Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 157

Commercial release of cotton genetically modified for insect resistance (COT102)

Applicant: Syngenta Australia Pty Ltd

February 2018

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR 157

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 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 the requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that this commercial release poses negligible risks to human health and safety and the environment and no specific risk treatment measures are imposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the release.

The application

Application number	DIR 157
Applicant	Syngenta Australia Pty Ltd (Syngenta)
Project title	Commercial release of cotton genetically modified for insect resistance (COT102)
Parent organism	*Gossypium hirsutum* L. (cotton)
Introduced gene and modified trait	**One insect resistance gene:** • *vip3Aa19* gene from *Bacillus thuringiensis (Bt)* **One selectable marker gene:** • *aph4* from *Escherichia coli* for resistance to hygromycin B
Proposed locations	Australia-wide
Primary purpose	Commercial release of the GM cotton

Risk assessment

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings, 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 are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short and long term risks are 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 other desirable organisms; proteins similar to the introduced proteins are widespread in the environment; the GM cotton was licenced for field trials in Australia between 2002 and 2010, with no reported adverse or unexpected effects; and the GM cotton has limited

capacity to survive in natural habitats. In addition, food made from the GM cotton has been assessed and approved by Food Standards Australia New Zealand as safe for human consumption.

Risk management

The risk management plan concludes that risks from the proposed dealings can be managed so as to protect people and the environment by imposing general conditions to ensure that there is ongoing oversight of the release.

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

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

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Abbreviations

aph4	*hygromycin B phosphotransferase* gene
AMR	Antimicrobial resistance
APVMA	Australian Pesticides and Veterinary Medicines Authority
ASTAG	Australian Strategic and Technical Advisory Group on Antimicrobial Resistance
BC	Backcross
bp	base pair
Bt	*Bacillus thuringiensis*
cm	Centimetre(s)
CRDC	Cotton Research and Development Corporation
Cry	Crystal protein
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
DT50	Time to dissipation of 50 % of initial bioactivity
ELISA	Enzyme-linked immunosorbent assay
f. sp.	Forma specialis
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
HGT	Horizontal gene transfer
HPT	Hygromycin B phosphotransferase
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IWM	Integrated Weed Management
km	Kilometre(s)
L.	Linnaeus
LOD	Limit of detection
m	Metre(s)
mg	Milligram(s)
mL	Millilitre(s)
NHMRC	National Health and Medical Research Council
ng	Nanogram(s)
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
OIE	World Organisation for Animal Health
PRR	Post release review
QLD DAFF	Queensland Department of Agriculture, Fisheries and Forestry
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator

RMP	Resistance management plan
spp.	Species
T-DNA	Transfer DNA
TGA	Therapeutic Goods Administration
the Act	The Gene Technology Act 2000
TIMS	Transgenic and Insect Management Strategy Committee
Vip	Vegetative insecticidal protein
vip3Aa19	*vip3Aa19* gene from *B. thuringiensis*
Vip3Aa	Vip3Aa crystal protein from *B. thuringiensis*
WHO	World Health Organization

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).

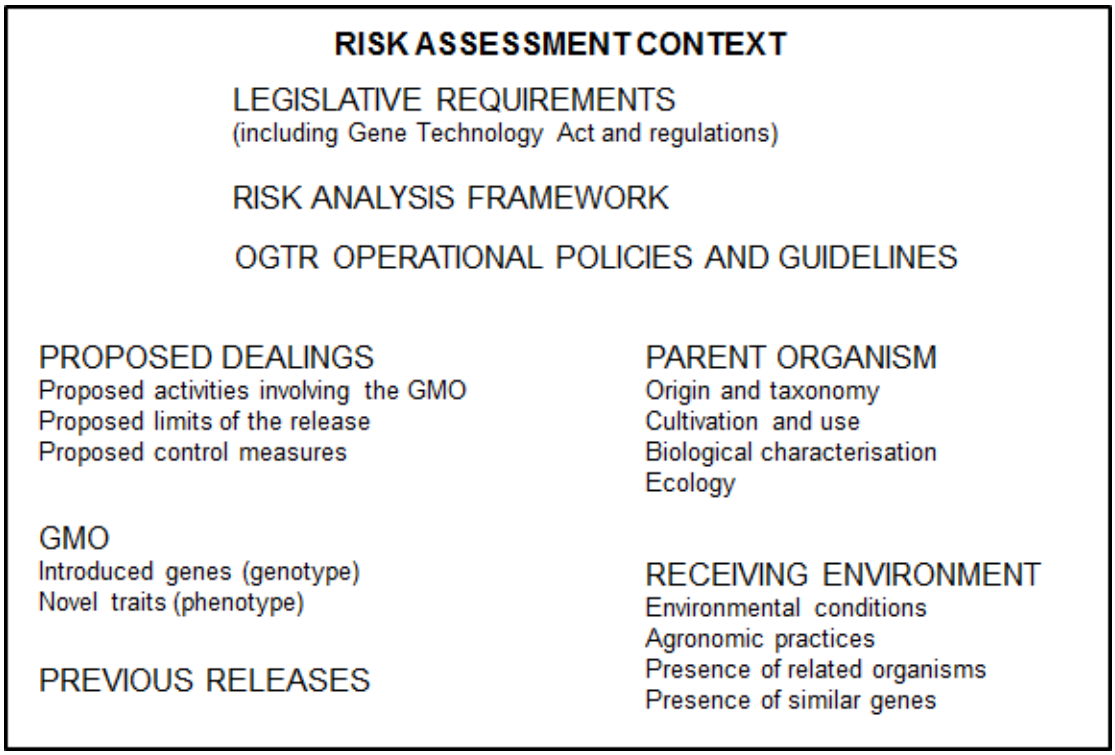

Figure 1 Summary of parameters used to establish the risk assessment context

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 councils 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. Four public submissions were received and their 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 the 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. Syngenta Australia Pty Ltd (Syngenta) proposes commercial cultivation of a genetically modified (GM) cotton line (COT102), which contains an introduced gene that confers insect resistance. This event is also known by the unique OECD identifier SYN-1R1Ø2-7.

10. The applicant is seeking approval for the release to occur Australia-wide, subject to any moratoria imposed by States and Territories for marketing purposes. COT102 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.

