



Australian Government

Department of Health

Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 143

Commercial release of cotton genetically modified
for insect resistance and herbicide tolerance
(GlyTol[®] (BCS-GH002-5) and
GlyTol TwinLink Plus[®] and (BCS-GH002-5 x
BCS-GH004-7 x BCS-GH005-8 x SYN-IR102-7))

Applicant: Bayer CropScience Pty Ltd

December 2016

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 143

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the intentional, commercial scale release of insect resistant and herbicide tolerant genetically modified (GM) cotton in Australia. 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 have been imposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the release.

The application

Application number:	DIR 143
Applicant:	Bayer CropScience Pty Ltd (Bayer)
Project Title:	Commercial release of cotton genetically modified for insect resistance and herbicide tolerance (GlyTol [®] (BCS-GH002-5) and GlyTol TwinLink Plus [®] (BCS-GH002-5 x BCS-GH004-7 x BCS-GH005-8 x SYN-IR102-7)) ¹
Parent organism:	Cotton (<i>Gossypium hirsutum</i> L.)
Introduced gene and modified trait:	<p>Three insect resistance genes</p> <ul style="list-style-type: none"> • <i>Cry1Ab</i> gene from <i>Bacillus thuringiensis</i> (Bt) • <i>Cry2Ae</i> gene from Bt • <i>Vip3Aa19</i> gene from Bt <p>Two herbicide tolerance genes</p> <ul style="list-style-type: none"> • <i>bar</i> gene from <i>Streptomyces hygroscopicus</i> for glufosinate tolerance • <i>2mepsps</i> gene from <i>Zea mays</i> (maize) for glyphosate tolerance <p>One selectable marker gene</p> <ul style="list-style-type: none"> • <i>aph4</i> from <i>Escherichia coli</i> for resistance to hygromycin B
Proposed locations:	Australia-wide

This commercial release follows field trial work conducted under licence DIR 133.

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed release, either in the short or long term, are negligible.

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 were characterised in relation to

¹ The title of the licence application submitted by Bayer is “Commercial release of GlyTol[®] cotton and GlyTol TwinLink Plus[®] cotton (*Gossypium Hirsutum* L.) for use in the Australian cropping system”.

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 impacts were considered.

Credible pathways to potential harm that were considered included: toxic and allergenic properties of the GM cotton; potential for 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 introduced proteins are not considered toxic or allergenic to people or toxic to vertebrates and most invertebrates; toxicity of the introduced insect-resistance proteins is limited to certain insects, including major pests of cotton; the GM cottons and other GM cotton lines containing the introduced genes have previously been assessed and authorised for field trial and/or commercial release in Australia and have a history of safe use overseas; the introduced genes and proteins are widespread in the environment; the GM cottons and their progeny can be controlled using integrated weed management; the GM cottons are susceptible to the biotic or abiotic stresses that normally restrict the geographic range and persistence of cotton; and the limited capacity of the GM cotton to spread and persist in undisturbed natural habitats. In addition, food made from the GM cottons has been approved by Food Standards Australia New Zealand as safe for human consumption.

Risk management

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

As the level of risk has been assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions 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

Act2	Actin 2
ADF	Acid detergent fibre
<i>aph4</i>	Hygromycin B phosphotransferase gene
APVMA	Australian Pesticides and Veterinary Medicines Authority
Bt	<i>Bacillus thuringiensis</i>
CaMV	Cauliflower mosaic virus
CFIA	Canadian Food Inspection Agency
<i>2mepsps</i>	<i>epsps</i> gene from maize with two mutated codons
Cry	Crystal protein
CsVMV	Cassava vein mosaic virus
2mEPSPS	EPSPS protein from maize with two mutated amino acids
DIR	Dealing involving intentional release
DNA	Deoxyribonucleic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ELISA	Enzyme-linked immunosorbent assay
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
FW	Fresh weight
g	Gram
GM	Genetically modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
HGT	Horizontal gene transfer
µg	Microgram
mRNA	Messenger ribonucleic acid
NDF	Neutral detergent fibre
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase chain reaction
PRR	Post release review
RARMP	Risk assessment and risk management plan
SCSV	Subterranean clover stunt virus
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
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 in States and Territories, 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).

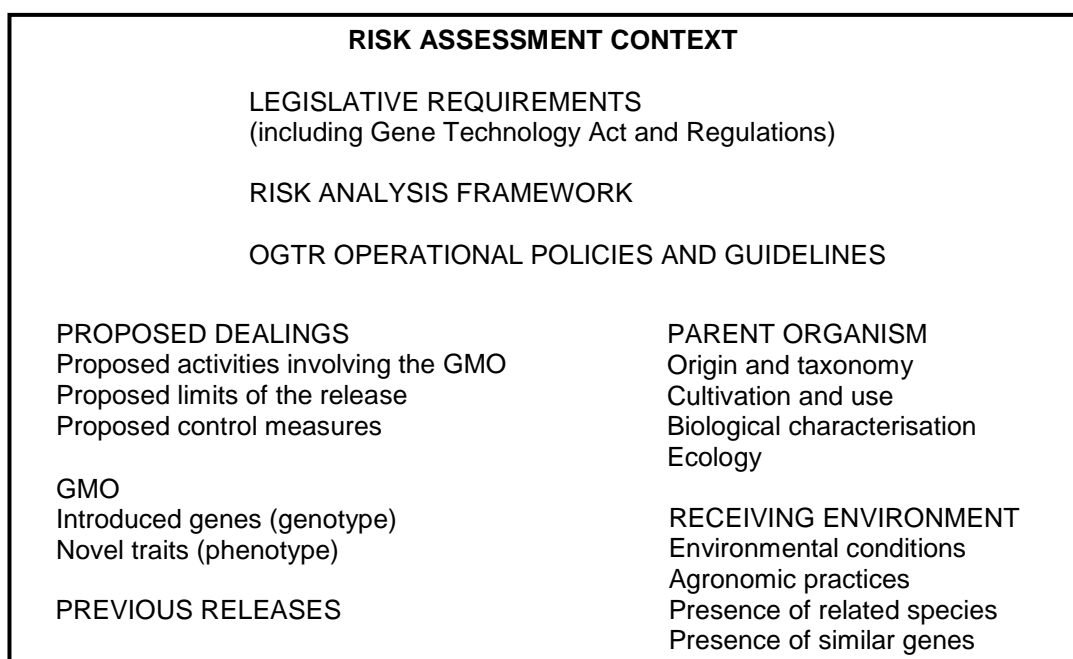


Figure 1. Summary of parameters used to establish the risk assessment context

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 who must be consulted, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the 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. Therefore, 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, all Australian local councils² 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 in the second round of consultation, and how it was taken into account, is summarised in Appendix B. One public submission was received and its consideration is summarised in Appendix C.

7. The Risk Analysis Framework (OGTR 2013) 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 and Department of Agriculture and Water Resources. 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 The proposed release

9. Bayer CropScience Pty Ltd (Bayer) proposes commercial cultivation of two types of GM cotton. The first type, GlyTol[®] cotton, confers tolerance to the herbicide glyphosate. The second type, GlyTol TwinLink Plus[®] cotton, contains three introduced genes that confer insect resistance, one gene that confers tolerance to the herbicide glufosinate and one gene for glyphosate tolerance.

10. GlyTol TwinLink Plus[®] cotton was produced by conventional breeding among four GM cotton lines GHB614, T304-40, GHB119 and COT102, and therefore is also known as GHB614 × T304-40 × GHB119 × COT102 cotton. GM cotton lines are identified by OECD unique identifiers as BCS-GH002-5 (GHB614), BCS-GH004-7 (T304-40), BCS-GH005-8 (GHB119) and SYN-IR102-7 (COT102).

11. The applicant is seeking approval for the release to occur Australia-wide, subject to any moratoria imposed by States and Territories for marketing purposes. The GM cottons could be grown in all commercial cotton growing areas, and products derived from the GM plants would enter general commerce, including use in human food and animal feed.

12. Initially, demonstration trials will be conducted at selected sites to introduce the GlyTol[®] cotton and GlyTol TwinLink Plus[®] cotton to Australian farmers, followed by full commercial release.

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

² Bayer is seeking approval for unrestricted commercial release of the GM cottons in all cotton growing areas of Australia. Cotton may be grown over a significant proportion of Australian agricultural land, and viable cotton seed may be transported out of the cotton growing areas. Therefore, the Regulator decided to consult with all of the local councils in Australia, except for those that have requested not to be consulted on such matters.

- (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 The parent organism

14. The parent organism is upland cotton (*Gossypium hirsutum* L.), which is the most commonly cultivated cotton species worldwide. Cotton is exotic to Australia and is grown as an agricultural crop in New South Wales and Queensland, with occasional trial or small-scale cultivation in Victoria, northern Western Australia and in the Northern Territory.

15. Cotton is grown as a source of textile and industrial fibre, cottonseed oil for food use, and cottonseed meal for animal feed. A brief description of relevant biological information about the parent organism is provided in the following sections. More detailed information is contained in a reference document, *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)*, which was produced to inform the risk assessment process for licence applications involving GM cotton plants (OGTR 2016b). The document is available from the [OGTR website](#) or on request from the OGTR.

4.1 Non-GM cotton as a crop

16. 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 2016b). It is a perennial plant that is cultivated as an annual.

17. A summary of climatic data and production systems for past 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 2006).

18. 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 (for further detail see discussion in RARMPs for DIR 066/2006 and DIR 124).

4.1.1 Non-GM cotton and herbicide resistance

19. 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.

20. 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 practices include cultivation or the application of herbicide treatments (OGTR 2016b). In addition, integrated weed management practices are used to avoid selection of resistant weed biotypes (CropLife Australia 2012). The Australian cotton industry uses such weed management practices to decrease the possibility that herbicide tolerant weeds will become a problem (Cotton Australia website).

21. At least 37 weed species from around the world are reported to have resistance to glyphosate and 12 of them are found in Australia³. However, no glufosinate-resistant weed species have been reported in Australia.

4.1.2 Management of pests in non-GM cotton crops

22. In non-GM 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). Historically, 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.

23. Shaw (1992) listed six major chemical groups for use in non-GM 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).

24. 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 et al. 1993). With the GM insect resistant cotton varieties being overwhelmingly grown, recent pest management also considers the impact of disruptive insecticides on beneficial predators and parasites and recommends that it is only sensible to control pests in the fields where they warrant control (Maas et al. 2015). This is based on the view that secondary pests, entering the crop after disruptive insecticide application that kills beneficial predators and parasitoids, would survive better and potentially cause more economic damage.

4.1.3 Management of volunteer cotton

25. Seedlings are easier to kill than older plants and volunteer seedlings that 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).

26. Glyphosate and glufosinate ammonium are registered for use on volunteer seedling cotton. Glyphosate is generally effective up to 6 leaf stage whereas glufosinate ammonium offers incomplete control at the 4 leaf stage and beyond. Glufosinate-ammonium is not recommended as effective for control of seedling cotton (Charles et al. 2013). Glyphosate and glufosinate herbicides are generally less effective on adult plants, requiring a number of applications in combination with other treatments to kill them (Roberts et al. 2002). Adult cotton plants can be controlled by other herbicides and mechanical means.

4.2 Non-GM cotton outside cultivation – weed risk potential

27. 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.

28. The Standards Australia National Post-Border Weed Risk Management Protocol rates the weed risk potential of plants according to properties that strongly correlate with weediness for

³ Sources: [International Survey of Herbicide Resistant Weeds website](#), accessed 15 June 2016; Green et al. (2008).

each relevant land use (Standards Australia Ltd et al. 2006). These properties relate to the plants' potential to cause harm (impact), to its invasiveness (spread and persistence) and to its potential distribution (scale). The weed risk potential of volunteer cotton has been assessed using methodology based on the National Post-Border Weed Risk Management Protocol (OGTR 2016b).

4.2.1 Potential to cause harm

29. 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.

30. 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 2016b).

31. 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). These compounds limit the use of cotton seed meal in human food and animal feed.

4.2.2 Invasiveness

32. 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 Spread

33. Cotton 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; a second phase of the survey in 2013 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 (CRDC 2013a).

4.2.4 Distribution

34. Volunteer cotton is not rated as a weed in agricultural settings (Groves et al. 2003). Modern cultivars usually do not possess seed dormancy and therefore do not persist in the field under normal conditions (OECD 2008).

35. Ephemeral populations of cotton volunteers can be found on cotton farms, by roadsides where cotton seed is transported, or in areas where cotton seed is used as livestock feed (Addison et al. 2007; Eastick & Hearnden 2006). As discussed in Section 4.2.3, a survey in 2012/13 in Qld and northern NSW showed that the majority of cotton volunteer plants were localised in close proximity to cotton farms or ginning facilities.

36. Naturalised populations of *G. hirsutum* have been found in a few non-agricultural areas in the north of Australia, indicating that it is possible for this species to establish outside cultivation, but cotton has a limited ability to spread and persist in undisturbed nature conservation areas (OECD 2008; OGTR 2016b). Most reports of naturalised *G. hirsutum* populations are from tropical areas of the Northern Territory ([Australia's Virtual Herbarium](#)). 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). Cotton is therefore considered to be a minor problem in natural undisturbed habitats in Australia (Groves et al. 2003).

4.3 Sexually compatible plants

37. In the natural environment, for successful hybridisation to occur, parent plants 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. 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*.

38. There are 17 native species of *Gossypium* in Australia, most of which are found in the NT and the north of WA (OGTR 2016b), but the likelihood that *G. hirsutum* could hybridise successfully with any of the native Australian cottons is extremely low, due to genetic incompatibility. This is discussed in greater detail in *The Biology of Gossypium hirsutum L. and Gossypium barbadense L.(cotton)* (OGTR 2016b) and the RARMP for DIR 124.

