However, while lepidopteran herbivory impacts adversely on productivity in commercial cotton crops, it is not considered an important limiting factor on the spread and persistence of cotton in nature reserves, including in northern Australia.

339. Although lepidopteran pests (mainly *H. armigera* and *H. punctigera*) are the main insect pests in cultivated cotton, they do not seem to be a major limiting factor in naturalised cotton populations. The RARMP for DIR 066/2006 (Bollgard II cotton) canvassed the potential for GM insect resistant cotton to become weedy, particularly in northern Australia, and concluded that insect pressure is not the critical factor limiting establishment and growth of cotton populations, and expression of the *cry* genes does not confer increased fitness. Rather, a range of other biotic and abiotic factors seem to be far more important in limiting the spread and persistence of cotton than lepidopteran herbivory.

340. In particular, monitoring of seven naturalised cotton populations in the Northern Territory revealed abundant seed production, suggesting that these cotton plants were not significantly affected by lepidopteran pests (Eastick 2002). The major insect herbivores observed, particularly over the wet season, were grasshoppers. Grasshoppers are considered to be the most important insect herbivores in tropical savanna ecosystems (Andersen & Lonsdale 1990) and are unaffected by the Cry or Vip proteins present in Bollgard® III cotton.

Potential harm

341. Increased spread and persistence of insect resistant GM cottons in nature reserves may give rise to adverse effects on desirable native vegetation, including reduced establishment of desirable native plants, thereby reducing native plant numbers and organisms reliant on those native plants. This could in turn reduce species richness, or cause undesirable changes in species biodiversity.

342. However, cotton has limited ability to reduce the establishment of other plants (OGTR 2013a) due to the lack of properties such as rambling growth or production of allelopathic compounds. The introduced genes do not result in phenotypic changes that indicate an increased potential to reduce establishment of desirable vegetation, except by displacement through greater numbers, which is considered unlikely. Therefore, any increased potential for the GM cotton to establish or persist in northern Australia due to reduced herbivory by lepidopterans is unlikely to significantly increase the invasiveness potential assessed as low according to the National Post-Border Weed Risk Management Protocol (OGTR 2013a).

Conclusion

343. Risk scenario 9 is not identified as a substantive risk due to limited potential for the insect resistance genes to move into feral cotton plants by pollen flow, restriction of the establishment of cotton populations outside of cultivation areas by abiotic factors and limited potential of cotton to reduce establishment of desirable vegetation. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.10 Risk Scenario 10

Risk source	*Causal pathway*	*Potential harm*
Introduced herbicide tolerance gene	Transfer of herbicide tolerance gene to other herbicide tolerant GM cottons by pollen flow ⬇ Establishment of volunteer cotton plants in agricultural areas ⬇ Reduced effectiveness of weed management measures to control volunteers	Reduced yield of desirable crop plants

Risk source

344. The source of potential harm for this postulated risk scenario is the introduced herbicide tolerance gene.

Causal pathway

345. The herbicide tolerance genes could potentially be transferred by pollen flow to other herbicide tolerant GM cotton plants. If hybrid progeny with dual herbicide tolerance were to establish in agricultural areas, there could be reduced effectiveness of existing weed management measures to control volunteer cotton.

346. As previously discussed, the GM cottons are sexually compatible with all *G. hirsutum* cultivars and *G. barbadense* (Chapter 1, section 4.3). Therefore, the introduced genes have potential to be transferred, by pollen flow, to cultivated cotton that is grown nearby. In Australia, two types of herbicide tolerant GM cotton are licenced for commercial cultivation: the parental GM cotton Roundup Ready Flex® which, together with Bollgard® II, comprises over 95% of the Australian commercial cotton crop, and a small amount of glufosinate ammonium tolerant LibertyLink® cotton (DIR 062/2005).

347. Expression of the *cp4 epsps* gene in combination with the *bar* gene (from Liberty Link® cotton) would result in hybrid offspring that are tolerant to both glyphosate and glufosinate

ammonium. However, agronomic practices for GM cotton require crops to be planted from new seed each season and the introduced genes are not expected to increase the persistence of any hybrid plants. Therefore the presence of the hybrids is expected to be transient and represent a small proportion of volunteers compared with the parental cottons. Nonetheless, there is a possibility that dual herbicide tolerance in volunteers could potentially lead to reduced choice of weed management measures for control of cotton volunteers. In this context, it should be noted that the RARMP for DIR 062/2005 (Liberty Link®) concluded that there was negligible risk to health and safety of people or the environment associated with the potential for reduced choice of herbicides to control cotton volunteers as a result of vertical gene transfer of the *bar* gene to other commercially approved GM cotton lines containing the *cp4 epsps* gene.