11. The dealings involved in the proposed intentional release are to:

(a) conduct experiments with the GMO

(b) breed the GMO

(c) propagate the GMO

(d) use the GMO in the course of manufacture of a thing that is not a GMO

(e) grow the GMO

(f) import the GMO

(g) transport the GMO

(h) dispose of 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

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

13. Cotton is grown as a source of textile and industrial fibre, cottonseed oil and linters for food use, and whole white ("fuzzy") cottonseed 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 can be found in *The Biology of* Gossypium hirsutum *L*. *and* Gossypium barbadense *L.*

(cotton) (OGTR, 2016), which was produced to inform the risk assessment process for licence applications involving GM cotton plants and is available from the OGTR Biology documents page.

14. In establishing the risk context, details of the parent organism form part of the baseline for a comparative risk assessment (Figure 1, OGTR, 2013). Non-GM cotton is the standard baseline for biological comparison, while noting that over 98% of the Australian commercial cotton crop is GM cotton (ABARES, 2017).

4.1 Cotton as a crop

15. 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 2016). It is a perennial plant that is cultivated as an annual.

16. 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. Commercial cultivation of cotton is also extensively reviewed in *The Biology of* Gossypium hirsutum *L. &* Gossypium barbadense *L. (cotton)* (OGTR, 2016).

17. Based on 2016/17 and 2017/18 estimates of commercial cropping areas in Australia, cotton is ranked as the crop with the sixth largest area of production. Estimated production area for 2016/17 was 557,000 ha, with a production value for cotton lint and cottonseed of $2,478 million (ABARES, 2017).

4.1.1 Management of pests in cotton crops

18. The major insect pests of non-GM cotton in Australia are the lepidopterans *Helicoverpa armigera* and *H. punctigera.* In the 1990s these were controlled by spraying chemical pesticides 8 – 15 times per season (Fitt, 2000). These broad-spectrum insecticides also control other pests, but disrupt beneficial insects that control secondary pests such as mites. The introduction of GM cotton, modified for insect resistance, in 1996 reduced the use of pesticides and is used as a tool in Integrated Pest Management (IPM) strategies in cotton (Whitehouse et al., 2005).

19. The use of IPM is promoted by the cotton industry as part of best management practice to reduce insect numbers, while reducing risks to the health of humans and the environment (myBMP website, accessed 11 July 2017). IPM involves using a range of tactics throughout the season to manage pest and beneficial insect populations in and around farms (CRDC and CottonInfo, 2017).

20. The cotton industry has implemented an Insecticide Resistance Management Strategy, which aims to minimise the development of insecticide resistance in aphids, mites, silverleaf whitefly and *Helicoverpa* spp. (CRDC and CottonInfo, 2017). Growers who plant GM cotton varieties with *Bt* insect resistance traits are required to adhere to a resistance management plan (RMP) developed by the seed company and the Transgenic and Insect Management Strategy (TIMS) Committee. The aim is to maintain the genetic diversity of *Helicoverpa* spp. and thus slow the development of resistance to the proteins produced by introduced *Bt* genes. The RMP specifies the use of refuge crops, planting windows or planting restrictions, pupae busting cultivation, control of volunteers and ratoon cotton, and spray limitations for GM crops (CRDC and CottonInfo, 2017).

4.1.2 Management of weeds

21. A number of agricultural practices are used to control weeds in fields prepared for the planting of cotton. These practices include cultivation or the application of herbicide treatments (OGTR, 2016). Integrated weed management (IWM) is used to avoid selection of resistant weed biotypes and reduce the likelihood of herbicide resistant weeds becoming a problem (CRDC and CottonInfo, 2017).

22. The control of cotton volunteers after harvest is usually achieved by mechanical means or use of a range of herbicides, preferably as part of IWM practices. Control of volunteer cotton by herbicides is most effective on seedling cotton and there are no herbicides currently registered for control of volunteer cotton larger than nine nodes in size (CRDC and CottonInfo, 2017).

4.2 Weed risk potential for cotton outside cultivation

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

24. *Gossypium hirsutum* is not recorded in the Australian government's *Weeds of National Significance* list (Department of Environment and Energy website, accessed 3 October 2017), the *National Environmental Alert List* (Department of Environment and Energy website, accessed 3 October 2017) or the *Noxious Weed List for Australian States and Territories* (Invasive Plants and Animals Committee, 2015).

25. The Standards Australia National Post-Border Weed Risk Management Protocol rates the weed risk potential of plants according to properties that correlate with weediness for each relevant land use (Standards Australia et al., 2006). These properties relate to the plants' potential to cause economic, environmental and/or social harm (impact); to spread, establish and reproduce (invasiveness); and to its potential distribution. The weed risk potential of volunteer cotton has been assessed using methodology based on the National Post-Border Weed Risk Management Protocol (OGTR, 2016).

4.2.1 Potential to cause harm

26. 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 (OGTR, 2016).

27. 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, 2016).

28. Mammals, including people, can be fatally poisoned when ingesting cotton plant parts, due to the presence of natural toxins in cotton. Gossypol, a terpenoid aldehyde, is a secondary metabolite with pesticidal activity that is produced in most parts of the cotton plant (Bell, 1986). The toxicity of gossypol to monogastric animals limits the use of cottonseed in human food and animal feed. Several other secondary metabolites, including flavonols and fatty acids, contribute to pest resistance. The toxicity of cotton plant parts to animals is enhanced by cyclopropene fatty acids, such as malvalic and sterculic acids (Bell, 1986).

4.2.2 Invasiveness

29. 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,
- 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 for spread by people from or to nature conservation areas (OGTR, 2016).

4.2.3 Spread and distribution

30. Cottonseed may be spread off-farm, primarily during transport of modules to gins. Seed is also dispersed through irrigation or stormwater runoff into common drainage channels. Ephemeral populations of cotton volunteers can be found on cotton farms, by roadsides where cottonseed is transported, or in areas where cottonseed is used as livestock feed (Addison et al., 2007). In 2012 and 2013, the Queensland Department of Agriculture, Fisheries and Forestry (QLD DAFF) conducted a survey of cotton plants throughout cropping areas in Qld and northern NSW. This study 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 within a 5 km radius of cotton farms and in close proximity to ginning facilities (CRDC, 2013a).