Section 5 The GMOs proposed for release

5.1 Introduction to the GMOs

39. The GM cottons proposed for release are:

- GlyTol[®] cotton (GHB614) and
- GlyTol TwinLink Plus[®] cotton.

40. GlyTol[®] is the commercial name for GHB614 cotton and is one of the parental GM cottons of GlyTol TwinLink Plus[®] cotton. GHB614 was evaluated and approved for field trials under licences DIR 113 and DIR 133

41. GlyTol TwinLink Plus[®] cotton is derived from conventional breeding using GM cotton lines GHB614, T304-40, GHB119 and COT102 (Table 1). T304-40 and GHB119 were approved for field trials under licences DIR 087, DIR 113 and DIR 133. COT102 (also known as VIP3A) cotton was evaluated as a parental GM cotton for Bollgard[®] III cotton, which was authorised for commercial release under licence DIR 124.

42. The RARMPs for DIR applications listed above provide a full description of the parental GM cottons. They include details relevant to this risk assessment such as molecular characterisation, toxicity, allergenicity, weediness and the potential for adverse effects upon outcrossing. Therefore, information for the individual parental GM cottons will be used as part of the characterisation of the GMOs themselves.

5.2 The introduced genetic materials and their effects

43. A detailed description of the genetic modifications is available in the RARMP for DIR 133. The introduced genetic material, source organisms and traits are summarised in Tables 1 and 2.

Table 1 Traits and their corresponding genes introduced into the GM cottons proposed for release

GM cotton	Parental GM cotton	Glyphosate tolerance	Glufosinate tolerance	Insect resistance	Antibiotic resistance
GlyTol® (GHB614)	-	<i>2mepsps</i>	-	-	-
GlyTol TwinLink Plus®	GHB614 (GlyTol®)	<i>2mepsps</i>	-	-	-
	T304-40	-	<i>bar</i>	<i>cry1Ab</i>	-
	GHB119	-	<i>bar</i>	<i>cry2Ae</i>	-
	COT102 (VIP3A)	-	-	<i>vip3Aa19</i>	<i>aph4</i>

Table 2 Genetic elements and their origin

Gene (source)	Protein produced	Function	Promoter (source)	Terminator (source)	Additional elements (source)
<i>2mepsps</i> (<i>Zea mays</i>)	5-enolpyruvylshikimate-3-phosphate synthase (double mutant)	Glyphosate tolerance	<i>Ph4a748At</i> (<i>Arabidopsis thaliana</i>)	<i>3'histonAt</i> (<i>A. thaliana</i>)	<i>intron1 h3A</i> (5' leader) (<i>A. thaliana</i>); <i>TPotp C</i> (transit peptide) (<i>Zea mays</i> & <i>Helianthus annuus</i>)
<i>bar</i> (<i>Streptomyces hygrosopicus</i>)	PAT (phosphinothricin acetyl transferase)	Glufosinate tolerance	<i>P35S3</i> (CaMV)	<i>3'-nos</i> (<i>Agrobacterium tumefaciens</i>)	-
			<i>PcsvmvXYZ</i> (CsVMV)	<i>3'-nos</i> (<i>A. tumefaciens</i>)	-
<i>cry1Ab</i> (<i>Bacillus thuringiensis</i>)	crystal protein 1Ab	Insect resistance	<i>Ps7s7</i> (SCSV)	<i>3'-me1</i> (<i>Flaveria bidentis</i>)	<i>5'e1</i> (5' leader) (<i>Oryza sativa</i>)
<i>cry2Ae</i> (<i>Bt</i>)	crystal protein 2Ae	Insect resistance	<i>P35S2</i> (CaMV)	<i>3'-35S</i> (CaMV)	<i>5'cab22L</i> (5' leader) (<i>P. hybrida</i>); <i>TPssuAt</i> (transit peptide) (<i>A. thaliana</i>)
<i>vip3Aa19</i> (<i>Bt</i>)	vegetative insecticidal protein 3A	Insect resistance	<i>PAct2</i> (<i>A. thaliana</i>)	<i>3'-nos</i> (<i>A. tumefaciens</i>)	<i>intron1 Act2</i> (5' leader) (<i>A. thaliana</i>)
<i>aph4</i> (<i>Escherichia coli</i>)	Hygromycin B phosphotransferase	Hygromycin B resistance	<i>PUBq3</i> (<i>A. thaliana</i>)	<i>3'-nos</i> (<i>A. tumefaciens</i>)	<i>intron1 Ubq3</i> (5' leader) (<i>A. thaliana</i>)

5.2.1 Herbicide tolerance

Glyphosate tolerance

44. Glyphosate is the active ingredient in a number of broad-spectrum systemic herbicides that have been approved for use in Australia (Australian Pesticides and Veterinary Medicine Authority). The mode of action of glyphosate is to specifically inhibit the function of the

enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Glyphosate binding to EPSPS in plants blocks biosynthesis of essential aromatic compounds, including the amino acids phenylalanine, tyrosine and tryptophan (Dill 2005).

45. The *2mepsps* gene in GHB614 cotton was developed from a maize (*Zea mays*) gene that encodes an EPSPS enzyme. Site-directed mutagenesis of the wild-type maize *epsps* gene resulted in two amino acid changes in the encoded protein (substitution of threonine by isoleucine at position 102 and substitution of proline by serine at position 106). These changes greatly reduce the affinity of this maize EPSPS enzyme for glyphosate, thus allowing sufficient enzyme activity for the plants to grow in the presence of glyphosate herbicide (Lebrun et al. 2003). Thus, GM cotton lines containing the *2mepsps* gene are tolerant to glyphosate.

Glufosinate tolerance

46. Glufosinate-ammonium is the active ingredient in a number of proprietary broad-spectrum herbicides that have been registered for use in Australia ([Australian Pesticides and Veterinary Medicine Authority](#)). These herbicides function in plants by inhibiting the enzyme glutamine synthase. Inhibition of this enzyme both prevents the synthesis of the amino acid glutamate and causes toxic accumulation of its precursor, ammonia, in plant tissues (Evstigneeva et al. 2003).

47. Glufosinate-ammonium is a synthetic analogue of an antimicrobial secondary metabolite called bialaphos that is produced naturally by the soil bacterium *Streptomyces hygroscopicus*. To avoid the toxicity associated with bialaphos production, *S. hygroscopicus* expresses a bialaphos resistance gene known as *bar*. The *bar* gene encodes phosphinothricin acetyltransferase (PAT), an enzyme that acetylates the free amino groups of glufosinate-ammonium and renders it inactive (Thompson et al. 1987). Thus, plants containing the *bar* gene are expected to tolerate glufosinate-ammonium herbicide. The gene sequence of the *bar* gene in the GM cotton lines was modified for expression in plants.

5.2.2 Insect resistance

48. The bacterium *Bacillus thuringiensis* (Bt) produces a range of insecticidal proteins, including the crystal (Cry) proteins (also known as delta-endotoxins) and vegetative insecticidal proteins (Vips). Vips are secreted 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). A survey of gene distribution in Bt strains found that 45% of the isolates contained a combination of *cry1A*, *cry2* and *vip3* genes (Hernandez-Rodriguez et al. 2009).

49. Both Cry proteins and Vips become active when ingested and cleaved by proteases in the insect midgut. In susceptible species, the activated toxins bind to specific receptors on the brush border membrane of the midgut epithelium, leading to formation of membrane pores, cell lysis, and eventual insect death (Bravo et al. 2007; Yu et al. 1997). Cry1A, Cry2A and Vip3A protein classes all bind to different specific binding sites on the epithelial membrane surface (Gouffon et al. 2011; Sena et al. 2009).

50. The *cry1Ab* gene in T304-40 cotton was isolated from the Bt subspecies berliner (Höfte et al. 1986) and the gene sequence has been modified for expression in plants. The amino acid sequence is identical to the native protein except that it is truncated at the C-terminal end and an alanine has been inserted at the N-terminal end. The truncated Cry1Ab contains the region responsible for insecticidal activity.

51. The *cry2Ae* gene in GHB119 cotton was isolated from Bt subspecies dakota and the gene sequence has been modified for expression in plants.

52. The *vip3Aa19* gene in COT102 cotton was isolated from Bt strain AB88 (Estruch et al. 1996) and the gene sequence was modified for expression in plants. The amino acid sequence

is identical to the native protein except that a glutamine residue has replaced a lysine residue at position 284.

5.2.3 Antibiotic resistance

53. The *aph4* gene (also known as *hph* or *hpt*) in COT 102 cotton was isolated from the common gut bacterium *Escherichia coli*. The gene encodes a hygromycin phosphotransferase (HPT) enzyme which inactivates the antibiotic hygromycin B. This antibiotic resistance trait was used as a selectable marker during plant transformation. Further information about this gene can be found in the document *Marker genes in GM plants* available from the [Risk Assessment References page](#) on the OGTR website.

5.2.4 Toxicity/allergenicity of the proteins encoded by the introduced genes

2mEPSPS protein

54. The *2mepsps* gene present in the GM cottons is a variant of the native maize *epsps* gene, and the modified maize EPSPS protein (designated as 2mEPSPS) is 99.5% identical to the native maize protein. Maize has been safely consumed by humans and other animals for centuries. The 2mEPSPS protein has no sequence similarity to known toxins or allergens, is rapidly degraded in simulated gastric or intestinal fluids and had no detrimental effect on mice when purified protein was administered orally or intravenously (Herouet-Guicheney et al. 2009). The 2mEPSPS protein has been approved for limited and controlled release in *Brassica napus* (DIR 032/2002, DIR 069/2006) and *Brassica juncea* (DIR 057/2004, DIR 069/2006). FSANZ has approved food derived from GM cotton varieties expressing 2mEPSPS as safe for human consumption (FSANZ 2008).

PAT protein

55. Purified PAT protein was not toxic to mice when administered intravenously at high doses. No sequence homology was found between PAT and any known toxic or allergenic proteins. PAT is rapidly degraded in simulated gastric or intestinal fluid (Herouet et al. 2005). FSANZ has approved food derived from GM cotton varieties expressing PAT protein as safe for human consumption (FSANZ 2005; FSANZ 2010a; FSANZ 2010b). The Regulator has also previously approved the commercial release of GM cotton lines expressing the PAT protein (DIR 062/2005 and DIR 091).

Cry1Ab, Cry2Ae and Vip3A proteins

56. The Cry1Ab, Cry2Ae and Vip3A proteins for insect resistance are derived from *Bacillus thuringiensis* (Bt). Bt is naturally found worldwide in soil, on plant surfaces and in animals, and microbial preparations of Bt have been used as a commercial pesticide for over 60 years (OECD 2007). Thus, people and other organisms have a long history of safe exposure to Bt insecticidal proteins.

57. Cry and Vip proteins are toxic to susceptible insects through a mechanism of binding to specific receptors found on the midgut epithelium (Bravo et al. 2007; Lee et al. 2006). 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). The proteins are not expected to be toxic to any organism lacking these specific receptors. The Cry2Ae and Vip3A proteins are only known to be toxic to lepidopteran insects. The Cry1Ab protein has confirmed toxicity to a range of lepidopteran insects and a hemipteran species. There are reports in the scientific literature that Cry1Ab is toxic to species in other insect orders, but the results were equivocal and/or not reproducible (van Frankenhuyzen 2013).

58. Acute oral toxicity studies of the purified Cry1Ab and Cry2Ae proteins reported no adverse effects in mice (Rouquie 2006; Rouquie 2007). Both proteins showed no detrimental effects on five representative non-target arthropods, including honeybee (*Apis mellifera*) (Richard 2008a; Richard 2008b), beetle (*Coleomegilla maculata*) (Patnaude 2007; Patnaude

2008c), springtail (*Folsomia candida*) (Patnaude 2008a; Patnaude 2008b), water flea (*Daphnia magna*) (Sayers 2008a; Sayers 2008b) and green lacewing (*Chrysoperla rufilabris*) (Patnaude 2009a; Patnaude 2009b). Both proteins were rapidly degraded in simulated gastric fluids (Mendelsohn et al. 2003; US EPA 2008a). Neither protein had amino acid sequence similarity to known allergens (Bushey et al. 2008).

59. For the Vip3A protein, toxicity and allergenicity to humans and toxicity to honey bees and other non-target invertebrates have been extensively discussed in the RARMP for DIR 124. Mice and ten other representative non-target organisms including honeybees showed no adverse effects following oral administration of high levels of purified Vip3A protein (Raybould & Vlachos 2011) and a recent review reported no harm to honey bees from exposure to purified Vip3A (CERA 2012 and references therein). The Vip3A protein had no sequence similarity to known protein allergens, and was degraded in simulated gastric fluid (Hill et al. 2003).

60. The effects of GM cotton expressing the Vip3A protein on arthropods were studied in Australian field trials. No major differences in species richness or diversity of beneficial and non-target arthropods were found in comparison to non-GM cotton (Whitehouse et al. 2007). Field measurements of insect abundance in GM corn crops expressing stacked Vip3A and Cry1Ab proteins showed no significant difference in overall biodiversity compared to non-GM crops without insecticide treatment. There were changes in density of some non-target taxa, for example due to reduced lepidopteran prey abundance, but these did not carry over to the subsequent growing season (Dively 2005).

61. Food (seed oil and linters) derived from cotton varieties containing the Cry1Ab, Cry2Ae and Vip3A proteins have been approved by FSANZ as safe for human consumption (FSANZ 2004; FSANZ 2010a; FSANZ 2010b). The Canadian Food Inspection Agency (CFIA) also determined that feed derived from such GM cottons does not present livestock feed safety concerns when compared to currently commercialised cotton varieties (CFIA 2008; CFIA 2011a; CFIA 2011b).