348. The control of cotton volunteers is important both in cotton fields and outside the fields such as along roadsides and drains. As previously mentioned, (Chapter 1, Section 4.2.3), herbicides can be used to control seedling cotton volunteers. Glyphosate has been the most common herbicide used to control these volunteers up to the 6 leaf stage but, with the uptake of Roundup Ready® GM cotton since 2000 alternative herbicides are being used, including glufosinate ammonium. However, the use of glufosinate ammonium is limited on cotton volunteers as it offers incomplete control on cotton seedlings at the 4 leaf stage and beyond. Other herbicides such as bromoxynil, carfentrazone and a combination of paraquat and diquat have been shown to be effective (Roberts et al. 2002). Cultivation is also a very effective method to control seedling cotton volunteers (Australian Cotton Cooperative Research Centre 2002a).

Potential harm

349. If left uncontrolled, volunteer cotton plants could establish and compete with other crops (Spotlight on Cotton, Winter 2013) or become host for pests and diseases, reducing yield from crop plants.

350. However, as noted in Risk Scenario 6, weed management is a farm stewardship issue that is not confined to herbicide tolerant cotton. Cropping areas are subject to standard weed management practices that would minimise the impact of volunteers on the establishment of desirable crop plants and reduce their potential to harbour pests and diseases. In addition, intensive use areas such as roadsides may be subject to management for aesthetic and practical purposes, removing large or invasive weeds.

Conclusion

351. Risk scenario 10 is not identified as a substantive risk, as the presence of the hybrids is expected to be transient and tolerance to both glyphosate and glufosinate ammonium is not likely to impact on the control of cotton volunteers, thus limiting their potential for increased ability to reduce crop yield. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

352. Uncertainty is an intrinsic part of risk analysis[3]. There can be uncertainty about identifying the risk source, the causal linkage to harm, the type and degree of harm, the chance of harm occurring or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls.

[3] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

353. Risk analysis can be considered as part of a first tier uncertainty analysis, namely a structured, transparent process to analyse and address uncertainty when identifying, characterising and evaluating risk. However, there is always some residual uncertainty that remains. If the residual uncertainty is important and critical to decision making, then this residual uncertainty may be subjected to further analysis (= second tier uncertainty analysis), such as building 'worst case' scenarios, or by using meta-analysis where results from several studies are combined.

354. There are several types of uncertainty in risk analysis (Bammer & Smithson 2008; Clark & Brinkley 2001; Hayes 2004). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity
  - perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

355. The RARMP for DIR 101 identified three points of additional information that may be required for a large scale or commercial release of Bollgard® III and Bollgard III®/Roundup Ready Flex® cotton. Information provided by the applicant in relation to these is outlined in Chapter 1, Section 6.1 and discussed in relevant sections of that Chapter.

356. Uncertainty can also arise from a lack of experience with the GMO itself. In regard to the parental cottons Bollgard® II and Roundup Ready Flex® cotton, the level of uncertainty is low given the several years of growing these GMOs in Australia and the US. None of these releases have resulted in concerns for human health, safety or the environment. However, Bollgard® III also contains the Vip3Aa protein, which has not previously been released on a commercial scale in Australia. Therefore, for the current application there is uncertainty with respect to the following:

- Australia has considerable experience in growing cotton (both GM and non-GM) in southern regions, but there is limited experience with commercial cotton growing in northern Australia. There were early unsuccessful attempts with non-GM cotton in the north and, more recently, small-scale experimental plantings of insect resistant GM cotton. The GM cottons proposed for release have been demonstrated to have agronomic and phenotypic characteristics comparable with non-GM and commercially approved GM cottons (see Chapter 1, Section 6.2). Therefore, they are expected to behave the same way in the environment, and be subject to the same biotic and abiotic constraints, as other commercially approved cottons. Wide-scale planting of cotton in northern Australia appears to be unlikely in the short term.
- Lack of Australian experience with commercial growing of cotton containing the Vip3A insect resistance protein in Australia. Vip3A (COT102) cotton has been released on a commercial scale for food and/or feed in the US (in 2005) and in Mexico (in 2010) and to date there have not been reports of adverse effects caused by these authorised releases. The risk assessment relies on results of Australian field

experiments with COT 102 and Bollgard® III cotton (Chapter 1, and Whitehouse 2007, 2014) as well as extensive information from international reports and regulatory assessments. Therefore, there is some uncertainty associated with commercial release into the Australian environment. However, based on the available information relating to non-target effects on vertebrates and invertebrates, phenotypic and agronomic characteristics, and potential for increased spread and persistence, no changes have been identified that would lead to increased estimate of risk associated with the release.