31. Volunteer cotton is present but not considered a weed in agricultural ecosystems (Groves et al., 2003). In natural Australian ecosystems, cotton is described by Groves et al. (2003) as a naturalised non-native plant with a weediness rating of 2. This rating indicates that cotton is naturalised and known to be a minor problem warranting control at three or fewer locations within a state or territory.

32. The establishment of cotton across most of Australia is limited by dry stress, cold temperatures and soil fertility. Establishment is further limited by canopy conditions of natural vegetation, as well as fire regimes and weed competition (Rogers et al., 2007). The addition of *Bt* insect resistance genes did not increase the fitness for weediness for cotton growing in non-cropping habitats in northern Australia (Eastick and Hearnden, 2006). Thus, although there are some naturalised populations in relatively natural areas of northern Australia, there is limited potential for *G. hirsutum* populations to spread and persist in undisturbed nature conservation areas.

33. Most reports of *G. hirsutum* volunteers or naturalized populations are from tropical regions of Australia, and cotton-growing areas throughout Queensland and New South Wales (Australia's Virtual Herbarium). Persistence of feral populations is limited, as *G. hirsutum* has little ability to invade undisturbed habitats (OGTR, 2016).

Section 5 The GM cotton – nature and effect of genetic modification

5.1 The genetic modification

34. The GM cotton line proposed for release is COT102. COT102 has been extensively evaluated in previous RARMPs for limited and controlled release, and has been approved for commercial release throughout Australia as a stack with other GM cotton lines under the DIR licences 124, 143 and 145.

5.1.1 Details of the introduced genetic elements

35. The genes introduced into COT102 are listed in Table 1.

Table 1 Introduced genes in cotton line COT102

Gene	Encoded protein	Source organism	Function
vip3Aa19	Vegetative insecticidal protein Vip3A	*Bacillus thuringiensis (Bt)*	Insect resistance
aph4	Hygromycin B phosphotransferase (HPT)	*Escherichia coli*	Marker - antibiotic resistance (hygromycin)

36. Short regulatory sequences that control expression of the introduced genes are also present in COT102. These regulatory elements are listed in Table 2. These sequences are derived from thale cress and a common soil-borne bacterium (*A. tumefaciens*).

37. Although two of these regulatory sequences are derived from a plant pathogen, by themselves they do not cause disease. The regulatory elements present in COT102 have been previously assessed by Australian and international regulators without identifying an increase in risk compared with endogenous regulatory elements in cotton.

Table 2 Introduced regulatory elements in COT102

Element	Function	Source
Pact2	*vip3Aa19* promoter	Promoter region and intron from the actin-2 gene from *Arabidopsis thaliana* (thale cress)
Tnos	*vip3Aa19* terminator	Terminator sequence from the nopaline synthase gene of *Agrobacterium tumefaciens*
Pubq3	*aph4* promoter	Promoter region plus the first intron from the ubiquitin 3 gene from *A. thaliana.*
Tnos	*aph4* terminator	Terminator sequence from the nopaline synthase gene of *A. tumefaciens*

5.1.2 Method of genetic modification

38. COT102 was produced using *Agrobacterium*–mediated transformation. This method has been widely used in Australia and overseas for introducing genes into plants. More information can be found in the document *Methods of Plant Genetic Modification* on the Risk Assessment References page on the OGTR website.

39. Genetic elements of the transformation plasmid pCOT1 were delivered into excised hypocotyls of cotton cultivar Coker 312 by *A. tumefaciens* (Burgin, 2014). The genes and regulatory elements listed in Table 1 and Table 2 were delivered as a single transfer DNA (T-DNA) insert. Genetic elements outside of the left and right border (the plasmid backbone) were not transferred (Section 5.3.1). Transformed

cotton cells were selected through their ability to grow in the presence of the appropriate selective agent, hygromycin B. GM cotton plants were regenerated from the selected cells.

5.2 The introduced genes, their encoded proteins and associated effects

5.2.1 The vip3Aa19 *gene and the encoded product*

40. *Bacillus thuringiensis* (*Bt*), a common soil bacterium, produces a range of insecticidal proteins, including the crystal (Cry) proteins (also known as δ-endotoxins) and vegetative insecticidal proteins (Vip) (Estruch et al., 1996). Vip proteins are secreted by various *Bacillus* species during vegetative growth stages and sporulation, whereas the Cry proteins are expressed by *Bt* only during sporulation and form crystalline inclusions in spores. Vip proteins do not exhibit any structural similarity with Cry toxins (Estruch et al., 1996) and bind to different receptors located on the brush border membrane in the insect midgut (Lee et al., 2003; Sena et al., 2009).

41. There are four Vip protein families. Vip1 and Vip2 proteins are toxic to some members of the Coleoptera and Hemiptera, while Vip3 proteins are toxic to many lepidopteran insect species. In particular, Vip3A proteins are toxic to species of *Agrotis* and *Spodoptera*, which have low susceptibility to Cry proteins (Chakroun et al., 2016) and are pests of cotton (CRDC and CottonInfo, 2017). Vip3A proteins are toxic to the major insect pests of cotton in Australia, *Helicoverpa armigera* and *H. punctigera* (Mahon et al., 2012). As discussed further in Section 6.3.3, there is a low level of background resistance to Vip3A present in these species in Australia.

42. To date, 54 *vip3Aa* genes have been reported (Chakroun et al., 2016). Approximately 50% of *Bt* strains surveyed globally carry *vip3* genes (Liu et al., 2007; Hernández-Rodríguez et al., 2009). A survey of 188 strains of *Bt* isolated from soil, grain dust and bird nest samples in Australia found that 72% of samples carried *vip3A* genes (Beard et al., 2008). Hernandez-Rodriguez et al. (2009) surveyed 507 strains of *Bt* and found that 91.5% of those with *vip3* genes also contained *cry1A* and *cry2* genes, speculating that these genes are encoded by the same plasmids.