HPT protein

62. HPT protein purified from *E. coli* had no adverse effects in an acute oral toxicity study on mice (Zhuo et al. 2009). The protein has no sequence similarity to known allergens and is rapidly digested by simulated gastric fluids (Lu et al. 2007). FSANZ has approved food derived from a GM cotton variety expressing the HPT protein as safe for human consumption (FSANZ 2004).

5.2.5 Toxicity of herbicide metabolites

Glyphosate metabolites

63. There is no expected difference in the metabolic fate of glyphosate in non-GM cotton and in GM cotton expressing the *2mepsps* gene. The 2mEPSPS protein encoded by the *2mepsps* gene is insensitive to the effects of glyphosate (Section 5.2.1). Consequently, in GM plant cells with the *2mepsps* gene, biosynthesis of aromatic amino acids is not inhibited in the presence of glyphosate. Therefore, no new metabolic products are formed in these GM plants as the only difference from the native EPSPS enzyme is the reduced affinity for glyphosate (OECD 1999a).

Glufosinate metabolites

64. The herbicide glufosinate comprises a racemic (equal) mixture of the L- and D-enantiomers. The L-enantiomer is the active constituent and acts by inhibiting the enzyme glutamine synthase. D-glufosinate does not exhibit herbicidal activity and is not metabolised by plants (Ruhland et al. 2002).

65. The PAT enzyme, encoded by the *bar* gene, inactivates the L-isomer of glufosinate by acetylating it to N-acetyl- L- glufosinate (NAG), which does not inhibit glutamine synthase (Dröge-Laser et al. 1994; OECD 2002). This metabolite is not found in non-GM plants.

66. The metabolism of glufosinate in tolerant GM plants and in non-GM (non-tolerant) plants has been reviewed (FAO/WHO 1998; OECD 2002). In non-GM plants the metabolism of glufosinate is low to non-existent because of plant death due to the herbicidal activity. However, some metabolism does occur (Müller et al. 2001) and is different from that in GM plants expressing the PAT protein (Dröge et al. 1992).

67. Two pathways for the metabolism of glufosinate in non-GM plants have been identified. The first step, common to both pathways, is the rapid deamination of L-phosphinothricin to the unstable intermediate 4-methylphosphonico-2-oxo-butanoic acid, which is then metabolised to either:

- 3-methyl-phosphinico-propionic acid (MPP, sometimes referred to as 3-hydroxy-methyl phosphinoyl-propionic acid) which may be further converted to 2-methyl-phosphinico-acetic acid (MPA); or
- 4-methylphosphonico-2-hydroxy-butanoic acid (MHB), which may be further converted to 4-methylphosphonico-butanoic acid (MPB), a final and stable product (Dröge-Laser et al. 1994; Ruhland et al. 2002; Ruhland et al. 2004).

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

68. The metabolism of glufosinate has been investigated in GM herbicide-tolerant canola, maize, tomato, soybean and sugar beet (FAO/WHO 1998; OECD 2002). The major residue present in the GM crops after glufosinate herbicide application was N-acetyl-glufosinate (NAG), with lower concentrations of glufosinate and MPP. Studies using cell cultures of GM canola gave similar results, with NAG being the major metabolite (Ruhland et al. 2002).

69. Both NAG and MPP are less toxic than glufosinate, which itself has low toxicity (EFSA 2005; OECD 1999b; OECD 2002).

5.3 The regulatory sequences

70. In addition to the introduced genes, the GM cottons contain short regulatory elements which control expression of the genes (Table 2). These sequences are derived from common plants (*Arabidopsis thaliana*, *Petunia hybrida*, *Flavaria bidentis*, *Helianthus annuus* and *Oryza sativa*) and plant viruses: subterranean clover stunt virus (SCSV), cassava vein mosaic virus (CsVMV) and cauliflower mosaic virus (CaMV).

71. 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 GlyTol TwinLink Plus[®] and GlyTol[®] cottons can be found in the RARMPs for DIR 133 and DIR 124. 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 GM cottons have been previously assessed by Australian and international regulators without identifying an increase in risk compared to endogenous regulatory elements of cotton.

5.4 Method of genetic modification

72. GlyTol[®] cotton and the other parental GM cotton lines were developed using *Agrobacterium*-mediated plant transformation. This method 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. The lines were then conventionally crossed to produce GlyTol TwinLink Plus[®] cotton.

5.5 Characterisation of the GMOs

5.5.1 Molecular stability

73. Southern blot, PCR and sequencing analyses were used to determine the copy numbers and the flanking sequences surrounding the insertion locus in each GM parental cotton line. It was confirmed that the GHB614, GHB119 and COT102 cottons each contain one intact copy of the intended introduced DNA (Artim 2002; Habex 2008a; Habex 2008b; Habex 2011; Habex & Leclair 2014; Moens 2008; Verhaeghe & Habex 2008). However, as a result of rearrangement, the T304-40 cotton contains two partial copies of the *cry1Ab* gene cassette in a tail-to-tail orientation, with one nearly intact copy (3' me1 terminators is truncated) and one adjacent partial copy containing a truncated Ps7s7 promoter (Moens & Criel 2008; Moens & De Pestel 2008). It was also confirmed that no vector backbone sequences (i.e. beyond the T-DNA borders) are present in these GM parental cotton lines. In all parental GM cotton lines, the insertions were found to be stably located within the genome over multiple generations.

74. Southern blot and PCR analyses were used to demonstrate the molecular equivalence of the GHB614, T304-40, GHB119 and COT102 events in GlyTol TwinLink Plus[®] cotton to the same events in the individual parental lines. This confirms the intactness of the GM loci and their flanking regions in GlyTol TwinLink Plus[®] cotton, indicating that no rearrangement occurred during conventional breeding (Peeters 2014).

5.5.2 Expression of the introduced proteins

75. The applicant measured protein expression levels in GM cottons from field trials in 2013 in the USA (Chapman & Wu 2014). The levels of 2mEPSPS, PAT, Cry1Ab, Cry2Ab and Vip3A proteins in leaf, bolls, pollen, fuzzy seed (ginned seed) and whole plant tissues from GlyTol[®] cotton, and GlyTol TwinLink Plus[®] cotton and its parental GM cottons, were determined by enzyme-linked immunosorbent assays (ELISA).

76. Table 3 shows the means and ranges of expression levels of introduced 2mEPSPS protein in these tissues in GlyTol[®] cotton. It also shows that the ranges of expression levels of 2mEPSPS, Cry1Ab, Cry2Ab and Vip3A proteins in these tissues in GlyTol TwinLink Plus[®] cotton are comparable to the expression levels in the parental cotton lines. However, the expression of the PAT protein is higher in GlyTol TwinLink Plus[®] cotton than the parental cottons T304-40 and GHB119, reflecting that GlyTol TwinLink Plus[®] cotton contains two copies of the *bar* gene from the T304-40 and GHB119 parents. Expression levels of the introduced proteins in pollen are substantially lower than in other tissues.

Table 3 Expression levels of introduced proteins in the GM cottons grown in the USA during 2013

Protein	Cotton line	Leaf Mean (SD) Range (µg/g FW) ¹	Boll Mean (SD) Range (µg/g FW)	Pollen Mean (SD) Range (µg/g FW)	Fuzzy seed Mean (SD) Range (µg/g FW)	Whole plant Mean (SD) Range (µg/g FW)
2mEPSPS	GHB614 ²	90.11 (15.49) 69.04 – 122.74	24.62 (4.43) 16.16 – 32.07	6.34 (4.74) 1.72 – 16.38	123.01 (12.59) 98.37 – 142.28	62.13 (41.26) 35.43 – 189.05
	GLTC ³	80.89 (22.74) 48.50 – 110.11	21.51 (2.56) 18.01 – 25.76	5.96 (3.63) 1.38 – 12.70	106.78 (7.94) 95.09 – 119.67	68.31 (27.98) 40.57 – 121.70
PAT	T304-40	71.25 (13.43) 54.32 – 93.89	37.39 (8.79) 27.19 – 56.58	0.43 (0.57) 0.02 – 1.88	89.75 (9.64) 71.56 – 101.86	80.52 (27.13) 38.32 – 145.92
	GHB119	41.35 (12.71) 25.09 – 61.32	24.53 (4.04) 18.31 – 32.47	0.87 (0.57) 0.16 – 1.74	76.18 (9.93) 57.41 – 92.25	41.27 (3.79) 35.46 – 47.71
	GLTC	141.21 (63.01) 84.57 – 261.95	71.32 (12.06) 52.83 – 87.82	0.70 (0.52) 0.17 – 1.66	224.65 (22.11) 184.20 – 252.71	134.86 (28.10) 93.09 – 191.80
Cry1Ab	T304-40	2.84 (0.73) 2.02 – 3.88	1.51 (0.51) 0.94 – 2.51	0.22 (0.14) 0.07 – 0.51	4.93 (1.06) 3.92 – 7.09	2.51 (1.11) 1.59 – 5.51
	GLTC	2.93 (0.79) 1.77 – 3.87	0.95 (0.24) 0.56 – 1.27	0.13 (0.09) 0.04 – 0.29	3.41 (0.59) 2.56 – 4.33	1.51 (0.93) 0.19 – 3.10
Cry2Ae	GHB119	28.75 (4.19) 21.96 – 37.62	3.02 (0.82) 1.84 – 4.48	0.18 (0.26) 0.04 – 1.00	16.30 (4.13) 9.66 – 21.34	25.99 (8.91) 12.23 – 41.39
	GLTC	28.39 (5.24) 20.60 – 37.63	3.06 (0.94) 1.72 – 5.46	0.13 (0.06) 0.05 – 0.29	18.55 (2.17) 15.56 – 21.81	22.27 (7.12) 13.72 – 39.42
Vip3A	COT 102	44.06 (13.20) 29.05 – 72.27	13.22 (4.86) 6.55 – 21.88	0.56 (0.22) 0.18 – 1.11	8.97 (3.12) 6.04 – 14.88	27.12 (6.65) 14.33 – 35.76
	GLTC	37.11 (11.63) 25.59 – 57.62	10.63 (2.91) 7.08 – 15.53	0.42 (0.09) 0.29 – 0.57	5.79 (1.01) 4.34 – 7.45	17.78 (6.70) 9.82 – 34.10

¹Protein levels are expressed as the arithmetic mean, standard deviation (SD) and range (minimum and maximum value) in microgram (µg) of protein per gram (g) of tissue on a fresh weight (FW) basis, calculated for each tissue across three field trials and four plot replicates per trial (n=12); ²GHB614 = GlyTol[®]; ³GLTC = GlyTol TwinLink Plus[®]

5.5.3 Phenotypic characterisation and environmental interaction

Compositional analysis

77. Compositional analysis of seed from GlyTol[®] cotton grown in field trials in the USA during 2005 has been previously considered by FSANZ. The components analysed were proximates, fatty acids, amino acids, vitamins, minerals, gossypol, phytic acid and cyclopropenoid fatty acids. The GM seed was assessed to be compositionally equivalent to non-GM cotton (FSANZ 2008). A separate compositional study using GHB614 seed from eight field trials sites in Spain in 2007 analysed proximates, fibre compounds, minerals, total tocopherols (vitamin E compounds), anti-nutrients, total amino acids and total fatty acids (Oberdörfer 2010). The results supported FSANZ's conclusion.

78. The applicant has provided compositional data for seed from GlyTol TwinLink Plus[®] cotton (Chapman 2014b). Cottonseed analysed was grown at eight trial sites across cotton growing regions in the USA during the 2013 growing season. Compositional analyses were conducted on ginned (fuzzy) seed collected from GlyTol TwinLink Plus[®] cotton, its non-transformed parental line FiberMax 966 (FM966) (as non-GM control, named Sicala 40 in Australia) and six commercial non-GM varieties (reference varieties).

79. Analyses of the cottonseed samples were conducted for nutrients including proximates (ash, carbohydrates, moisture, protein and fat), fibre (acid detergent fibre ADF and neutral detergent fibre NDF), amino acids, fatty acids (C8-C24), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), tocopherols, and anti-nutrients (free and total gossypol, dihydrosterculic acid, malvalic acid and sterculic acid). In all, 71 different analytical components were measured. Of these, nineteen (eighteen fatty acids plus sodium) had more than one-third of the values 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 (Kenward & Roger 1997).

80. Statistical comparisons to the non-GM 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 varieties, grown concurrently in the same trial as test substances and the non-GM control FM966, were combined across all sites and used to calculate a 99% tolerance interval for each component to estimate the natural variability in cotton varieties with a history of safe consumption. If the statistical analysis of a component resulted in a significant difference, the mean was then compared to the range of the commercial reference varieties, the 99% commercial varieties tolerance interval and a cited literature range obtained from publicly available reports and the [ILSI Crop Composition Database \(ILSI-CCDB\)](#) (Chapman 2014b)

81. The combined-site analysis did not detect statistically significant differences between GlyTol TwinLink Plus[®] cotton and FM966 cotton in moisture, protein, ash, ADF, NDF, alpha or total tocopherols, calcium, iron, magnesium, manganese, phosphorus, zinc, all eighteen amino acids tested and five fatty acids. Statistically significant differences were noted for fat, carbohydrates, copper, potassium, gamma tocopherol and six fatty acids. However, the observed values fell within the range of the commercial reference varieties, the established 99% tolerance intervals and/or within the cited literature ranges. Therefore, no biological relevance to the statistical differences of these components was identified.