- Presence of feral cottons. There is some uncertainty associated with the possibility that feral cottons acquiring insect resistance genes may show increased spread and persistence. The likelihood of vertical gene transfer of the three insect resistance genes to feral cottons in northern Australia is taken into account in risk scenario 9 and the risk assessed as negligible. Current information suggests that lepidopteran herbivory is not a limiting factor on spread and persistence of cotton.

357. Overall, the level of uncertainty in this risk assessment is considered low.

Section 4 Risk evaluation

358. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

359. Factors used to determine which risks need treatment may include:

- risk criteria
- level of risk
- uncertainty associated with risk characterisation
- interactions between substantive risks.

360. Ten risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. The level of risk for each scenario was considered negligible in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, and considering both the short and long term. The principal reasons for these conclusions are summarised in Table 13.

361. The *Risk Analysis Framework* (OGTR 2013a), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. Therefore, no controls are required to treat these negligible risks. Therefore, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management

Section 1 Background

362. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management addresses risks evaluated as requiring treatment, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through licence conditions.

363. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

364. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

365. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and to manage risk to people or the environment. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Risk treatment measures for identified risks

366. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed release of Bollgard® III and Bollgard® III x Roundup Ready Flex® cottons. These risk scenarios were considered in the context of the large scale of the proposed release and the receiving environment. The risk evaluation concluded that no controls are required to treat these negligible risks.

Section 3 General risk management

367. All DIR licences issued by the Regulator contain a number of conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- identification of the persons or classes of persons covered by the licence reporting structures
- a requirement that the applicant allows access to specified sites for purpose of monitoring or auditing.

3.1 Applicant suitability

368. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant (both individuals and the body corporate)

- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

369. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers Monsanto suitable to hold a licence.

370. The licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

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

3.2 Testing methodology

372. Monsanto is required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This instrument is required prior to conducting any dealings with the GMOs.

3.3 Identification of the persons or classes of persons covered by the licence

373. Any person, including the licence holder, may conduct any permitted dealing with the GMOs.

3.4 Reporting requirements

374. The licence obliges the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the dealings
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the release.

375. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

376. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

3.5 Monitoring for Compliance

377. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

378. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to health and safety of people or the environment could result.

Section 4 Post release review

379. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator does not fix durations, but takes account of the likelihood and

impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

380. For the current application for a DIR licence, the Regulator has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. This ongoing oversight will be achieved through post release review (PRR) activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3).

381. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

382. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), fax (02 6271 4202), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see 4.3 below) as well as the risk assessment of future applications involving similar GMO(s).

4.2 Requirement to monitor specific indicators of harm

383. Additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

384. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. If specific indicators of harm were identified, the licence holder would be required to monitor these as mandated by the licence.

385. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

386. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks that could be greater than negligible. Therefore, they did not warrant further detailed assessment. No specific indicators of harm have been identified in this RARMP for application DIR 124. However, specific indicators of harm may also be identified after a licence is issued, eg through either of the other components of PRR.

387. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

388. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings

of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s), or identified new risks to people or to the environment that needed managing, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

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

390. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, general conditions have been imposed to ensure that there is ongoing oversight of the release.

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Appendix A Summary of advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the consultation RARMP[4]

The Regulator received a number of submissions from prescribed experts, agencies and authorities on matters considered relevant to the preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. The issues raised, and where they are addressed in the consultation RARMP, are summarised below.

Summary of issues raised	Comment
Some concern about crops with inserted antibiotic resistance genes being used for animal fodder.	The antibiotic genes and their products have been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by regulatory agencies in Australia and overseas (Chapter 2, Section 2.1). The potential for these genes to pose risks (eg through reduction of therapeutic efficiency of antibiotics, or an increase in bacterial antibiotic resistance) is also addressed in the document Marker genes in GM plants available from the Risk Assessment References page on the OGTR website. Cotton is limited in its use as animal fodder, due to the presence of endogenous toxins such as gossypol.
A major proportion of the Australian cotton industry is already GM for the Bt toxin; the new transgene is a variant from a different strain of Bt. The use of GM cotton with Bt toxins has radically reduced pesticide usage in the cotton industry. The glyphosate resistance gene is one of the most commonly deployed transgenes in the world and has been deployed in Australia in GM canola. Australia does have many native *Gossypium* but they are not weeds, are confined to the arid inland or NW Australia, and do not naturally hybridise with *G. hirsutum.* No justifiable objections to this release.	Noted
Limited knowledge in the area, but cannot see any problems with this application.	Noted
Recommends that northern Australia be identified as a specific risk assessment context and that a separate risk identification process be carried out for this region of Australia.	Chapter 1 discusses the history of cotton growing in northern Australia and identifies differences in environmental conditions and agronomic practices specific to those areas. Reference is made to this material where relevant to a causal pathway and/or potential harm (see risk scenarios 5 and 9).