43. COT102 contains the *vip3Aa19* gene (NCBI accession number DQ539887), a synthetic copy of the naturally occurring *vip3Aa1* gene (NCBI accession number L48811) modified to accommodate the preferred codon usage in plants (Murray et al., 1989). The *vip3Aa1* gene was derived from *Bt* strain AB88, which was isolated from sour milk (Estruch et al., 1996). An error was made during the original sequencing of this gene, resulting in a single amino acid difference at position 284 (Hill et al., 2003). Thus the protein expressed by *vip3Aa19* (and *vip3Aa1*) contains a glutamine residue at position 284, whereas the native protein contains lysine. All other amino acid residues are identical. The substitution is conservative as lysine and glutamine are polar amino acids having a molecular weight of 146 g/mol (Hill et al., 2003). The amino acid substitution does not appear to have changed the function of the protein (USDA-APHIS, 2005).

5.2.2 The antibiotic resistance gene

44. The *aph4* gene (also known as *hph* or *hpt*) in COT102 was isolated from the common gut bacterium *Escherichia coli* (strain K-12). The gene encodes a hygromycin B phosphotransferase (HPT) enzyme, which inactivates the antibiotic hygromycin B (NCBI protein accession number CAA85741) (US EPA, 2008). 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.

5.2.3 Toxicity and allergenicity of the proteins encoded by the introduced genes

45. FSANZ has approved food derived from COT102 expressing the Vip3Aa19 and HPT proteins as safe for human consumption (FSANZ, 2004, 2006).

46. The Canadian Food Inspection Agency (CFIA) determined that livestock feed derived from COT102 does not present safety concerns when compared with currently commercialised cotton varieties (CFIA, 2011).

Vip3Aa19 protein

47. The Vip3A protein for insect resistance is 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 70 years (CERA, 2012). Thus, people and other organisms have a long history of safe exposure to *Bt* insecticidal proteins.

48. A review of Vip proteins found that the Vip3A proteins are only known to be toxic to lepidopteran insects (Chakroun et al., 2016). Table 3 summarises the measured effect of Vip3Aa and Vip3Aa19 proteins on lepidopteran species. The effect of a further 14 variants of the Vip3Aa subfamily, e.g. Vip3Aa1, Vip3Aa7, etc., are given in Chakroun et al. (2016). Some variability in the toxicity of Vip3Aa variants to different insect species has been reported by different researchers. It is expected that these differences may in some instances be attributed to differences in protein preparation or experimental procedures, including differences between laboratories, purification procedures, batch-to-batch variation and days to scoring insect mortality.

49. Vip3A proteins become toxic after being activated by insect midgut proteases. After crossing the peritrophic membrane, proteins bind with specific receptors on the midgut epithelium, leading to pore formation and cell death (Lee et al., 2003).

50. The safety of the Vip3A protein was extensively discussed in the RARMP for DIR 124. All evidence indicates that Vip3A is highly unlikely to be toxic or allergenic to humans. Vip3A proteins have a narrow spectrum of pesticidal activity specific to lepidopteran insects. They are not expected to be toxic to humans, livestock or other vertebrates that lack the receptors to which Vip3A binds, such as those found on the brush border membrane vesicles in the midguts of some lepidopteran larvae (CERA, 2012; Chakroun et al., 2016).

51. It is unlikely that the Vip3A protein would be allergenic to humans, as it has no sequence similarity to known protein allergens, and is degraded in simulated gastric fluid (Hill et al., 2003).

52. No observable effect of Vip3A was found in ecotoxicological tests carried out on the non-target vertebrates bobwhite quails, mice, channel catfish and chickens (Raybould and Vlachos, 2011; CERA, 2012).

53. The effect of Vip3A on non-target invertebrates is discussed in Section 5.3.6.

HPT protein

54. The potential risks of the hygromycin B phosphotransferase (HPT) protein are discussed in the document *Marker Genes in GM Plants* available from the Risk Assessment References page on the OGTR website. There is no evidence that HPT is toxic or allergenic to humans.

55. In an acute oral toxicity study, HPT protein purified from *E. coli* was administered to mice at doses of 1, 5 and 10 g/kg body weight, with no observed adverse effects (Zhuo et al., 2009). In a similar study Vlachos (2002) reported no evidence of toxicity in mice receiving HPT at 774 mg /kg body weight for 14 days.

56. No significant amino acid sequence homology has been found between HPT and known or putative allergenic proteins or proteins known to be toxins (Hill et al., 2003; US EPA, 2008).

57. Lu et al. (2007) tested the potential allergenicity of the HPT protein. HPT was rapidly digested by simulated and intestinal gastric fluids. Injection of HPT protein did not elicit an immune response in rats. Based on these results, and that the protein has no sequence similarity to known allergens, the authors concluded that HPT has a low probability of inducing allergenicity.

Table 3 Effect of Vip3Aa and Vip3Aa19 proteins on lepidopteran species

Species	Common name	Protein	LC_{50} (ng/cm^2)	Reference
Lepidoptera: Noctuidae				
Agrotis ipsilon	Black cutworm	Vip3Aa	<200	(Yu et al., 1997)
		Vip3Aa	17.1	(Lee et al., 2003)
		Vip3Aa	63.4	(Gayen et al., 2012)
Helicoverpa armigera	Cotton bollworm	Vip3Aa	155	(Liao et al., 2002)[a]
		Vip3Aa19	24.1 ng/mg	(Liu et al., 2007)
		Vip3Aa	89.1	(Gayen et al., 2012)
		Vip3Aa	68.3 µg/g	(Mahon et al., 2012)
Helicoverpa punctigera	Native budworm	Vip3Aa	22	(Liao et al., 2002)[a]
		Vip3Aa	55.2 µg/g	(Mahon et al., 2012)
Helicoverpa zea	Corn earworm	Vip3Aa	active	(Liao et al., 2002)
		Vip3Aa	113	(Lee et al., 2003)
		Vip3Aa19	500	(Welch et al., 2015)
Heliothis virescens	Tobacco budworm	Vip3Aa	active	(Liao et al., 2002)[a]
		Vip3Aa19	1.35 µg/mL	(Gulzar and Wright, 2015)
Spodoptera exigua	Beet armyworm	Vip3Aa19	1.4 ng/mL	(Liu et al., 2007)
Spodoptera frugiperda	Fall armyworm	Vip3Aa	<200	(Yu et al., 1997)
		Vip3Aa	55.9	(Lee et al., 2003)
Spodoptera littoralis	Cotton leafworm	Vip3Aa	35.8	(Gayen et al., 2012)
Lepidoptera: Pyralidae				
Elasmopalpus lignosellus	Lesser cornstalk borer	Vip3Aa	49.9	(Lemes et al., 2017)
Lepidoptera: Plutellidae				
Plutella xylostella	Diamondback moth	Vip3Aa19	59.8 µg/mL	(Liu et al., 2007)
		Vip3Aa19	2.24 mg/mL	(Gulzar and Wright, 2015)
Lepidoptera: Crambidae				
Diatraea flavipennella	Sugarcane borer	Vip3Aa	495	(Lemes et al., 2017)
Ostrinia furnacalis	Asian corn borer	Vip3Aa19	>100 µg/mL	(Liu et al., 2007)
Ostrinia nubilalis	European corn borer	Vip3Aa	not active	(Yu et al., 1997)
		Vip3Aa	not active	(Lee et al., 2003)
Scirpophaga incertulas	Yellow stem borer	Vip3Aa	60.2	(Gayen et al., 2012)
Lepidoptera: Sphingidae				
Manduca sexta	Tobacco hornworm	Vip3Aa	176	(Lee et al., 2003)
Lepidoptera: Nymphalidae				
Danaus plexippus	Monarch butterfly	Vip3Aa	not active	(Lee et al., 2003)