82. For the anti-nutrients, the combined-site analysis showed statistically significant differences in free and total gossypol, dihydrosterculic acid, malvalic acid and sterculic acid. However, the observed values still fell within the range of the commercial reference varieties, the 99% tolerance intervals and the cited literature ranges for cotton anti-nutrients. Therefore, it is unlikely that there is biological relevance associated with the statistical differences.

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

Phenotypic and agronomic characterisation

84. GlyTol[®] cotton has been granted nonregulated status in the USA (APHIS 2009). In its petition document submitted to APHIS-USDA, Bayer provided substantive assessment data for agronomic performance of GlyTol[®] cotton (Scott 2006). The data were obtained from field trials carried out at 17 locations in 5 states in the USA over the 2004 and 2005 growing seasons to compare agronomic performance of GlyTol[®] cotton with its parent variety Coker 312. Evaluations were made on 30 agronomic parameters (such as seed weight, seed dormancy, plant growth, height to node ratio, days to bloom, fertility ratings, lodging, susceptibility to diseases, yield) to assess the growth habit and phenotype, and lint quantification and quality measurements. These data showed that there are no agronomically meaningful differences between GlyTol[®] cotton and other non-GM cotton varieties. Evaluations were also made on composition of the GlyTol[®] cotton seed, including gossypol and other antinutrient levels, and

no significant difference was detected when compared to other non-GM cotton varieties. Therefore, GlyTol[®] cotton is agronomically and compositionally similar to non-GM cottons.

85. GlyTol TwinLink Plus[®] cotton has been assessed by the applicant for plant growth and development characteristics in field trials and laboratory studies in the USA and Australia to identify any unintended phenotypic effects relative to non-GM cotton. These include field trials conducted at eight sites in the cotton growing regions of the USA in 2013 (Chapman 2014a) and four sites in Queensland and NSW in the 2013/14 and 2014/15 growing seasons (Addison 2014a; Addison 2014b; Addison 2015a; Addison 2015b; Eulenstein 2014a; Eulenstein 2014b; Eulenstein 2015a; Eulenstein 2015b).

86. In the 2013 trials in the USA, the agronomic performance of GlyTol TwinLink Plus[®] cotton (with or without glyphosate and glufosinate ammonium treatment) was compared with its non-transformed parental line FM966 and six non-GM reference varieties. Observations for the comparative analysis included days to emergence, early stand count, days to first flower, days to first open bolls, disease incidence, plant lodging, boll type, percent of open bolls, lint yield, total harvest weight, fibre properties, number of seeds per boll, boll size, seed index, percent lint, and end of season plant mapping (plant height, number of nodes, first fruiting branch, bolls per plant, and height to node ratio, and fruit retention at 1st and 2nd positions).

87. In a combined-site analysis, statistically significant differences were detected in the number of nodes per plant, height to node ratio, five boll properties (weight of 25 bolls, average boll weight, lint weight for 25 bolls, seed weight for 25 bolls, and seed index), three fibre properties (micronaire, length, and elongation), plant vigor and plant lodging between FM966 and both herbicide-treated and non-treated GlyTol TwinLink Plus[®] cotton. Significant differences ($p < 0.05$) were also detected in fibre strength between the FM966 and the non-treated GlyTol TwinLink Plus[®] cotton, and average number of bolls per plant between FM966 and the herbicide treated GlyTol TwinLink Plus[®] cotton. However, all mean values were within the range of the commercial reference varieties. This suggests that the significant differences detected are not biologically significant. No significant differences were observed for the remaining agronomic parameters.

88. In the field trials in Australia, replicated trials were carried out at four locations in 2013/14 and four locations in 2014/15 in New South Wales and Queensland to compare the agronomic performance of GlyTol TwinLink Plus[®] cotton and its non-transformed parental line FM966 (Sicala 40), as well as its near isogenic component lines including GHB614, GHB614 × T304-40, GHB614 × GHB119, GHB614 × COT102 and GHB614 × T304-40 × GHB119. Phenotypic characteristics assessed included plant stand (seedling emergence), plant height, number of nodes, height/node ratio, internode length, maturity (percentage of flowering), boll count, seed cotton yield, lint yield and gin turn-out (weight ratio of lint to seed cotton). However, not all listed parameters of the characteristics were assessed in all eight field trials. Statistical differences were identified at a 5% level of significance ($p < 0.05$) and the results of the agronomic performance assessment are summarised as follows:

- Plant emergence was assessed in all eight trials, with no significant differences recorded
- Plant height was assessed in all eight trials, with seven trials recording no significant differences
- Number of nodes was assessed in 6 trials, with 5 trials recording no significant differences
- Height to node ratio was assessed in 4 trials, with 3 trials recording no significant differences
- Internode length was assessed in 4 trials, with 2 trials recording no significant differences

- Plant maturity (% flowering) was assessed in 4 trials, with all trials recording significant differences as FM966 was less mature than GlyTol TwinLink Plus[®] cotton
- Number of bolls was assessed in four trials, with two trials recording no significant differences and other two showed that FM966 has significantly less bolls than GlyTol TwinLink Plus[®] cotton
- Seed cotton yield was assessed in 6 trials, with 4 trials recording no significant differences but the other two showed significantly less yield of FM966 than GlyTol TwinLink Plus[®] cotton
- Lint yield was assessed in all 8 trials, with 5 trials recording no significant differences
- Gin turn-out was assessed in 4 trials, with all four trials recording no significant differences

89. All eight field trials showed that GlyTol TwinLink Plus[®] cotton had less infestation with *Helicoverpa sp.* than FM966. However, the presence of *Helicoverpa sp.* during field trials at different locations and time fluctuated (see discussion in the Environmental interaction section below) and the significant differences detected in performance parameters may reflect this variation in insect pressure. Although significant differences were observed for some agronomic parameters such as internode length, plant maturity and number of bolls at some trial sites, these were most likely related to the insect damage to the non-GM control plants compared to the GM insect resistant plants. Furthermore, the majority of the tested agronomical parameters (plant emergence, plant height, number of nodes, height to node ratio, seed cotton yield, lint yield and gin turn-out) showed no significant difference between GlyTol TwinLink Plus[®] cotton and the non GM control, indicating that GlyTol TwinLink Plus[®] is phenotypically and agronomically similar to GM parental and non-GM cottons.

Environmental interaction

90. Environmental interaction refers to the interaction between the crop plants and their receiving environment, which may include plant response to abiotic stressors, disease and arthropod damage. APHIS-USDA has assessed GlyTol[®] cotton using the data supplied by Bayer (Scott 2006) for its environmental interaction characteristics in the respective environments, including interactions with symbiotic nitrogen-fixing bacteria, response to naturally occurring abiotic stresses, and susceptibility to diseases and pests. The conclusion was that GlyTol[®] cotton did not display any such characteristics differently compared to non-GM plants (APHIS 2009).

91. The parental GM cotton lines T304-40, GHB119 and COT102 have also been assessed from USA field data as no different from non-GM cotton varieties in terms of their environmental interaction such as disease ratings and response to less than optimal growth conditions (APHIS 2005; APHIS 2011).

92. In field trials conducted in Australia during the 2013/14 and 2014/15 growing seasons, the interactions between GlyTol TwinLink Plus[®] cotton and cotton bollworm (*Helicoverpa armigera*) were assessed by both laboratory bioassays and field observations across all eight trial sites in NSW and Queensland. Bioassays were conducted weekly for 6 weeks using a single neonate (one-day old) fed bollworm larva per leaf (ten plants in each plot and a total of 40 leaves per treatment). Comparison was made between GlyTol TwinLink Plus[®] cotton and its parental variety FM966, as well as its near isogenic component lines including GHB614, GHB614 × T304-40, GHB614 × GHB119, GHB614 × COT102 and GHB614 × T304-40 × GHB119. The bioassay results from samples collected from all eight trials showed that all GM lines containing Bt toxin genes have effects on larval development compared to the parental variety FM966. However, the lines containing the GHB119 event (with the *cry2Ae* gene), which includes GlyTol TwinLink Plus[®] cotton, provided significantly

better control of larval development than the lines containing only the T304-40 event or COT102 event, with the line GHB614 × T304-40 displayed the least effect. No significant difference was detected between GlyTol TwinLink Plus[®] cotton and other lines containing the GHB119 event (e.g. GHB614 × GHB119 and GHB614 × T304-40 × GHB119).

93. The field observations reflected results obtained from the bioassays, which showed that the GM lines containing different Bt toxin genes displayed varied levels of effects on larval development. Due to fluctuation of the presence of *Helicoverpa sp.* during field trials at different locations and time, the field observations varied. In the field trials where there was a lack of natural infestations of *Helicoverpa* (low *Helicoverpa sp.* pressure), there were no significant differences ($P < 0.05$) in plant part damage (square, flower and boll) by *Helicoverpa* larvae between the Bt lines and the control parental variety, although the control parental variety and GHB614 cotton generally showed a higher level of damage than the Bt lines (Addison 2014a; Addison 2015a; Eulenstein 2014a; Eulenstein 2014b; Eulenstein 2015a; Eulenstein 2015b). However, in the field trials with sufficient *Helicoverpa sp.* pressure, significant difference in plant part damage between the Bt lines and the control parental variety or GHB614 cotton was detected (Addison 2014b; Addison 2015b). Among the Bt lines, GlyTol TwinLink Plus[®] cotton and other lines containing the GHB119 event received much less damage, followed by GHB614 × COT102 and then GHB614 × T304-40. This is consistent with the results from the bioassays.

5.5.4 Effect on non-target invertebrates

94. A number of overseas regulatory agencies have assessed whether the parental GM cotton lines have any increased toxicity to non-target organisms as a result of the genetic modifications. In its assessments of GHB614, COT102 and TwinLink[™] (GHB119 × T304-40) cottons, the USDA-APHIS determined that the GM cottons would not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture due to the lack of known toxicity of the introduced proteins (APHIS 2005; APHIS 2009; APHIS 2011). In addition, the COT102 X COT67B cotton (trademark name of VipCot), which contains the same *vip3Aa19* and *cry1Ab* genes, was assessed as safe for non-target organisms and registered as pesticide product in the USA (US EPA 2008b). Similarly, in assessing these GM cottons for feed use, CFIA also determined that use of these GM cottons will not result in altered impacts on interacting organisms (CFIA 2008; CFIA 2011a; CFIA 2011b). Data provided on oral toxicity of the individual proteins to representative invertebrates showed no adverse effects (Section 5.2.4).

95. In addition, as described in section 5.5.3, there are no demonstrated synergistic or antagonistic interactions among Cry1Ab, Cry2Ae and Vip3A proteins in GlyTol TwinLink Plus[®] cotton leading to a significantly changed level of effect on target *Helicoverpa sp.* Therefore, an effect on non-target organisms resulting from a combination of the three proteins is unlikely. To confirm this, the applicant conducted tests on honeybees (*Apis mellifera* L.) and springtails (collembola) using tissue from GlyTol TwinLink Plus[®] cotton plants.

96. In the honeybee study (Patnaude 2014), the toxicity of GlyTol TwinLink Plus[®] cotton pollen to honey bee larval survival was evaluated in an artificial *in vitro* testing system over 21 days. The study used a nominal concentration of 46mg pollen/g diet for both GlyTol TwinLink Plus[®] cotton and the non-GM cotton variety FM966, as well as an untreated control diet and a reference diet containing dimethoate as the toxicant, to feed the second instar larvae. At test termination, 74% mortality was observed among the larvae exposed to the dimethoate reference toxicant, while 31%, 33% and 33% mortality was observed for the larvae fed on the control diet and the diet containing pollen from GlyTol TwinLink Plus[®] and FM966 cottons, respectively. Therefore, no statistically significant difference ($p < 0.05$) on larval mortality was detected between GlyTol TwinLink Plus[®] cotton and FM966.

97. In the study on springtails, the effect of lyophilised leaf material from GlyTol TwinLink Plus[®] cotton on survival and reproduction of *Folsomia candida* was assessed during an exposure of 28 days (Frommholz 2014). This study compared mortality and reproduction of adult *F. candida* feeding on diets containing 5%, 20% and 50% lyophilized leaf material from GlyTol TwinLink Plus[®] cotton, control diet containing 50% lyophilized leaf material from FM966 and untreated control diet. No significant difference ($P < 0.05$) was detected between the treatment diet and the control diets on both survival and reproduction of *F. candida*.

98. These confirmatory studies demonstrated that stacking the events via conventional breeding did not lead to adverse effects on beneficial non-target invertebrates such as bees.

Section 6 The receiving environment

99. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. Relevant information about the receiving environment includes abiotic and biotic interactions of the crop with the environment where the release would occur; agronomic practices for the crop; presence of plants that are sexually compatible with the GMO; and background presence of the gene(s) used in the genetic modification (OGTR 2013).

100. The applicant has proposed to release GlyTol[®] cotton and GlyTol TwinLink Plus[®] cotton in all commercial cotton growing areas, Australia-wide. Therefore, for this licence application, it is considered that the receiving environment is all of Australia but in particular agricultural areas that are suitable to cultivate cotton. 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. The actual locations, number of sites and area of land used in the proposed release would depend on factors such as field conditions, grower demand and seed availability.

6.1 Relevant agronomic practices

101. It is anticipated that the agronomic practices for the cultivation of the GM cottons will not differ significantly from industry best practices used in Australia. All cotton plants would be grown following standard cotton agricultural management practices and would receive applications of water, fertilisers, and herbicides similar to commercially grown non-GM and current GM cotton crops. Cultivation practices for cotton are discussed in more detail in *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)* (OGTR 2016b).