[4] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Summary of issues raised	Comment
In northern Australia, transfer of GM insect resistant traits to established and naturalised feral cotton populations that are well-adapted to local conditions could result in 'GM cotton hybrids'. The OGTR should carefully consider information provided in the application in relation to GM cotton hybrids, particularly with respect to: • the ability of GM cotton hybrids to survive and establish self-perpetuating populations • likelihood that GM cotton hybrids will have increased weediness traits that will lead to environmental harm as compared to non-GM feral cotton.	For transfer of GM insect resistant traits to naturalised feral cottons to occur, a plausible causal pathway must be established. Spatial limitations on potential for gene transfer by pollen flow are discussed in risk scenario 9. Risk scenario 9 also considers the potential for any GM cotton hybrids to spread and persist outside cultivation and their potential to reduce establishment of desirable vegetation.
The importance of insect damage to the weediness profile of naturalised and established populations of feral cotton is unclear. Introduction of the insect resistance genes to established populations of feral cottons could lead to weediness by removing lepidopteran herbivory as a factor limiting spread and persistence of naturalised and established feral cotton.	A general overview of abiotic factors relevant to cotton production growing areas can be found in Chapter 1, section 2.1. Areas where cotton can be grown in Australia are mainly limited by water availability, the suitability of the soil, temperature and the length of the growing season. Section 6.2.5 includes observations of Bollgard® III responses to abiotic stressors, disease damage, and arthropod damage at ten sites in northern and southern Australia. No differences were observed between Bollgard® III and the conventional control. The potential role of lepidopteran herbivory in limiting spread and persistence of cotton in southern and northern Australia is discussed in Risk scenarios 5 and 9.
Pectinophera gossypiella is a targeted pest of Bollgard III and evidence of active insect predation of naturalised feral cotton by *P. gossypiella* was recorded in and near Kakadu National Park. It is therefore likely that lepidopteran damage is one factor that limits the weediness of the existing feral cotton.	In the Kakadu study, caterpillars collected from the bolls were identified as *P. gossypiella*. However, evidence of insect herbivory does not per se support the notion that *Pectinophera* is a limiting factor on spread of feral cotton Insect herbivory can occur at all stages in the plant life cycle, with different insects preferring different stages (OGTR 2013b); no information was gathered in the Kakadu study on herbivory at other stages of the cotton lifecycle. The potential role of lepidopteran herbivory in limiting spread and persistence of cotton in southern and northern Australia is discussed in Risk scenarios 5 and 9.
No data on specificity of Vip3A protein toxicity to Australian species has been provided with the application. In particular, grasshoppers (order *Orthoptera*) have been noted as a significant cotton pest in Northern Australia and there is no data in the application relating to effect of Vip3A on grasshoppers.	Australian field observations of effects of Bt cottons on non-target insects (including grasshoppers) are discussed in Chapter 1, Section 6.2.3. Results of US field trials that included arthropod damage evaluations for a range of arthropod stressor including grasshoppers are also outlined.
The presence of widely but sparsely dispersed naturalised feral cotton populations over northern Australia is evidence of the ability for naturalised feral cotton seed to disperse into the Australian environment. GM hybrid cotton seed could retain these attributes.	The weediness potential of non-GM cotton is discussed in Chapter 1, Section 4.2. The potential for feral cotton to establish and persist in nature reserves is discussed in risk scenarios 5 and 9.