[a] as cited in Chakroun et al. (2016).

5.3 Characterisation of the GMO

5.3.1 Molecular stability

58. A number of molecular analyses of COT102 cotton were provided by the applicant. Southern blot analyses of digested leaf DNA confirmed 1) the absence of unintended backbone sequences from plasmid pCOT1 in the GM plants, 2) that a single intact copy of the COT102 insert had integrated into a single locus on the cotton genome, and 3) that the insert was stably inherited over five generations from BC_3F_3 to BC_3F_7 (Burgin, 2014).

59. The frequency of the presence of the *vip3Aa19* and *aph4* genes was measured in three generations (F_1, BC_1F_1, BC_4F_1) of COT102 backcrossed with non-GM cotton. The two genes co-segregated and were inherited in a predictable manner, according to Mendelian principles (Burgin, 2016).

60. Sequencing of the insert and flanking regions of the T-DNA insertion in COT102 showed that 1) an 86 bp deletion of the cotton genome occurred during integration of the COT102 T-DNA, and 2) that filler DNA sequences with similarity to *Gossypium* DNA sequences, with lengths of 4 bp and 690 bp, were inserted at the 5' and 3' insert-to-genome junctions, respectively, and 3) that 24 bp of the right border and 19 bp of the left border were truncated (Burgin, 2016). Truncation of a portion of the border region is expected and has no effect on the functionality of the insert.

5.3.2 Expression of the introduced proteins

61. In the USA field trials were carried out across six states to measure protein expression in COT102 (Hill, 2015). Enzyme-linked immunosorbent assay (ELISA) results are shown in Table 4. Vip3A expression was highest in leaves of younger cotton, decreasing as plants matured. Pollen, roots and cottonseed had lower levels of Vip3A protein than leaves, flowers and bolls. In Australian field experiments by Llewellyn et al. (2007), the relative concentrations of Vip3A protein measured in COT102 plant tissues were different, with squares having approximately 15 % more Vip3A than leaves and flower buds.

Table 4 Expression levels of introduced proteins in COT102 grown in the USA (Hill, 2015)

Tissue (plant growth stage)	Protein expression (ng/mg dry weight, standard deviation)	
	Vip3A	**HPT**
Leaf (4-leaf)	460.78 ± 216.49	ND [<0.5 ± 0.48][a]
Leaf (1st white bloom)	98.12 ± 54.22	ND [<0.5 ± 0.24]
Leaf (1st open boll)	78.85 ± 64.41	ND [<0.5 ± 0.20]
Bolls (peak bloom)	46.03 ± 10.07	ND [<0.5]
Flower (peak bloom)	106.99 ± 27.85	ND [<0.5]
Pollen (early bloom)	2.15 ± 1.82	-[b]
Root (maturity)	5.19 ± 3.91	ND [<0.05 ± 0.03]
Cottonseed (maturity)	10.65 ± 3.47	ND [<0.5]
Squares (1st white bloom)	55.17 ± 15.73	ND [<0.25 ± 0.07]
Whole plant (maturity)	7.42 ± 6.92	ND [<0.45]

ND, result was below the limit of detection for the method.

[a] Numbers in square brackets indicate the limit of detection (LOD). Standard deviations are given when the individual sample range extended above the LOD, but the arithmetic mean was below the LOD.

[b] HPT expression in pollen could not be measured by ELISA, due to matrix effects. HPT expression was detected by Western Blotting in all COT102 samples.

62. On average, HPT protein expression by the *aph4* gene was below the limit of detection in all plant tissues. Expression of HPT in pollen was detected, but could not be quantified in this study. In previous studies, assessed by the US EPA (2008), HPT protein has been measured at higher concentrations in COT102 pollen than in leaf, root or reproductive tissues. Pollen concentrations of HPT were reported at 2.25 ng/mg and 64.3 ng/mg.

5.3.3 Germination and dormancy

63. The applicant provided data from seed germination and dormancy tests carried out in the USA in 2015 (Potter, 2016). Field grown seeds from COT102, a non-GM near-isogenic control line and three reference lines were incubated at two temperature regimes. Seeds or seedlings were rated as normally germinated, abnormally germinated, dead, dormant or hard.

64. At a constant 10 °C temperature regime, the majority of seeds were still dormant after 12 days, with some dead or hard. There was no significant difference in percent dormant or dead between COT102, control and reference lines. Hard seed was only observed for the control line.

65. At an alternating 20-30 °C temperature regime, over 90% of seeds germinated within 12 days, with the remainder germinated but abnormal, or dead. There was no significant difference in seed rating between COT102, control and reference lines.

66. In summary, there were no measurable differences in germination, dormancy or viability between COT102 and comparable non-GM lines.