102. Glyphosate and/or glufosinate may be applied over the top of the GM cotton crop to control weeds, in the same manner that herbicides are applied over other herbicide tolerant cotton or canola varieties grown in Australia. Herbicides would be applied according to label directions approved by the APVMA. The APVMA assesses all herbicides used in Australia and sets their conditions of use. It should be noted that the Regulator will not consider issues relating to efficacy of the herbicide or resistance management as these issues most appropriately fall under the *Agricultural and Veterinary Chemicals Code Act 1994*, and as such are the responsibility of the APVMA.

103. The applicant has developed a Crop Management Plans (CMP) for both GlyTol[®] cotton and GlyTol TwinLink Plus[®] cotton, and a Resistance Management Plan (RMP) for GlyTol TwinLink Plus[®] cotton. The CMP is designed for weed resistance management to aid in minimising the risk of the evolution of glyphosate and glufosinate-ammonium resistant weeds in the Australian cotton production system. The RMP is designed for insect resistance management, which provides measures to minimise the exposure of *Helicoverpa sp.* to the introduced Bt proteins in GlyTol TwinLink Plus[®] cotton, and dilute and remove resistant *Helicoverpa sp.* Growers of the GM cottons would be required, under a Technology User Agreement and conditions of Registration under the *Agricultural and Veterinary Chemicals*

Act 1994, to practice preventative herbicide and insect resistance management as set out in the CMP and RMP.

6.2 Relevant abiotic factors

104. The abiotic factors relevant to the growth and distribution of commercial cotton in Australia are discussed in *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)* (OGTR 2016b). To summarise, factors restricting where cotton can be grown in Australia are water availability (through rainfall or irrigation), soil suitability and, most importantly, temperature. Cotton seedlings may be killed by frost, growth and development of cotton plants below 12°C is minimal, and a long, hot growing season is crucial for achieving good yields.

6.3 Relevant biotic factors

6.3.1 Presence of related plants in the receiving environment

105. Cotton is largely self-pollinating and no self-incompatibility mechanisms exist. Where cross-pollination does occur it is likely facilitated by honeybees.

106. Commercial cotton grown in Australia is either *Gossypium hirsutum* or *Gossypium barbadense*, with 99% of cotton planted in 2006 being *G. hirsutum* (OGTR 2016b). The GM *G. hirsutum* proposed for release is capable of crossing with both species of commercially grown cotton. In the 2013-14 growing season more than 99% of the Australian cotton crop was genetically modified, and more than 95% of the national cotton crop contained stacked insect resistance and herbicide tolerance GM traits (Roth 2014). These cottons are the commercially approved Bollgard[®] II and Bollgard II/RoundupReady Flex[®].

107. As discussed in Section 4.3, there are 17 native species of *Gossypium* in Australia. Only three of these species are likely to occur in the regions of Australia where cotton is cultivated: *G. sturtianum*, *G. nandewarensense*, and *G. australe*. However, native *Gossypium* species prefer well-drained sandy loams and are rarely found on heavy clay soils favoured by cultivated cotton.

108. 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 2016b).

6.3.2 Presence of other biotic factors

109. Lepidopteran pests are the major cotton insect pests. In Australia, the most damaging lepidopteran pests are cotton bollworm (*Helicoverpa armigera*) and native budworm (*H. punctigera*). In addition, beet armyworm (*Spodoptera exigua*), cluster caterpillar (*Spodoptera litura*) and pink bollworm (*Pectinophora gossypiella*) can also affect cotton production (OGTR 2016b). These lepidopteran pests are now managed through the widespread adoption of GM cotton varieties with Bt toxin genes that specifically target these insect pests.

110. In many cotton growing areas across Australia, there are some major non-lepidopteran pests. These include: spider mites – two-spotted spider mite (*Tetranychus uticae*), bean spider mite (*T. ludeni*) and strawberry spider mite (*T. lambi*); mirids – green mirid (*Creontiades dilutus*) and brown mirid (*C. pacificus*); cotton aphids (*Aphis gossypii*); and whiteflies (*Bemisia tabaci*). There are also other minor pests, such as, tobacco thrips (*Thrips tabaci*), tomato thrips (*Frankliniella schultzei* Trybom), green vegetable bug (*Nezara viridula*), pale cotton stainer bug (*Dysdercus sidae*) and cotton harlequin bug (*Tectocoris diophthalmus*) (Queensland Department of Agriculture and Fisheries) (CottonInfo 2015).

111. Australian cotton is affected by a number of bacterial, and soil-borne and foliar fungal diseases, with viral diseases a less problem (OGTR 2016b). The main bacterial disease is the bacterial blight caused by *Xanthomonas campestris* pv. *Malvacearum*. Two soil-borne wilt pathogens *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) and *Verticillium dahlia* are the main fungal diseases of cotton. Fusarium wilt is now considered as the most important constraint to sustainable cotton production in Australia. In addition, several other fungi, including *Pythium* and *Rhizoctonia* can cause cotton diseases at seedling stage (Queensland Department of Agriculture and Fisheries).

112. Cotton is susceptible to competition from weeds. The problematic weeds range from large plants such as Noogoora burr (*Xanthium occidentale*), Bathurst burr (*X. spinosum*) and thornapples (*Datura* spp.), plants with vine such as cow vine (*Ipomoea lonchophylla*) and yellow vine or spine-less caltrop (*Tribulus micrococcus*) to grass such as nut grass (*Cyperus rotundus*) (Charles 2002) .

6.4 Presence of the introduced or similar genes and proteins in the receiving environment

113. The introduced genes and regulatory sequences were originally isolated from naturally occurring organisms that are already widespread and prevalent in the environment.

114. The *2mepsps* gene was isolated from *Zea mays* (maize), which is widely grown as a food crop in Australia. The DNA sequence of the maize EPSPS-encoding gene was modified to result in the *2mepsps* gene. However, the encoded protein has only two amino acids that differ from the native maize protein.

115. The *bar* gene was isolated from *Streptomyces hygroscopicus*, which is a natural soil bacterium in Australia. The *bar* gene is also present in Liberty Link[®] GM cotton, which has been commercially planted in Australia since 2008.

116. The *cry1Ab*, *cry2Ae* and *vip3Aa19* genes were isolated from the bacterium *Bacillus thuringiensis*. Bt is a natural soil bacterium in Australia. Also, microbial preparations of Bt are used as insecticide sprays in Australia, particularly in organic agriculture and domestic gardening (Australian Pesticides and Veterinary Medicine Authority). Therefore, these genes and their encoded proteins are widespread in the Australian environment.

117. The *aph4* gene was isolated from the common bacterium *E. coli*, which is part of the normal flora of human and animal guts. Therefore, these genes and their encoded proteins are widespread in the Australian environment.

Section 7 Previous releases

7.1 Australian approvals

7.1.1 GMOs proposed for release

118. GlyTol[®] cotton (GHB614) and GlyTol TwinLink Plus[®] cotton have been approved by the Regulator for limited and controlled release under licence DIR 113 and DIR 133 and have been grown in field trials since 2012.

7.1.2 Parental GM cotton lines

119. All parental GM cotton lines have been previously approved by the Regulator for release in Australia. The relevant authorisations are shown in Table 4.

Table 4 Previous releases of the parental GM cotton lines in Australia

Event	Field trial licence	Commercial licence
T304-40	DIR 087, DIR 113	
GHB119	DIR 087, DIR 113	
COT102	DIR 017/2002, DIR 025/2002, DIR 034/2003, DIR 036/2003, DIR 058/2005, DIR 065/2006, DIR 073/2007, DIR 101, DIR 113, DIR 120, DIR 133	DIR 124

7.1.3 Other relevant GM cottons

120. The Regulator has issued licences for the commercial release of other herbicide tolerant and/or insect resistant cottons (Table 5).

Table 5 Other relevant GM cottons in Australia

GM cotton	Field trial licence	Commercial licence	Comment
Liberty Link®	DIR 038/2003	DIR 062/2005	Containing the <i>bar</i> gene for glufosinate ammonium tolerance
Widestrike™	DIR 044/2003, DIR 040/2003	DIR 091	Containing the Bt <i>cry1F</i> gene and <i>cry1Ac</i> gene for insect resistance
Bollgard® II (MON15985)		DIR012/2002	Containing the <i>cry1Ac</i> and <i>cry2Ab</i> genes for insect resistance
Bollgard II/Roundup Ready Flex®		DIR012/2002	Containing the <i>cry1Ac</i> and <i>cry2Ab</i> genes for insect resistance and <i>cp4 epsps</i> gene for glyphosate tolerance
Bollgard® III	DIR 101	DIR 124	Containing the <i>cry1Ac</i> , <i>cry2Ab</i> and <i>vip3Aa19</i> genes for insect resistance
Bollgard III/Roundup Ready Flex®	DIR 101	DIR 124	Containing the <i>cry1Ac</i> , <i>cry2Ab</i> and <i>vip3Aa19</i> genes for insect resistance and <i>cp4 epsps</i> gene for glyphosate tolerance

121. Information on these licences is available from the [GMO Record](#) on the OGTR website. Bollgard® II and BollgardII/RoundupReady Flex® cottons constitute over 95% of the Australian commercial cotton crop and there have been no reports of adverse effects on human health or the environment resulting from any of these releases.

7.2 Approvals by other Australian agencies

122. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved the use of food derived from GlyTol® (GHB614) cotton and all other three parental GM cotton lines of GlyTol TwinLink Plus® cotton. Assessments of GHB614 (application A614), GHB119 (application A1040), T304-40 (application A1028) and COT102 (application A509) are available from the FSANZ website. These approvals include food made from any offspring produced through conventional breeding, and therefore no further approvals are required for the GlyTol TwinLink Plus® cotton.

123. APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. GlyTol TwinLink Plus® 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. The applicant is required to complete APVMA registration of this GM cotton as an insecticidal product prior to commercialisation and will

need to comply with an approved insect resistance management plan and any other relevant conditions that may be imposed.

124. It is intended that glyphosate herbicide be applied to GlyTol[®] cotton, and both glyphosate and glufosinate herbicides be applied to GlyTol TwinLink Plus[®] cotton. This would also be subject to regulation by the APVMA. Therefore, the applicant is also required to obtain full APVMA registrations for the use of these herbicides on the GM cottons prior to commercialisation.

7.3 International approvals

125. GlyTol[®] cotton has been approved for commercial cultivation in the USA in 2009 and Brazil in 2012. GlyTol TwinLink Plus[®] cotton has been approved for commercial cultivation in the USA in 2015.

126. Import permissions have been granted for GlyTol[®] cotton and GlyTol TwinLink Plus[®] cotton in Japan, Korea and Mexico. Import permissions have also been granted for GlyTol[®] cotton in Canada and China.

Chapter 2 Risk assessment

Section 1 Introduction

127. 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.

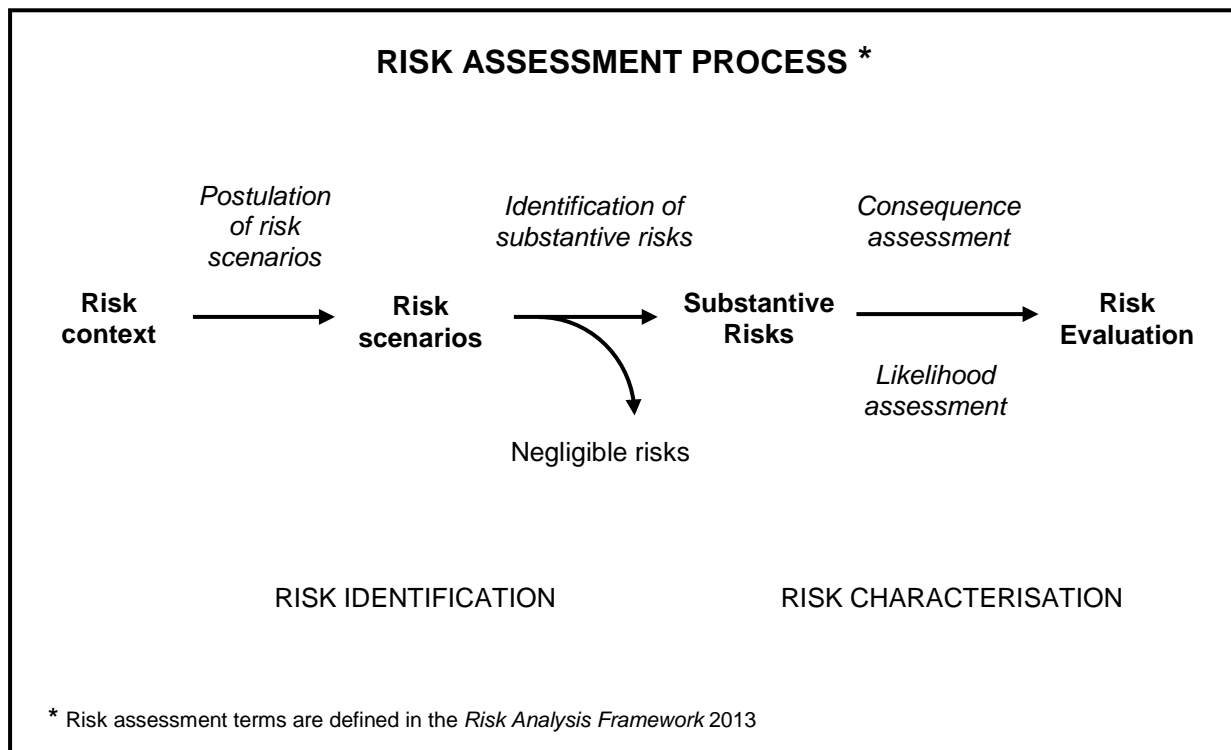


Figure 2. The risk assessment process

128. 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.