Summary of issues raised	Comment
Effects of abiotic factors (such as temperature, flood, fire and water availability) on potential establishment of GM cotton hybrids in northern Australia should be taken into account.	Abiotic factors that limit the spread and persistence of cotton are discussed in Chapter 1.
The potential for hybrids to occur between the GM cottons and native *Gossypium* species should be addressed in the risk assessment.	Chapter 1, Section 4.3 discusses sexual compatibility of cultivated cottons with native *Gossypium* species. Genetic differences make the possibility of hybridisation extremely low.
Rather than providing primary data, summaries by governments from other countries are included in the application. This applies to non-target Vip3A protein testing, germination and dormancy data and bioactivity studies of Vip3A in soil. It is recommended that any issues or deficiencies in these be identified.	The Regulator is required to consider relevant previous assessment by a regulatory authority in Australia or overseas [Regulations 10(1)(a)]. The data mentioned are not the sole pieces of evidence relating to the risk to human health or the environment from the introduced Vip3A protein. Field studies conducted in Australia provide highly relevant and useful information. The Regulator considers the weight of evidence available.
Should acknowledge limitations of non-target toxicity testing with Vip3A protein derived from GM maize or bacteria. Uncertainty around the effect of Vip3A on non-target species in the Australian environment should be addressed.	As discussed in Chapter 1, Section 5.3.4, laboratory studies of purified proteins or tissues from GM insecticidal crops show effects that are either consistent with, or more conservative than, those found in field studies. The data mentioned are not the sole pieces of evidence relating to the risk to human health or the environment from the introduced Vip3A protein. Field studies conducted in Australia provide highly relevant and useful information. The Regulator considers the weight of evidence available. The uncertainty regarding Vip3A in Australia has been discussed in Chapter 2, Section 3.
When preparing the RARMP, the Regulator should consider potential for stacking with other commercial GM cottons.	The potential for harm resulting from stacking with commercially approved herbicide tolerant LibertyLink® cotton is assessed in Risk scenario 10. The potential for harm resulting from stacking with commercially approved insect resistant WideStrike™ cotton is assessed in Risk Scenario 8.

Appendix B Summary of advice from prescribed experts, agencies and authorities on the consultation RARMP[5]

The Regulator received several submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

Summary of issues raised	Comment
The shire voted in September 2012 to adopt a GM Crops Policy. This states that council does not support the growing of genetically modified crops within its district. This policy is based on the notion that there is an absence of conclusive evidence that GM crops are safe for people or the environment. Council is concerned that acceptability of risks is considered rather than accepting only safe products. Council acknowledges the commercial pressures in the context of the proposed release. Council urges to withhold approval until safety can be proven rather than the risks of the products are considered acceptable.	The Act requires the Regulator to protect human health and safety and the environment by identifying and managing risks posed by or as a result of gene technology. FSANZ conducts safety assessments of GM foods and has approved the use in foods of the GM parent cottons. The GM cottons proposed for release are the result of conventional breeding and therefore covered by these existing FSANZ approvals. Marketing issues are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. However, areas may be designated under State or Territory law for the purpose of preserving the identity of GM or non-GM crops (or both) for marketing purposes. The licence contains a preamble and condition 3 which specify that dealings with GMOs are not authorised if otherwise prohibited as a result of such State legislation. South Australia currently has in place a moratorium on GM food crops.
Notes that addition of a third type of Bt toxin should diminish the chances of Bt resistance developing in *Helicoverpa* grubs and also simplify herbicide treatments.	Noted.
Assumes that non-GM cotton refuges will be recommended/mandated as a condition of growing the GM cotton, although no reference to this is made in the RARMP. These refuges are a precaution to prevent Bt resistance developing, as at least some of the moth population are not under selection pressure and can interbreed.	As noted in Section 6.2.5, the GM cottons would also be subject to regulation by the Australian Pesticides and Veterinary Medicines Authority (APVMA), which assesses all herbicides and insecticidal products used in Australia and sets their conditions of use, including resistance management. Cultivation of GM insect resistant cotton varieties need to comply with an approved insect resistance management plan and any other relevant conditions that may be imposed by the APVMA. The requirements under existing resistance management plans include mandatory growing of refuges to produce susceptible insects.
Sees no problems with the commercial release.	Noted.