5.3.4 Compositional analysis of cottonseed

67. Compositional analysis of seed from COT102, alone or in combination with other GM traits, has been previously considered by the OGTR (DIR 101, DIR 124, DIR 143, DIR 145) and FSANZ (FSANZ, 2004). The GM seed was assessed to be compositionally equivalent to non-GM cotton.

68. The applicant provided compositional data for cottonseed harvested from experimental field plots of COT102 cotton and corresponding non-GM near-isogenic cotton (Coker 312) grown in six states of the USA in 2007 (McDonald, 2017). Cottonseed was acid-delinted and analysed for food and feed nutrients and antinutrients based on recommendations of the Organisation for Economic Co-operation and Development (OECD, 2004). The moisture content of COT102 cottonseed was significantly higher than for the non-GM comparator, and both moisture contents were higher than the range of values reported for acid-delinted cottonseed in the ILSI Crop Composition Database (ILSI-CCDB) (accessed 21 July 2017). The values for all other COT102 and non-GM cotton analytes fell within the ILSI-CCDB range of values for acid-delinted cottonseed.

69. For the remaining proximate analyses, COT102 ash content was significantly lower than non-GM ash; however protein, fat, carbohydrates, acid detergent fibre, neutral detergent fibre and total dietary fibre did not vary significantly (McDonald, 2017). Mineral analyses showed that COT102 had significantly lower concentrations of copper, magnesium, manganese, phosphorus and zinc than the non-GM comparator. Calcium, iron, potassium and sodium values did not vary significantly. There was no significant varietal difference in the concentration of Vitamin E or amino acids. COT102 had significantly higher oleic acid content than non-GM. There was no significant difference for the remaining measured fatty acids. COT102 had significantly lower concentrations of all antinutrients than the non-GM comparator. Measured antinutrients were free gossypol, total gossypol, sterculic acid, malvalic acid and dihydrosterculic acid.

70. In summary, the compositional data analysis supports the compositional equivalence of COT102 with non-GM cotton (McDonald, 2017). The component values that were statistically significantly different between COT102 cottonseed and non-GM cottonseed were not considered biologically significant.

5.3.5 Phenotypic and agronomic characterisation

71. Assessment of phenotypic and agronomic traits for COT102 in previous licences has not found any unintended or pleiotropic[1] effects of the inserted genes (OGTR: DIR 034/2003, DIR 036/2003, DIR 065/2006, DIR 101, DIR 120, DIR 124, DIR 143, DIR 145).

72. Data for plant growth and development of Bollgard® 3 compared with non-GM cotton was previously evaluated in the RARMP for DIR 124. Bollgard® 3 is a combination of two GM cotton lines, including COT102. Agronomic data was supplied from field trials in the USA and Australia. No statistically significant differences in growth and development were detected between Bollgard® 3 and the non-GM control.

73. The applicant provided agronomic performance data for COT102 and corresponding non-GM, near-isogenic cotton (Coker 312) grown in eight locations across seven states of the USA in 2002 (Negrotto and Potter, 2011). Overall, six of the seven measured characteristics varied significantly between COT102 and the non-GM cotton; however none of the characteristics varied significantly at all locations, as there were significant location-by-genotype interactions. Many of these differences were attributed to differences in lepidopteran pest damage. Early and final stand counts were significantly lower for COT102 than non-GM cotton; but these differences were not significant in plots that had been sprayed with insecticide. The position of the first fruiting branch was at a significantly lower node number in COT102 than non-GM cotton, although the total number of nodes per plant did not vary significantly between lines. COT102 plants at late bloom stage were significantly shorter than non-GM and, thus, the ratio of plant height to total number of nodes was also significantly lower in COT102. Yield of COT102 was significantly greater than non-GM in both sprayed and unsprayed treatments. In summary, although COT102 cotton had a yield advantage due to insect resistance, plants did not grow more vigorously than non-GM cotton and were unlikely to have a higher weediness potential.

74. While conducting experiments on differences in invertebrate communities, Whitehouse et al. (2007) measured the phenotypes of COT102, and non-GM Coker 312 and Sicala 40 varieties grown in field plots in Australia. In the Narrabri experiment there were no differences in height or node number between COT102 and the non-GM varieties; however, COT102 retained more bolls than the other two varieties. In the experiment at Kununurra, unsprayed COT102 plants were taller, and retained more squares and bolls, than Sicala 40 plants (the near-isogenic comparator of COT102, Coker 312, was not included in the Kununurra experiment). An increase in boll production was also recorded in an experiment with cotton containing a *Bt* gene for insect resistance (*Cry1Ac*), at some sites near Kununurra (Eastick and Hearnden, 2006); however the authors concluded that cotton genetically modified for insect resistance did not have greater weediness potential than non-GM cotton in northern Australia.

5.3.6 Effect on non-target invertebrates

75. The toxicity of the Vip3A protein expressed by COT102 to honey bees and other non-target invertebrates was discussed in the RARMP for DIR 124. A dietary exposure assay showed that there was no significant effect of the Vip3Aa protein on the Asian ladybird beetle *Harmonia axyridis* (Ali et al., 2017). Eleven representative non-target organisms including the invertebrates seven-spot ladybirds, pink-spotted ladybirds, insidious flower bugs, green lacewings, rove beetles, springtails, earthworms and honeybees, showed no adverse effects following oral administration of high levels of purified Vip3A protein (Raybould and Vlachos, 2011). A significant effect of Vip3A protein on the fecundity of water fleas (*Daphnia magna*) was attributed to reduced feeding due to an elevation of dietary protein content, rather than toxicity of the specific protein (Raybould et al., 2014).

[1] Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated multiple phenotypic traits (Kahl, 2001).

76. Although Vip3A is toxic to a range of lepidopteran insects (Table 3), the protein does not affect monarch butterfly larvae (Lee et al., 2003).

77. In their assessment of the environmental degradation of the Vip3A protein, the APVMA (2016) concluded that the protein degrades rapidly and does not persist in the soil. They reported that the estimated DT50 (time to dissipation of 50 % of initial bioactivity) towards black cutworm (*Agrotis ipsilon*) was 6.0–12.6 days at a soil concentration of 16 mg/g.