129. 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.

130. 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 2013) 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.

131. 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

132. 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).

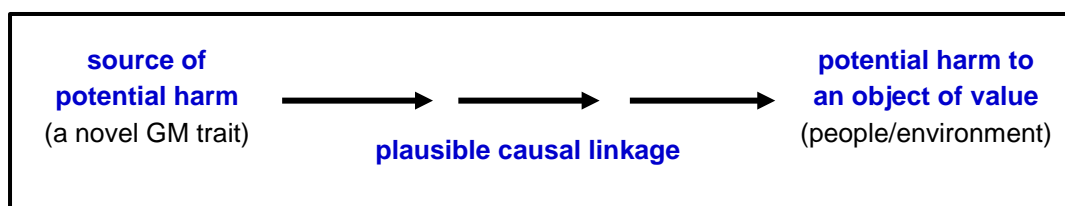


Figure 3. Risk scenario

133. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors:

- 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
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMO
- characteristics of the parent organism(s).

2.1 Risk source

134. 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.

135. As discussed in Chapter 1, GlyTol[®] cotton has been modified by the introduction of a glyphosate herbicide tolerance gene, and the GlyTol TwinLink Plus[®] cotton has been modified by the introduction of three insect resistance genes and two genes for tolerance to glyphosate and glufosinate herbicides, respectively. GlyTol[®] cotton is a parent of GlyTol TwinLink Plus[®] cotton, so will not be assessed individually.

136. GlyTol TwinLink Plus[®] cotton also contains the *aph4* antibiotic resistance selectable marker gene (also known as *hph* or *hpt*, see Chapter 1, 5.2.3). This gene and its product 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 by other regulatory agencies

in Australia and overseas. Further information about this gene can be found in the document *Marker genes in GM plants* available from the [Risk Assessment References page](#) on the OGTR website. As this gene has not been found to pose a substantive risk to either people or the environment, its potential effects will not be further considered for this application.

137. The introduced genes are controlled by introduced regulatory sequences. These regulatory sequences are derived from plants, bacteria and plant viruses (see Table 2). Regulatory sequences are naturally present in plants, and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein, and dietary DNA has no toxicity (Society of Toxicology 2003). As described in Chapter 1, these sequences have been widely used in other GMOs, including the parental GM lines that are grown commercially overseas, without reports of adverse effects. Hence, risks from these regulatory sequences will not be further assessed for this application.

138. The genetic modifications have the potential to cause unintended effects in several ways including altered expression of endogenous 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. However, the range of unintended effects produced by genetic modification is not likely to be greater than that from accepted traditional breeding techniques. These types of effects also occur spontaneously and in plants generated by conventional breeding (Bradford et al. 2005; Ladics et al. 2015; Schnell et al. 2015). In general, the crossing of plants, each of which will possess a range of innate traits, does not lead to the generation of progeny that have health or environmental effects significantly different from the parents (Steiner et al. 2013; Weber et al. 2012). Therefore, unintended effects resulting from the process of genetic modification will not be considered further.

2.2 Causal pathway

139. 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 (e.g. reproductive characteristics, dispersal pathways and establishment potential)
- tolerance to abiotic conditions (e.g. climate, soil and rainfall patterns)
- tolerance to biotic stressors (e.g. pest, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organism
- gene transfer by horizontal gene transfer (HGT)
- unauthorised activities.

140. 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 (see sections 2.2.1 to 2.2.5 below).

2.2.1 Tolerance to abiotic factors

141. 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 (OGTR 2016b). 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. As discussed in Chapter 1 (Section 5), there was no significant difference between the GM cottons and non-GM cotton varieties in their response to a number of abiotic factors. Therefore, tolerance to abiotic stresses will not be assessed further.

2.2.2 Weed management measures

142. Extensive practices (including use of herbicides) are used in agriculture to control cotton volunteer plants (see Chapter 1, Section 4.1.3). As discussed there, glyphosate and glufosinate herbicides are not generally used to control adult cotton plants. Therefore, weed management and volunteer control measures for GlyTol TwinLink Plus[®] would not be expected to differ markedly from standard management practices.

143. Some feral cotton does occur outside of cultivation in northern Australia, including in nature reserves. However, as previously discussed in the RARMP for DIR 124, these plants are not routinely subjected to control measures such as the use of herbicide. If gene transfer from the GM cottons to feral cottons was to occur, 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.

2.2.3 Gene transfer to sexually compatible relatives

144. 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 2016b). In summary, cotton is predominantly self-pollinating with no self-incompatibility mechanisms present. It does not reproduce by asexual mechanisms, although root cuttings can be propagated under laboratory conditions. Expression of the introduced genes is not expected to change the pollination characteristics of the GM cotton compared to non-GM cotton.

145. As discussed in Chapter 1, Section 6.3.1, *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.

146. It should be noted that GlyTol TwinLink Plus[®] cotton was generated by conventional crossing between four GM lines, so the introduced genes have inserted into different regions of the plant genome and segregate independently of one another. Therefore, after any initial outcrossing of GlyTol TwinLink Plus[®] cotton to other cotton, subsequent generations of cotton volunteer plants may contain either all genes from GlyTol TwinLink Plus[®] cotton, genes from one of the GM parental cottons, or genes from combinations of some of the parental lines of GlyTol TwinLink Plus[®]. The resulting cottons will have equivalent or less insecticidal efficacy and or herbicide tolerance than a GM cotton volunteer plant with all genes, so there are no additional risks from segregation and the assessment for weediness as a

result of gene transfer of the introduced genes to other cottons is not affected. Therefore, segregation of the inserted genes will not be considered further.

2.2.4 Gene transfer by HGT

147. 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 the RARMP for DIR 108. 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. Unauthorised activities

148. The potential for unauthorised activities to lead to harm 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

149. 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 (e.g. providing food or shelter for pests or pathogens) or abiotic environment (e.g. negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table).

150. These harms are based on those used to assess risk from weeds (Keese et al. 2014; Standards Australia Ltd et al. 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

151. 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).

152. 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).

153. 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 2mEPSPS and PAT proteins and associated metabolites

154. The introduced herbicide tolerance genes encode the 2mEPSPS and PAT proteins, which have been rigorously assessed for toxicity and allergenicity in humans and for toxicity in a range of other organisms. As discussed in Chapter 1 Sections 5.2.4, an extensive body of experimental work has produced no evidence that the 2mEPSPS or PAT proteins are toxic or allergenic to people or toxic to other organisms. In Australia, the applicant has received approval from FSANZ for food derived from GHB614, GHB119 and T304-40 cottons expressing these proteins. The assessments by FSANZ note that there is no evidence of toxic and allergenic properties associated with these proteins.

155. In addition, no new herbicide metabolic products have been identified in GM plants expressing 2mEPSPS. While new metabolic products are produced in GM plants expressing the PAT protein, they are less toxic than glufosinate itself, which has low toxicity (Chapter 1, Section 5.2.5). A GM canola (InVigor[®] × TruFlex[™] Roundup Ready[®]) containing the same *bar* gene for glufosinate tolerance and a *cp4 epsps* gene from a soil bacterium for glyphosate tolerance has previously been assessed and approved by the Regulator for commercial cultivation in Australia under DIR 138 (OGTR 2016a).

156. In the event of hybrids being produced between Roundup Ready Flex[®] cotton (containing the *cp4 epsps* gene) and GlyTol TwinLink Plus[®] cotton (refer to Risk scenario 8), the stack of three herbicide tolerance genes *2mepsps*, *cp4 epsps* and *bar* is not expected to have increased toxicity.

157. Therefore, on the basis of the substantial knowledge base relating to the 2mEPSPS and PAT proteins, the toxicity and allergenicity of the 2mEPSPS and PAT proteins will not be considered further.

Endogenous cotton toxins

158. 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).

159. 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 (Kandyliis 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).

160. 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 5.5.3) showing that gossypol levels in seed from the GM cottons lie within the recorded range of non-GM 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

161. Eight risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 7 and further discussion of each scenario 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.

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

Table 6 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	Commercial cultivation of GM cottons expressing these introduced genes ↓ Exposure of people or other organisms through contact or ingestion of the GM plants or products	Increased toxicity or allergenicity for people or increased toxicity for other desirable organisms	No	<ul style="list-style-type: none"> There is limited exposure of humans to the expressed proteins The Cry1Ab, Cry2Ae and Vip3A proteins have no demonstrated toxicity or allergenicity to humans Consumption of cotton by livestock is limited Low toxicity of Cry 1Ab, Cry2Ae and Vip3A proteins to organisms other than certain insects The introduced genes and proteins are widespread in the environment.
2	Introduced insect resistance genes	Commercial cultivation of GM cottons expressing these introduced genes ↓ Exposure of non-target insects to GM plant material through contact or ingestion	Increased toxicity for non-target insects	No	<ul style="list-style-type: none"> There is no demonstrated toxicity of Cry1Ab, Cry2Ae and Vip3A on non-target insects There is no demonstrated increase in adverse effects on desirable insects compared to existing commercial GM cottons in Australia.
3	Introduced insect resistance genes	Dispersal of GM cottonseed to nature reserves ↓ Establishment of GM plants in nature reserves ↓ Reduced insect herbivory of GM plants, leading to increased spread and persistence	Reduced establishment of desirable native vegetation	No	<ul style="list-style-type: none"> 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.
4	Introduced insect resistance genes	Expression of insect resistance genes in GM plants ↓ Reduced populations of target pest insects ↓ Reduced use of insecticides ↓ Increased populations of other insect pests	Reduced establishment of desirable agricultural crops	No	<ul style="list-style-type: none"> Standard agronomic practice for cotton cultivation includes practices for effective management of secondary pests.
5	Introduced herbicide tolerance genes	Commercial cultivation of GM cotton lines expressing these introduced genes ↓ Establishment of volunteer GM cotton plants in agricultural areas ↓ Reduced effectiveness of weed management measures to control the volunteer GM cotton plants	Reduced establishment or yield of desirable agricultural crops or increased reservoir for pathogens	No	<ul style="list-style-type: none"> Standard agronomic practice for cotton cultivation includes integrated weed management practices that will effectively reduce volunteer populations Glyphosate and glufosinate herbicides are of limited usefulness in controlling cotton volunteers Cotton volunteer with dual herbicide tolerance can be controlled using alternative weed management strategies.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
6	Introduced insect resistance genes	Transfer of insect resistance genes to other cultivated 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	<ul style="list-style-type: none"> Transfer of the introduced genes to other cultivated cottons by pollen flow is likely to be limited The Cry 1Ab, Cry2Ae and Vip3A proteins have no demonstrated toxicity to humans or other desirable organisms or allergenicity to humans Stacking of these genes is not expected to increase toxicity for non-target invertebrates.
7	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	<ul style="list-style-type: none"> Spatial limitations on potential for movement of the insect resistance genes into feral cotton plants by pollen flow Restrictions on establishment of cotton populations by abiotic factors outside of cultivation areas Low numbers of feral cottons and limited potential to reduce establishment of desirable vegetation.
8	Introduced herbicide tolerance genes	Transfer of herbicide tolerance genes 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 or yield of desirable agricultural crops	No	<ul style="list-style-type: none"> No new herbicide tolerance traits will be generated in hybrids 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

<i>Risk source</i>	Introduced insect resistance genes
<i>Causal pathway</i>	Commercial cultivation of GM cotton expressing these introduced genes ↓ Exposure of people or other organisms through contact or ingestion of the GM plants or products ↓
<i>Potential harm</i>	Increased toxicity or allergenicity for people or increased toxicity for other desirable organisms

Risk source

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

Causal pathway

164. The insect resistance genes *cry1Ab*, *cry2Ae* and *vip3Aa19* 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 majority of 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.

165. Expression of the insect resistance genes in cultivated GM cotton plants, or in volunteer GM cottons, may expose other organisms including livestock to the GM plant material through contact or ingestion. Livestock are exposed to cotton in the form of cottonseed meal or through limited grazing of stubble. Apart from presence in all parts of the GM cotton plants including cottonseed and leaves, the insecticidal proteins may also occur at low levels in the soil from plant material left after harvesting and exudates from roots.

166. The exposure of insects to GM plant material is addressed in Risk scenario 2. Livestock would be exposed when consuming the GM cotton as forage, whole seed or seed meal. 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. Other organisms, including other mammals, birds, 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.

167. Cotton volunteers are commonly found along roadsides neighbouring cultivation sites and some transport routes, which 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

168. 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 involved in cultivating or processing the GM cotton, or using GM cotton meal as animal feed.

169. The introduced insect resistance genes were individually isolated from the soil bacterium Bt, which is widespread and prevalent in the environment (Chapter 1, Section 6.4). Microbial preparations of Bt are also used as insecticide sprays in Australia. The potential for harm to humans, and other desirable organisms through exposure to Bt toxins has been discussed in detail in the RARMP for DIR 124. It was concluded that 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). Inhalation and ingestion of Bt is not known to cause allergic reactions (International Programme on Chemical Safety 1999).

170. As discussed in Chapter 1, Section 5.2.4, FSANZ assessed the safety of human food derived from linters and cotton seed oil from the parental GM cottons GHB119, T304-40 and VIP3A (containing Cry1Ab, Cry2Ae and Vip3A proteins, respectively) and concluded that they were safe for human consumption.

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

172. The introduced insecticidal gene products are also not expected to be toxic to other organisms, apart from certain insects (addressed in Risk Scenarios 1 and 2). It is expected that microorganisms, especially soil microorganisms, are regularly exposed to the Cry1Ab, Cry2Ae or Vip3A proteins and there is no evidence from currently available literature to suggest that the Cry1Ab, Cry2Ae or Vip3A proteins or similar proteins are toxic to microorganisms including various species of protozoa, bacteria, fungi, algae and diatoms.