[5] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Summary of issues raised	Comment
The Q&A mentions "Combining 3 different insecticidal genes is expected to reduce the chance of insects developing resistance". This is an unsubstantiated claim and should have experimental evidence to back it up.	The expectation that Bollgard® III will reduce the chance of susceptible insects developing resistance is based on the independent modes of action of the three Bt toxins and the proposition that it is unlikely that insects will be resistant to more than one toxin. References to current research in this field have now been added to Chapter 1, Section 6.2.5. The applicant has undertaken a review of resistance risks associated with Bt cottons in conjunction with Cotton Australia's Transgenic and Insect Management Strategies (TIMS) Committee. Monsanto will submit a Bollgard® III Resistance Management Plan to the APVMA for approval.
If the combined roundup ready and 3X resistant product saturates the market, then what happens if resistance does develop to all three mechanisms? What is the market fallback position?	The Act requires the Regulator to protect human health and safety and the environment by identifying and managing risks posed by or as a result of gene technology. Marketing issues are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. The applicant has undertaken a review of resistance risks associated with Bt cottons in conjunction with Cotton Australia's Transgenic and Insect Management Strategies (TIMS) Committee. Monsanto will submit a Bollgard® III Resistance Management Plan to the APVMA for approval.
Notes that this is just an evolution of the existing Bollgard® II product to include a third Bt gene (VIP) on top of the existing Cry1Ac and Cry2Ab technologies. This will significantly increase the robustness of the product from a resistance point of view; an appropriate resistance management strategy for this next generation of Bt cotton is under development and consultation with Industry. Cannot see any issues with commercial release of this technology which is very similar to the existing Bollgard® II traits already licensed.	The GM cottons would also be subject to regulation by the Australian Pesticides and Veterinary Medicines Authority (APVMA), which assesses all herbicides and insecticidal products used in Australia and sets their conditions of use. Cultivation of GM insect resistant cotton varieties needs to comply with an approved insect resistance management plan and any other relevant conditions that may be imposed by the APVMA. Noted.
The new cottons will strengthen the sustainability of the existing and proposed traits. The main issue is the robustness of the RMP for the technology which is likely to be similar to Bollgard II.	Cultivation of GM insect resistant cotton varieties needs to comply with an approved insect resistance management plan and any other relevant conditions that may be imposed by the APVMA.
In northern Australia, there is likely to be transfer of GM insect resistant traits to established and naturalised feral cotton populations that are well-adapted to local conditions. This could result in 'GM cotton hybrids'.	For transfer of GM insect resistant traits to naturalised feral cottons to occur, a plausible causal pathway must be established. There are spatial limitations on potential for gene transfer by pollen flow; these are discussed in risk scenario 9. Risk scenario 9 also considers the potential for any GM cotton hybrids to spread and persist outside cultivation and their potential to reduce establishment of desirable vegetation. Risk scenario 9 was not identified as a substantive risk.

Summary of issues raised	Comment
Chapter 2, Section 2.4.9 of the RARMP states that lepidopteran herbivory is not considered an important limiting factor on the spread and persistence of cotton in Northern Australia. However, lepidopterans such as *Pectinophera gossypiella* are common insects in northern Australia and it is unclear as to their influence on feral cotton in this part of Australia. The RARMP should reflect the uncertainty of the influence of lepidopteran insects on the weediness profile of naturalised and established populations of GM cotton hybrids in northern Australia.	A general overview of abiotic factors relevant to cotton production growing areas can be found in Chapter 1, section 2.1. Areas where cotton can be grown in Australia are mainly limited by water availability, the suitability of the soil, temperature and the length of the growing season. The potential role of lepidopteran herbivory in limiting spread and persistence of cotton in southern and northern Australia is discussed in Risk scenarios 5 and 9. Section 6.2.5 includes a summary of observations of Bollgard® III responses to abiotic stressors, disease damage, and arthropod damage at ten sites in northern and southern Australia. No differences were observed between Bollgard® III and the conventional cotton control. Uncertainty is explicitly acknowledged in Chapter 2 Section 3 but is not considered sufficient to increase the risk from the relevant scenarios above negligible.
Some non-lepidopteran insects, such as grasshoppers (order Orthoptera) are considered to be the most important herbivores in northern Australia. Based on a field study by Whitehouse (2014) that measured abundance of invertebrate communities in the GM insect resistant cottons, the RARMP states that grasshoppers are unaffected by the Cry or Vip proteins present in Bollgard III cotton. However, no specific data regarding the toxicity of Vip3A proteins (alone and stacked) to non-target insects such as grasshoppers is available. The conclusion of the RARMP should reflect uncertainty associated with this lack of data on the toxicity of the proteins to non-target insects that may restrain the feral cotton populations in northern Australia.	No laboratory testing data is available for effects of Vip 3A on specific non-target insects such as grasshoppers. However, the Australian field studies by Whitehouse are also consistent with results from US field studies (Galadima & Bommireddy 2013). In the latter studies, no differences were observed between Bollgard® III and the conventional control for any of 91 comparisons for the assessed arthropod stressors, including grasshoppers (Ch 1, Section 6.2.3). As discussed in the RARMP, studies on cotton show that other (abiotic) factors are more important in limiting spread and persistence. There are very few feral cottons in northern Australia; where they exist they are mostly associated with waterways, supporting the idea that water is more likely to be a limiting factor than insect predation. Even if lepidopteran herbivory were a restraining factor, the likelihood of contact between any planting of commercially approved GM cotton and cotton in nature reserves is low (Risk scenario 9). Nonetheless, were these feral cottons to acquire and express the introduced genes there is some uncertainty associated with whether this would confer an advantage, given limited knowledge of insect pressures in those areas. This uncertainty is acknowledged in Chapter 2, Section 3.
For at least one experimental field, Whitehouse et al (2014), reported greater diversity in the invertebrate communities in the non-Bt cotton than in the Bt. The reduction in diversity was attributed to factors such as flooding or waterlogging. Such events commonly occur in the potential cotton growing areas in northern Australia and feral cotton populations are found near water courses that are subject to flooding and water logging. This may reduce the populations of insects that restrain these feral cottons and contribute to their weediness. This could be addressed under Risk Scenario 9.	Reduction in insect numbers associated with waterlogging or flooding would apply to both GM and non-GM cotton. Risk scenario 9 concluded that the likelihood of feral cottons acquiring the insect resistance genes, reproducing and establishing was very low. Nonetheless, if waterlogging was a significant factor in reducing insect populations, feral insect-resistant GM cottons would have no competitive advantage over non-GM cottons during periods of flooding, ie insect numbers and diversity would in any case be reduced. If a reduction in invertebrate diversity was associated with plants experiencing flooding or waterlogging, existing feral cottons growing near water courses in the northern Australia would in theory be associated with a reduced population of insects. There are few reports of feral cotton in northern Australia and, while these tend to be associated with water courses, there is no suggestion that these populations display enhanced weediness due to reduced insect pressures during flooding.
Some Australian native lepidopterans, especially tortricids, are considered to be beneficial to the environment. It is recommended that the effect of the GM cotton hybrids on these insects should be addressed in the RARMP.	Risk scenario 9 concluded that there was only limited potential for the insect resistance genes to move into feral cotton plants by gene flow. In addition, given the low numbers of feral cotton plants, the likelihood of building up sufficiently large populations to affect specific lepidopteran species is low.