78. The effect of Vip3A expression by COT102 on arthropods was studied by Whitehouse et al. (2007) in Australian field experiments. The addition of insect resistance has an effect on the composition of invertebrate communities in the cotton crop, which in turn can affect the growth of crop plants. Although no major difference in species richness and biodiversity of beneficial or non-target arthropods was found between insect resistant and non-GM crops, the abundance of different insect species changed. Vip3A expression did not affect egg lay by *Helicoverpa* spp.; however larval numbers were reduced. Lower activity of Lepidoptera in COT102 crops reduces shedding of squares and bolls, and can result in larger plants (Section 5.3.5). The greater abundance of fruiting structures may lead to increased abundance of insect species, such as mirids and pollen beetles, for which these are a food source. The dynamics of insect populations in cotton crops, including predator and prey interactions, is complex and variable.

79. Similarly, field measurements of insect abundance in GM corn crops expressing stacked Vip3A and Cry1Ab proteins showed no significant difference in overall biodiversity compared with non-GM corn 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).

80. More broadly, the introduction of Cry protein *Bt* insect resistance traits has been associated with variations in the diversity of pest and beneficial insects in Chinese cotton fields. The abundance of mirids (Heteroptera: Miridae) increased in cotton crops and surrounding fruit crops following the introduction of *Bt* cotton, due to a reduction in insecticide spray applications (Lu et al., 2010). In response, farmers increased the frequency of insecticide sprays to control mirids in cotton; however the total number of insecticide sprays per season remained lower than prior to the introduction of *Bt* cotton.

81. Conversely, Lu et al. (2012) showed that the abundance of predators, namely ladybirds, spiders and lacewings, increased with the introduction of *Bt* cotton. These predators controlled aphid populations, which decreased in *Bt* crops. Yao et al. (2016) compared the abundance of aphids and their natural enemies on *Bt* cotton (containing Cry1Ac and Cowpea Trypsin Inhibitor, CpTI) and non-GM cotton. They found that the number of aphids and predators, including ladybirds, spiders and other arthropods, did not vary significantly between the two cultivars, with the exception of parasitoids mummies.

82. Han et al. (2016) reviewed the effect of insect resistance traits in GM crops on the behaviour of target and non-target arthropods. For example, the spatial distribution of insects may vary between crops, e.g. aphids are found on different parts of the plant in *Bt* cotton, compared with non-*Bt* cotton. Non-target species are often reported to preferentially feed on *Bt* crops. This may be due to *Bt* plants experiencing less herbivory from target insects and thus producing lower levels of defensive secondary metabolites. In general, behavioural changes were more likely to affect target insect species, with limited effects on the natural enemies of arthropods.

Section 6 The receiving environment

83. 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).

84. The applicant has proposed to release COT102 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. Commercial cotton production occurs mainly in New South Wales, southern and central Queensland, and northern Victoria, and on a trial basis in northern Queensland, northern Western Australia and the Northern Territory. 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

85. It is anticipated that the agronomic practices for the cultivation of the GM cotton 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 current commercially grown non-GM and 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, 2016).

6.2 Relevant abiotic factors

86. 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, 2016). 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 sexually compatible plants in the receiving environment

87. 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 must occur and progeny must 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. Cotton does not reproduce by asexual mechanisms, although root cuttings can be propagated under laboratory conditions (OGTR, 2016).

88. There are 17 native species of *Gossypium* in Australia, most of which are found in the NT and the north of WA (OGTR, 2016). Only three of these species are likely to occur in the regions of Australia where cotton is cultivated: *G. sturtianum, G. nandewarense*, and *G. australe*. However, native *Gossypium* species prefer well-drained sandy loams and are rarely found on heavy clay soils favoured by cultivated cotton.

89. Furthermore, the likelihood that *G. hirsutum* could hybridise successfully with any of the native Australian cottons is extremely low, due to genetic incompatibility. 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, 2016).

90. *Gossypium hirsutum* is sexually compatible with the other species of cultivated cotton, *G. barbadense* (Pima cotton). Commercial cotton grown in Australia is predominantly *G. hirsutum.* The amount of *G. barbadense* cotton grown in Australia has declined, making up around 1% of cotton planted in 2006 (OGTR, 2016), with no *G. barbadense* varieties being sold in the 2017/18 season (CSD, 2017). The GM *G. hirsutum* proposed for release is capable of crossing with both species of commercially grown cotton.

91. More than 98% of the Australian cotton crop is genetically modified (ABARES, 2017). Currently licenced GM cotton varieties are listed in Table 5; however not all varieties are available to growers in the 2017/18 season (CSD, 2017). Bollgard® 3 varieties made up 92% of the national cotton crop in the 2016/17 season (Monsanto Company website, accessed 13 July 2017). Bollgard® 3 contains stacked insect resistance GM traits, including COT102, while Bollgard® 3 x Roundup Ready Flex® contains an additional GM herbicide tolerance trait.

Table 5 Current commercial releases of GM cotton in Australia

DIR licence number	Cotton variety	GM agronomic traits
062/2005	Liberty Link®	Contains the *bar* gene for herbicide tolerance
066/2006	Bollgard II® (BGII), Roundup Ready® (RR), Roundup Ready Flex® (RRF), RR/BGII, RRF/BGII (north of latitude 22° South)	Contains *cry1Ac* and *cry2Ab* for insect resistance, and *cp4 epsps* for herbicide tolerance
091	WideStrike™	Contains *cry1Ac* (*synpro*) and *cry1F* (*synpro*) for insect resistance
118	Roundup Ready Flex® *Gossypium barbadense*	Contains *cp4 epsps* for herbicide tolerance
124	Bollgard® 3, Bollgard® 3 Roundup Ready Flex®	Contains *cry1Ac, cry2Ab* and ***vip3Aa19*** for insect resistance, and *cp4 epsps* for herbicide tolerance
143	GlyTol®, GlyTol TwinLink Plus®	Contains *cry1Ab, cry2Ae* and ***vip3Aa19*** for insect resistance, and *2mepsps* and *bar* for herbicide tolerance
145	Bollgard® 3 XtendFlex™, XtendFlex™	Contains *cry1Ac, cry2Ab* and ***vip3Aa19*** for insect resistance, and *cp4 epsps*, *dmo* and *bar* for herbicide tolerance

6.3.2 Presence of other biotic factors

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

93. Many cotton growing areas across Australia also have important non-lepidopteran insect pests. These include cotton aphids (*Aphis gossypii*), green mirids (*Creontiades dilutus*), brown mirids (*C. pacificus*), two-spotted spider mites (*Tetranychus urticae*), silverleaf whitefly (*Bemisia tabaci*), thrips (*Thrips tabaci*, *Frankliniella schultzei* and *F. occidentalis*), green vegetable bugs (*Nezara viridula*) and solenopsis mealybugs (*Phenacoccus solenopsis*) (CRDC and CottonInfo, 2017).