Conclusion

173. Risk scenario 1 is not identified as a substantive risk, due to limited exposure of humans to the expressed Cry1Ab, Cry2Ae and Vip3A proteins, and the lack of toxicity or allergenicity of the proteins to humans. Also, these proteins showed low toxicity to organisms other than certain insects, and are widespread in the environment. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

<i>Risk source</i>	Introduced insect resistance genes
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Commercial cultivation of GM cottons expressing these introduced genes</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Exposure of non-target insects to GM plant material through contact or ingestion</p> <p style="text-align: center;">↓</p>
<i>Potential harm</i>	Increased toxicity for non-target insects

Risk source

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

Causal pathway

175. 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. Pollinators would be exposed to nectar and pollen from the GM cotton. Soil borne insects such as springtails would contact root exudates or decomposing plant material after harvest.

Potential harm

176. Exposure of non-target insects to the Cry1Ab, Cry2Ae 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.

177. GlyTol TwinLink Plus[®] cotton contains the 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, Section 5.2.4, Cry1Ab and Cry2Ae proteins have been assessed for potential toxicity to non-target invertebrates through testing of a range of representative arthropods (including bees, beetles, springtails, water fleas and green lacewings); Vip3A protein has also been subject to similar assessment (OGTR 2014). 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-lepidopterans. The three insecticidal proteins Cry1Ab, Cry2Ae and Vip3A expressed in GlyTol TwinLink Plus[®] cotton bind to different receptors and are expected to have additive effects but no synergistic effects (Chapter 1 Section 5.5.4). 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. The primary effect is toxicity to lepidopterans that feed on cotton. However, most of these organisms, including *H. armigera*, *H. punctigera*, *Spodoptera litura* and *Pectinophora*

gossypiella are considered pests of cotton that warrant control by farmers (Cotton Catchment communities CRC 2006; Strickland et al. 2003; Strickland et al. 2000). These control measures include spraying with broad spectrum insecticides.

178. The potential impact of a range of Bt crops on non-target insects has been widely examined in both laboratory and field studies. A discussion of the published literature was included in the RARMP for DIR 124 and, as summarised there, the impact ranges from no detrimental effect, to minimal adverse effects (e.g. on beneficial predator insects), to an increase in abundance of beneficial insects.

179. 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. Over 99% of cotton production in Australia is GM cottons with Bt toxin genes (Chapter 1, Section 6.3.1) and no adverse effects on non-target insects have been reported. Since the insecticidal genes contained in GlyTol TwinLink Plus[®] cotton are very similar to those present in the commercially grown GM cottons, it is not expected that GlyTol TwinLink Plus[®] cotton will have increased adverse effects on non-target insects. This has been confirmed by lab studies on honey bees and collembola (Chapter 1, Section 5.5.4).

Conclusion

180. Risk scenario 2 is not identified as a substantive risk due to the lack of toxicity of Cry1Ab, Cry2Ae and Vip3A proteins to non-target insects, and no increase in adverse effects on non-target insects compared with commercially grown insect resistant cottons, or with standard control measures applied to non-GM cottons. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

<i>Risk source</i>	Introduced insect resistance genes
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Dispersal of GM cottonseed to nature reserves</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Establishment of GM plants in nature reserves</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Reduced insect herbivory of GM plants, leading to increased spread and persistence</p> <p style="text-align: center;">↓</p>
<i>Potential harm</i>	Reduced establishment of desirable native vegetation

Risk source

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

Causal pathway

182. 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.

183. 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) and DIR 124 (Bollgard III and Bollgard III/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 2016b). Although cotton has limited ability to establish amongst existing vegetation, there is the possibility of establishment after disturbances such as flooding.

184. 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.

185. 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 4.2.4). 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).

186. Although lepidopteran pests (mainly *H. armigera* and *H. punctigera*) are the main insect pests in cultivated cotton, they are not a major limiting factor in naturalised cotton populations as assessed in the RARMPs for DIR 066/2006 (Bollgard II cotton) and DIR 124 (Bollgard III). These RARMPs considered 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* or *vip* genes does not confer increased fitness. Rather, a range of other biotic and abiotic factors, such as water and nutrient availability, temperature and soil type, seem to be far more important in limiting the spread and persistence of cotton than lepidopteran herbivory.

187. Evaluation of a number of phenotypic and agronomic characteristics (Chapter 1, Section 5.5.3) for GlyTol TwinLink Plus[®] cotton indicates that it is comparable with the cottons currently commercially produced in the Australian cotton industry, so the abiotic factors limiting other commercial cotton plants will also limit the ability of GlyTol TwinLink Plus[®] cotton to spread and persist.

188. 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 2016b).

189. 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. 2014).

Potential harm

190. 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.

191. However, cotton has limited ability to reduce the establishment of other plants (OGTR 2016b) 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

192. Risk scenario 3 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.

2.4.4 Risk Scenario 4

<i>Risk source</i>	Introduced insect resistance genes
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Expression of insect resistance genes in GM plants</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Reduced populations of target pest insects</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Reduced use of insecticides</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Increased populations of other insect pests</p> <p style="text-align: center;">↓</p>
<i>Potential harm</i>	Reduced yield of desirable agricultural crops

Risk source

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

Causal pathway

194. 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 insecticides, which may lead to an increase in populations of other insect pests which are otherwise controlled by the same pesticides.

195. Similar to Bollgard III cotton, GlyTol TwinLink Plus[®] cotton 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 prey or hosts.

196. Since Bollgard II cotton expressing the Cry1Ac and Cry2Ab proteins was introduced into Australian cotton cropping in 2003/4, pesticide usage in cotton production has been reduced by approximately 85% (ICAC 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. However, there have also been substantial increases in beneficial arthropod populations in GM cotton crops which have helped to manage other insects (Mansfield 2006). If GlyTol TwinLink Plus[®] cotton were released for commercial production, it is expected that overall pesticide usage patterns will be similar to those used for Bollgard II cotton.

197. 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 does not directly affect the levels of endogenous toxins (Chapter 1, Section 5.5.3), 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. 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.

198. In summary, adoption of GlyTol TwinLink Plus[®] cotton 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 as compared to current agronomic practice.

Potential harm

199. 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 since the introduction of existing GM insect resistant cottons. These management practices would be the same for cultivation of GlyTol TwinLink Plus[®] cotton.

Conclusion

200. Risk scenario 4 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 GlyTol TwinLink Plus[®] cotton compared to other GM cottons (Bollgard II or Bollgard III). Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.5 Risk Scenario 5

<i>Risk source</i>	Introduced herbicide tolerance genes
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Commercial cultivation of GM cotton expressing these introduced genes</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Establishment of volunteer GM cotton plants in agricultural areas</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Reduced effectiveness of weed management measures to control the volunteer GM cotton plants</p> <p style="text-align: center;">↓</p>
<i>Potential harm</i>	<p style="text-align: center;">Reduced establishment or yield of desirable agricultural crops</p> <p style="text-align: center;">or</p> <p style="text-align: center;">Increased reservoir for pathogens</p>

Risk source

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

Causal pathway

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

204. 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.

205. If glyphosate or glufosinate herbicides were the primary means of weed control, expression of dual herbicide tolerance genes in volunteer cotton plants could reduce the effectiveness of weed management measures and enhance the possibility of survival and establishment of these volunteer cottons.

206. However, as noted in Chapter 1, Section 4.1.3, glyphosate and glufosinate herbicides are not generally used to control established cotton as it usually fails to kill mature cotton plants. Other herbicides such as bromoxynil, carfentrazone and a combination of paraquat and diquat have been shown to be effective (Roberts et al. 2002), but there are no herbicides registered for seedlings beyond nine nodes of growth. Mechanical removal is the preferred option for older plants.

207. Cotton volunteers in intensive use areas such as roadsides are not known to give rise to self-perpetuating feral populations. Such volunteers may be subject to roadside management practices (e.g. appropriate herbicide treatment or slashing/mowing) and/or grazed by livestock, thereby limiting their potential to reproduce.

Potential harm

208. If left uncontrolled, volunteer cotton plants could establish and compete with other crops (CRDC 2013b) or become host for pests and diseases, reducing establishment or 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 (CropLife Australia 2012). In

addition, intensive use areas such as roadsides may be routinely managed for aesthetic and practical purposes by removal of large or invasive weeds.

Conclusion

209. Risk scenario 5 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 greater than negligible and does not warrant further detailed assessment.

2.4.6 Risk Scenario 6

Risk source	Introduced insect resistance genes
Causal pathway	<p style="text-align: center;">↓</p> <p style="text-align: center;">Transfer of insect resistance genes to other cultivated cottons by pollen flow</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Expression of insect resistance genes in the stacked GM cottons</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Exposure of people or other organisms by contact or ingestion, or inhalation of cotton pollen</p> <p style="text-align: center;">↓</p>
Potential harm	Increased toxicity or allergenicity for people or desirable organisms

Risk source

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

Causal pathway

211. The GM cotton is sexually compatible with all *G. hirsutum* cultivars and *G. barbadense*, but not with native cotton species (Chapter 1, Section 4.3). Therefore, the introduced genes have the potential to be transferred by pollen flow to cultivated cotton that is grown nearby.

212. Most of these cultivated cottons are likely to be Bollgard® II and Roundup Ready Flex®, which constitute the majority of Australian commercial cotton production (Chapter 1, Section 6.3.1). A limited amount of Bollgard® III and LibertyLink® (glufosinate herbicide tolerant cotton) is also grown. Roundup Ready Flex® and LibertyLink® cottons contain only herbicide tolerance genes and will be considered in Risk scenario 8.

213. Bollgard® II contains the *cry1Ac* and *cry2Ab* genes and Bollgard® III contains these two *cry* genes plus the *vip3Aa19* gene. Bollgard® III was approved for commercial cultivation in 2014 and only small scale demonstration planting has been carried out since then. However, it is expected that large scale commercial production will start from the 2016/17 growing season. In addition, Widestrike™ insect resistant cotton containing the *cry1Ac* gene and *cry1F* gene is approved for commercial cultivation in areas south of latitude 22°S, but there have been no commercial plantings to date. Therefore, in the near future, the potential exists for Bollgard® II or Bollgard® III to cross with GlyTol TwinLink Plus® cotton, resulting in hybrid progeny that expresses five *Bt* proteins Cry1Ac, Cry2Ab, Cry1Ab, Cry2Ae and Vip3A.

214. 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 stacked cottons.

215. However, for reasons discussed in Section 4.2.4, cotton volunteers do not persist in the field under normal conditions and expression of the introduced genes is not expected to increase the persistence of the hybrid plants. Therefore, the presence of the hybrids is expected to be transient and represent a small proportion of volunteers compared with the

planted GM cottons. Nonetheless, 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

216. 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 and 2, the Cry 1Ab, Cry2Ae and Vip3A proteins have no demonstrated toxicity or allergenicity to humans or toxicity to other desirable or non-target organisms.

217. The toxicity of Cry 1Ab, Cry2Ae 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 Cry1Ac and Cry2Ab proteins expressed by Bollgard II and Bollgard III, which are toxic to a similar range of lepidopteran species, but have not been shown to be toxic or allergenic to humans or toxic to other animals (OGTR 2014).

218. Expression of all these Bt proteins in a stacked hybrid may lead to additive toxic effects against lepidopteran pest species (Hilbeck & Otto 2015). However, 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 GlyTol TwinLink Plus[®] cotton crossing with Bollgard[®] II or Bollgard[®] III in the field, it would be predicted that the Cry1Ac and Cry1Ab proteins would compete for binding sites. Similarly the Cry2Ab and Cry2Ae proteins would compete for binding sites, resulting in an antagonistic interaction.

219. 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. However, 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 (see also De Schrijver et al. 2015). 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.

Conclusion

220. Risk scenario 6 is not identified as a substantive risk as transfer of the introduced genes to other cultivated GM insect-resistant 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 greater than negligible and does not warrant further detailed assessment.

2.4.7 Risk Scenario 7

<i>Risk source</i>	Introduced insect resistance genes
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Transfer of insect resistance genes to feral cotton plants in nature reserves by pollen flow</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Reduced insect herbivory of GM feral cotton, leading to increased establishment and reproduction of GM feral cotton in nature reserves</p>

	↓
Potential harm	Reduced establishment of desirable native vegetation

Risk source

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

Causal pathway

222. 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.

223. 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 Risk scenario 6). 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 only at low frequency and almost solely to cultivated cotton varieties or feral cottons that occur close by.

224. In assessing the possible impact of commercial release of Bollgard III cotton on gene flow to feral cottons, the RARMP for DIR 124 (OGTR 2014) also considered other factors that may limit the potential for gene flow from GM cottons to feral cotton. This included the amount and distribution of naturalised *G. hirsutum* and *G. barbadense* populations and spatial isolation of these feral cottons with cultivated GM cottons (including the state ban on cotton cultivation in Northern Territory, where most feral cotton populations have been reported). The RARMP concluded that the potential for pollen mediated gene flow between GM cotton in commercial cropping areas and feral cotton populations in nature reserves is very low in the short term, and a similar conclusion is valid for GlyTol TwinLink Plus[®] cotton.

225. 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. 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.

226. 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.

227. However, as discussed in Risk scenario 3, 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.

Potential harm

228. Increased spread and persistence of insect resistant feral cottons in nature reserves may give rise to adverse effects on desirable native vegetation, 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.