Summary of issues raised	Comment
Supportive of the application as the consultation RARMP indicates that the proposed commercial release would pose negligible risks to human health or the environment.	Noted.
FSANZ has already assessed and approved the use of food derived from the three parent GM cottons (Bollgard® II, VIP3A and Roundup Ready Flex®).This approval covers food produced from any offspring resulting from conventional breeding. Therefore, FSANZ has already approved food produced from the new GM cottons.	Noted.
The GM cottons were generated by conventional crossing of GM cottons already grown in Australia. Under the GT Act, the Regulator is required to conduct a risk assessment of these GM cottons, while FSANZ does not.	While the parental GM cottons Bollgard® II and Roundup Ready® Flex cotton have been approved for commercial release, Vip3A cotton has only been grown during limited and controlled release in Australia.
The GM cottons are tetraploids and Australian native cottons are diploids. It is very unlikely that crosspollination would result in introgression of the introduced genes into native cottons.	Noted.
Support the conclusion of the RARMP that the proposed release poses negligible risks to human health and the environment.	Noted.
The committee agrees with the overall conclusions of the RARMP. The committee agrees that the RARMP identifies all plausible risk scenarios by which the proposed release could give rise to risks to human health and safety or the environment.	Noted.
The Regulator should further consider the potential for interaction between the introduced Cry and Vip proteins.	Additional discussion regarding potential for interaction between the introduced Cry and Vip proteins has been added to Risk Scenario 3.
The Regulator should consider clarifying the wording in the RARMP regarding Vip3A specificity and conclusions about potential toxicity.	The wording was changed to better reflect the likelihood of toxicity.
The Regulator should consider clarifying the description in the RARMP of weed management practices undertaken by Councils on roadsides.	The wording was changed to better reflect current roadside weed management practices in Risk Scenarios 6 and 10.

Appendix C Summary of submissions from the public on the consultation RARMP

The Regulator received four submissions from the public on the consultation RARMP. The issues raised in these submissions are summarised in the table below. All issues raised in the submissions that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Abbreviations:

View (general tone): **n** = neutral; **x** = do not support; **y** = support.

Issues raised: **E**: Environment; **H**: Human health; **HU**: Herbicide use

Other abbreviations: **APVMA**: Australian Pesticides and Veterinary Medicines Authority; **FSANZ**: Food Standards Australia New Zealand; **GM**: Genetically Modified; **RARMP**: Risk Assessment and Risk Management Plan.

Sub-mission No:	View	Issue	Summary of issues raised	Comment
1	x	H	Strongly opposed to GM cotton (and any other GMO's) being released to the general public. Monsanto researchers claim to have "proven" GMOs are safe but independent third party research (particularly in America and Europe) shows it is in fact extremely unsafe for humans.	The RARMP for this release considered information provided by the applicant as well as currently available scientific information from Australian and international sources. This information was considered in the context of the large scale of the proposed release, and the RARMP concluded that risks to human health and the environment are negligible. FSANZ conducts safety assessments of GM foods and has approved the use in foods of the GM parent cottons. The GM cottons proposed for release are the result of conventional breeding and therefore covered by these existing FSANZ approvals.
2	x	H, E, HU	Considers that release of the GM cotton will lead to increased use of glyphosate, which is undesirable for the sake of human and ecological health, especially soil, insect and bird populations.	The APVMA has regulatory responsibility for the registration of agricultural chemicals, including herbicides, in Australia. The APVMA considers a range of issues in assessing agricultural chemicals for registration, including efficacy, resistance management and human health and environmental impacts. The APVMA will not register a chemical product unless satisfied that its approved use would not be likely to have an effect that is harmful to people or the environment.

Sub-mission No:	View	Issue	Summary of issues raised	Comment
3	x	H, E	There are questions that need to be answered before more GM seed is released into the Australian environment and into the food chain: • What are the effects of the insecticidal proteins on animals that ingest them? • What are the carryon effects to humans who ingest meat that is grown on feed containing this GM cottonseed and or oil? • How do the new proteins or toxins in this cotton react in the human body?	Risk scenarios 1-4, and 8 discuss the potential for the introduced insect resistance genes to result in increased toxicity for people, livestock, non-target insects or other desirable organisms. The level of risk for each scenario was considered negligible; the principal reasons for these conclusions are summarised in Chapter 2, Table 13. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has previously given approval for the use in food of cotton seed oil and linters derived from the parental GM cottons Bollgard® II, Roundup Ready® Flex and Vip3A; these approvals also cover material derived from Bollgard® III and Bollgard® III x Roundup Ready® Flex cotton. People have been consuming Bollgard® II and Roundup Ready® Flex cotton products in Australia since 2002 and 2005, respectively, without any reported adverse health effects. In addition, cotton seed and meal from Bollgard® II and Roundup Ready® Flex have been fed to domestic animals since their commercial release in Australia. The three parental GM cottons have also been approved for use as food and/or feed in a number of other countries; to date, there have been no reports of adverse effects on human health or the environment caused by any of these authorised releases.
	x	H	Does the transgenic DNA in cotton products have any long term, pervasive or accumulative effects?	As noted in Risk Scenario 1, the introduced genes and expressed proteins are not present in cotton products such as cottonseed oil, fibres and linters which are consumed or used by people. In addition, all of the introduced genes are already widespread in the environment.
	x	E	What effects does this strain of insecticidal cotton have on bees and other beneficial pollinators in the environment?	As discussed in Chapter 1, Cry 1Ac, Cry2Ab and Vip3A have only a narrow range of target specificity within Lepidopteran species and are unlikely to harm non-lepidopteran arthropods. Chapter 1 (Table 3 and paragraph 154) presents data on ecotoxicological testing of Cry 1Ac, Cry 2Ab and Vip3A on honeybees, which support a conclusion of no harm to honey bees from exposure to the purified proteins.

Sub-mission No:	View	Issue	Summary of issues raised	Comment
	x	H, E, HU	The OGTR also needs to consider the impact of glyphosate herbicide on human and environmental health: • What does Roundup do in the systems of the animals that ingest it with their feed? Are there any residual effects for humans of eating this meat? • What does Roundup do in the human body? • What are the impacts of increased herbicide use on the soils, groundwater and the future fertility of the soil?	The APVMA has regulatory responsibility for the registration of agricultural chemicals, including herbicides, in Australia. The APVMA considers a range of issues in assessing agricultural chemicals for registration, including efficacy, resistance management and human health and environmental impacts. The APVMA will not register a chemical product unless satisfied that its approved use would not be likely to have an effect that is harmful to people or the environment.
	n	H,E	Have all human and environmental safety issues surrounding this organism been thoroughly evaluated?	The RARMP for this release considered information provided by the applicant and the currently available scientific information in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible.
4	y	-	Supports the general release of this technology and notes that GM cotton lines containing three of the introduced genes for insect resistance and herbicide tolerance have been previously approved for commercial release in Australia. Notes that this support is based on industry experience with GM cotton since 1996.	Noted.