229. However, cotton has limited ability to reduce the establishment of other plants as discussed in Risk scenario 3. The introduced Bt toxin genes do not result in phenotypic changes that indicate an increased potential to reduce establishment of desirable vegetation. Therefore, any increased potential for feral cottons acquiring these genes to establish or persist in northern Australia due to reduced herbivory by lepidopterans is unlikely.

Conclusion

230. Risk scenario 7 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 greater than negligible and does not warrant further detailed assessment.

2.4.8 Risk Scenario 8

<i>Risk source</i>	Introduced herbicide tolerance genes
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Transfer of herbicide tolerance genes to other herbicide tolerant GM cotton plants by pollen flow</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Establishment of volunteer GM cotton plants in agricultural areas</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Reduced effectiveness of weed management measures to control the volunteer GM cotton plants</p> <p style="text-align: center;">↓</p>
<i>Potential harm</i>	Reduced establishment or yield of desirable agricultural crops

Risk source

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

Causal pathway

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

233. As discussed in Risk scenario 6, the introduced genes in GlyTol TwinLink Plus[®] cotton 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: Roundup Ready Flex[®] which, together with Bollgard[®] II and Bollgard[®] III (DIR 059/2005 and DIR 124) comprises over 95% of the Australian commercial cotton crop, and a small amount of glufosinate ammonium tolerant LibertyLink[®] cotton (DIR 062/2005). The potential exists for Roundup Ready Flex[®] cotton or LibertyLink[®] cotton to cross with GlyTol TwinLink Plus[®] cotton, resulting in hybrid progeny that expresses multiple herbicide tolerance genes.

234. Roundup Ready Flex[®] cotton contains the *cp4 epsps* gene from *Agrobacterium* sp. strain CP4. This gene has the same function as the maize *2mepsps* gene in GlyTol TwinLink Plus[®] cotton conferring tolerance to glyphosate herbicides with no new metabolites produced. LibertyLink[®] cotton contains the same *bar* gene as that in GlyTol TwinLink Plus[®] cotton. Therefore, in the event of hybrids being produced, no new herbicide tolerance traits will be generated. However, there could be additive effect that the hybrids could tolerate higher rates of herbicide application for both glyphosate and glufosinate-ammonium.

235. The control of cotton volunteers is important both in cotton fields and outside the fields such as along roadsides and drains. As discussed in Risk scenario 5, glyphosate and glufosinate herbicides are not generally used to control established cotton but other herbicides may be used. Cultivation is also a very effective method to control seedling cotton volunteers.

Potential harm

236. If left uncontrolled, volunteer cotton plants could establish and compete with other crops or become host for pests and diseases, reducing yield from crop plants.

237. However, as noted in Risk Scenario 5, 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

238. Risk scenario 8 is not identified as a substantive risk, as the presence of the hybrids is not expected to have any impact on standard agronomic practices for the control of cotton volunteers, or on their 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

239. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis⁴.

240. 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.

⁴ 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.

241. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk the Regulator will take this uncertainty into account in making decisions.

242. GlyTol[®] and GlyTol TwinLink Plus[®] cottons have been approved by the Regulator for limited and controlled release under licence DIR 133. The RARMP for DIR 133 identified two points of additional information that may be required for a large scale or commercial release of GlyTol TwinLink Plus[®] cotton. Information provided by the applicant in relation to these is outlined in Chapter 1, Section 5 and discussed in relevant sections of that Chapter.

243. Uncertainty can also arise from a lack of experience with the GMO itself. The level of uncertainty for the current application is considered to be low given that the GM cottons proposed to release have been commercially grown in the United States and field trialled in Australia. None of these releases have resulted in concerns for human health, safety or the environment. However, although Australia has considerable experience in growing cotton (both GM and non-GM) in southern regions, there is a lack of experience with commercial cotton growing in northern Australia. 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 5.5). 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. Widescale planting of cotton in northern Australia appears to be unlikely in the short term. Similarly, were feral cottons to acquire and express the introduced insect resistance genes there is some uncertainty associated with whether this would confer an advantage, given limited knowledge of insect pressures in areas of northern Australia. 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 7 and the risk assessed as negligible. Current information suggests that lepidopteran herbivory is not a limiting factor on spread and persistence of cotton.

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

245. For commercial releases of GMOs, which typically do not have limited duration, uncertainty regarding any future changes to knowledge about the GMO is addressed through post release review (Chapter 3, Section 4).

Section 4 Risk evaluation

246. 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.

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

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

248. Eight 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, and by considering both the short and long term. The principal reasons for these conclusions are summarised in Table 6.

249. The *Risk Analysis Framework* (OGTR 2013), 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.⁵

⁵ As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to 8 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Chapter 3 Risk management plan

Section 1 Background

250. 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 addresses risks evaluated as requiring treatment, and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through imposed licence conditions.

251. 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.

252. 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 allow the Regulator, or a person authorised by the Regulator, to enter premises 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.

253. 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 substantive risks

254. 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. 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 containment measures are required to treat these negligible risks.

Section 3 General risk management

255. 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
- testing methodology
- identification of the persons or classes of persons covered by the licence reporting structures
- access for the purpose of monitoring for compliance.

3.1 Applicant suitability

256. 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.

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

258. 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

259. Bayer is required to provide a method to the Regulator for the reliable detection of the GMOs and the presence of 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

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

3.4 Reporting requirements

261. 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.

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

263. 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

264. 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 the Regulator, or a person authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

265. 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 the health and safety of people or the environment could result.

Section 4 Post release review

266. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator 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.

267. 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).

268. 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

269. 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 Section 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

270. Collection of 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.

271. 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.

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

273. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warrant further detailed assessment. Uncertainty is considered to be low. No specific indicators of harm have been identified in this RARMP for application DIR 143. However, specific indicators of harm may also be identified during later stages, *e.g.* following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

274. 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

275. 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 require management, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

276. 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.

277. 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 submissions from prescribed experts, agencies and authorities

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.

Submission	Summary of issues raised	Comment
1	The cotton industry is of vital importance to the Shire. Any support provided to the industry to minimise chemical use can only be an advantage.	Noted.
2	Agrees with the issues identified by the office for consideration in the RARMP and no new issues were identified for consideration.	Noted.
3	Believes that there will be minimal risk associated with the proposed commercial cultivation of GM cotton. However, Council does not have staff that are experienced in this field to formally comment	Noted.
4	Council has declared the Shire a GMO free zone and opposes the release of GM cotton into the environment. GM foods are an area of concern for the council, including labelling.	Some areas may be declared GM free under State or Territory law for marketing purposes. This is a decision that falls under the jurisdiction of individual State or Territory governments. FSANZ has regulatory responsibility for food safety assessment and labelling, including for GM food.
5	<p>Noted that all four events that are the subject of DIR 143 have been assessed as part of the limited and controlled release under DIR 133 and one of the events has been assessed as part of the commercial release under DIR 124. The conclusions of the RARMPs for DIR 133 and DIR 124 regarding toxicity and the effects on the environment should be broadly applicable to DIR 143.</p> <p>For GlyTol TwinLink Plus cotton, there appears to be no plausible pathway for the interaction of the herbicide tolerance (PAT, 2mEPSPS) and insect resistance (Cry1Ab, Cry2Ae, Vip3Aa19) proteins in a single plant that would lead to a 'novel' adverse trait (ie a trait unrelated to herbicide tolerance and insect resistance). Where there is no basis for a potential interaction of genes (or at least their products) in a GM stack, or if there is experimental evidence or good reason to believe that predicted interactions will not affect risk, the risk assessment of individual parental GM plants containing one of the events will likely be sufficient to assess the risks of the GM stack.</p> <p>The RARMP should address two issues concerning the presence in the GM plants of multiple proteins for each separate trait (herbicide tolerance and insect resistance). Firstly, the presence of more than one insecticidal</p>	<p>Noted.</p> <p>Noted. The RARMP takes into account information relating to the individual parental cottons as part of considerations for risk assessment.</p> <p>The possible synergistic effect of the three</p>

Submission	Summary of issues raised	Comment
	<p>protein in the GM plants could lead to harm to non-target insects that are not adversely affected by the parent plants.</p> <p>Secondly, it is possible that the volunteers from a cotton plant with dual herbicide tolerance could be more difficult to control than those arising from a plant possessing single herbicide tolerance, leading to a higher potential for invasion of native vegetation. The susceptibility of the GM plants of this application to commonly used herbicides, other than glufosinate-ammonium and glyphosate, and their potential for management by agricultural methods commonly used with cotton, should be discussed in the RARMP, together with the importance of the use of an integrated weed management programme.</p> <p>In general, for conventional breeding, the crossing of plants, each of which will possess a range of innate traits, does not lead to the generation of progeny that have health or environmental effects significantly different from the parents. This view may be relevant to the generation of the stacked GM plants in this application, and could be referred to in the RARMP.</p> <p>There are indigenous <i>Gossypium</i> species in Australia. Due to different genome compositions, hybridisation between these Australian species and <i>Gossypium hirsutum</i> is unlikely. Although this topic has been considered in previous cotton RARMPs, it should be summarised in the RARMP for DIR 143.</p> <p>In Australia, cotton is grown predominately in NSW and southern QLD. However, the map on page 163 of the application (figure 36) indicates that there are "new opportunities" in northern Australia, and cotton has been sporadically grown in the designated northern areas.</p> <p>The issue of the growth of cotton in northern Australia has been discussed in previous RARMPs (DIR 066/2006 and DIR 091), and should be revisited in the RARMP for DIR 143. Evidence that lepidopteran pests of cotton, such as <i>Helicoverpa armigera</i> and <i>H. punctigera</i>, act to limit the spread and persistence of weedy populations of cotton is limited. Theories relating the spread and persistence of plants to the presence or absence of natural enemies, such as so-called Enemy Release Hypothesis, remain controversial, especially with respect to their experimental evaluation.</p>	<p>introduced Bt proteins to non-target insects is discussed in Chapter 2, Risk scenario 2 of the RARMP.</p> <p>The use of herbicides in control of cotton volunteers is discussed in Chapter 1, Section 4.1.3 and Chapter 2, Risk scenario 4, with reference to integrated weed management practices.</p> <p>This is discussed in Chapter 2, Section 2.1 of the RARMP.</p> <p>The potential for hybridisation with native <i>Gossypium</i> species is summarised in Chapter 1, Sections 4.3 and 7.3.1 of the RARMP.</p> <p>Current information suggests that lepidopteran herbivory is not a limiting factor on spread and persistence of cotton, but evidence is limited. It has been identified as an area of uncertainty in the risk analysis (Chapter 2, Section 3 of the RARMP).</p>
6	<p>Notes that there is no information provided on the safety of the insecticide-producing traits of the cotton, and its impact on use in human food or animal feed, or on the ecology of the cropping system. Understands that this should theoretically be covered by a different part of the application process.</p>	<p>This issue is addressed in Chapter 2, Risk Scenarios 1 and 4 of the RARMP. The RARMP concludes that risks to human health and the environment are negligible.</p>

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

The Regulator received a number of submissions from prescribed experts, agencies and authorities⁶ 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.

Submission	Summary of issues raised	Consideration in RARMP	Comment
1	No comment		-
2	Do not have specialist scientific advisors, so no comment		-
3	No comments or issues		Noted
4	Supports the conclusion that DIR 143 poses negligible risk of harm to human health and safety and the environment.		Noted
5	FSANZ has approved food made from the GM cottons as safe for human consumption. No further comment.		Noted
6	Supportive of the application. Notes the RARMP conclusions of negligible risks to people or the environment. Understands there are licence conditions to ensure continued oversight of the release. Notes that food from the GM cottons has been approved by FSANZ.		Noted
7	No further comment. Supports the conclusions of the RARMP.		Noted
8	Agrees with the overall conclusions of the RARMP. The risk assessment identifies all plausible risk scenarios. Suggests modifying the section on uncertainty to clarify that it relates to lack of experience in growing cotton in northern Australia.	Chapter 2, Section 3	Noted Wording has been amended to clarify this point

⁶ Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

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

The Regulator received one submission from the public on the consultation RARMP. The issues raised in this submission are summarised in the table below. All issues 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.

Submission	Summary of issues raised	Consideration in RARMP	Comment
1	<p>Concerns about reports by the International Agency for Research on Cancer, regarding glyphosate and its assessment of glyphosate as ‘probably carcinogenic to humans’.</p> <p>Asks how the use of glyphosate on food for human consumption or animal feed can possibly be considered.</p> <p>Use of glyphosate in the production of foodstuffs should not be sanctioned while there is any possibility it could be carcinogenic.</p>	-	<p>The RARMP for this GM cotton considers the risks to human health and the environment from the GM cotton only.</p> <p>Issues relating to herbicide use are outside the scope of the Gene Technology Regulator’s assessments. The APVMA are responsible for assessing an application for use of glyphosate on the GM cotton. The APVMA considers risks to human health, animals and the environment in assessing agricultural chemicals for registration and in setting maximum application rates. Further information on the safety assessment of glyphosate is available on the APVMA website.</p> <p>A number of countries have completed reviews of glyphosate and concluded that glyphosate is unlikely to cause cancer in humans. More information about findings in other countries is available on the APVMA website with links to individual countries’ decisions.</p> <p>The APVMA’s current assessment is that products containing glyphosate are safe to use as per the label instructions.</p> <p>APVMA and FSANZ have shared responsibilities in setting maximum residue limits (MRLs) for agricultural chemicals in food. At the time the MRLs are set, a dietary exposure evaluation is undertaken to ensure that the levels do not pose an undue hazard to human health. The FSANZ website has an information page regarding herbicide use, herbicide tolerance and herbicide residues in GM foods.</p>