94. Many other arthropods are associated with cotton fields, including beneficial organisms such as spiders, ladybird beetles, earwigs, hoverflies, bugs, bees, parasitoid wasps and flies, and lacewings (Whitehouse et al., 2005).

95. Australian cotton is affected by a number of soil-borne and foliar fungal diseases, along with oomycete, bacterial and viral diseases. Fungal pathogens cause the major diseases Verticillium wilt (*Verticillium dahliae*) and Fusarium wilt (*Fusarium oxysporum* f. sp. *vasinfectum*; FOV). Common seedling diseases of cotton are black root rot (*Thielaviopsis basicola*) and damping off (caused by *Rhizoctonia solani*, *Pythium* spp. and *Phytophthora* spp.). Leaves may be affected by Alternaria leaf spot (*Alternaria* spp.) and cotton bunchy top virus spread by aphids. Boll rots are caused by different pathogens, including fungi, bacteria and oomycetes (CRDC and CottonInfo, 2017).

96. Reniform nematode (*Rotylenchulus reniformis*) emerged as a new pest in Central Queensland in 2012. The soil-borne plant parasite has a wide host range and is found in a broad range of climatic conditions (CRDC and CottonInfo, 2017).

97. Cotton is susceptible to competition from weeds. Problematic weeds range from large plants such as Noogoora burr (*Xanthium occidentale*), Bathurst burr (*X. spinosum*), thornapples (*Datura* spp.) and sesbania (*Sesbania canabina*), to vines such as cowvine and bellvine (*Ipomoea* spp.), yellow vine or spine-less caltrop (*Tribulus* spp.), to grasses such as nut grass (*Cyperus rotundus*) (CRDC, 2013b). Some weed species are alternate hosts for diseases of cotton, e.g. many weeds are hosts for *Verticillium dahliae* (CRDC and CottonInfo, 2017).

6.3.3 Presence of resistance to the Vip3A protein in lepidopteran pests

98. Prior to the introduction of GM crop varieties incorporating *vip3A* insect resistance genes, Vip3A resistant alleles were found to be present at frequencies of 0.027 and 0.008 in Australian populations of *Helicoverpa armigera* and *H. punctigera,* respectively (Mahon et al., 2012). Resistance was generally recessive, although heterozygous colonies had slightly increased tolerance to Vip3A treatment than homozygous susceptible colonies.

6.3.4 Use of hygromycin B in agriculture and medicine

99. Internationally, hygromycin B is used in animal production as a feed additive for swine and chickens to kill parasitic worms, e.g. in Hygromix® products registered by the U.S. Food & Drug Administration (US FDA website, accessed 3 August 2017). Hygromycin B is currently not registered for use as a veterinary medicine in Australia (APVMA PubCRIS database, accessed 5 July 2017) and is not on the international *OIE List of Antimicrobial Agents of Veterinary Importance* (OIE, 2015).

100. Hygromycin B is not used in human medicine in Australia and is currently not listed in the Australian Register of Therapeutic Goods (TGA website, accessed 17 August 2017). Furthermore, the antibiotic is not considered high priority for managing the development of antibiotic resistance: it is not listed in the Australian Strategic and Technical Advisory Group on Antimicrobial Resistance's *Importance Ratings and Summary of Antibacterial Uses in Humans in Australia* (ASTAG, 2015) or the *World Health Organization list of Critically Important Antimicrobials for Human Medicine* (WHO, 2017).

101. In addition to hygromycin B, the HPT protein phosphorylates the closely related compounds hygromycin B_2, destomycin A and destomycin B (Rao et al., 1983; FSANZ, 2006). These compounds are not generally used in human or veterinary medicine.

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

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

103. The *vip3Aa19* gene was isolated from a bacterium *Bacillus thuringiensis* (*Bt*) that is common in soil worldwide. Microbial preparations of *Bt* are used as insecticide sprays in Australia, particularly in organic agriculture and domestic gardening (APVMA PubCRIS database, accessed 5 July 2017).

104. The *aph4* gene was isolated from the common bacterium *E. coli*, which is part of the normal flora of human and animal guts.

Section 7 Previous authorisations

7.1 Australian authorisations of COT102

105. The Regulator has issued fourteen licences for COT102 cotton for limited and controlled, and commercial releases (Table 6). These licences have been issued for the COT102 event alone or in combination with other insect resistance and, in some cases, herbicide tolerance traits. Previous assessments of COT102 concluded that the event poses negligible risks to human health and safety, and the environment.

106. In 2014, DIR 124 licenced the use of the COT102 trait (*vip3Aa19* gene) in combination with the *cry1Ac* and *cry2Ab* insect resistance genes in the lines Bollgard® 3 and Bollgard® 3 x Roundup Ready Flex® (with the addition of a herbicide tolerance gene). Bollgard® 3 varieties made up 92% of the cotton planted in Australia in the 2016/17 season (Monsanto Company website, accessed 13 July 2017). As such, experience with Bollgard® 3 is important to the risk context for this RARMP.

107. To date, the Regulator has not received any reports of adverse effects on human health, animal health or the environment caused by any releases of COT102 cotton. There are no scientific studies showing adverse effects of COT102 cotton grown as a crop on human health or the environment in Australia.

Table 6 Previous releases of COT102 in Australia

DIR licence number	Licence type	Title	Additional GM agronomic traits
017/2002	L&C[a]	Agronomic assessments and efficacy studies of transgenic cotton expressing a new insecticidal gene	
025/2002	L&C	Seed increase and efficacy studies in Northern Australia of transgenic cotton expressing a new insecticidal protein gene *(vip3A)*	
034/2003	L&C	Field Trial of Genetically Modified Cotton *(Gossypium hirsutum)* Expressing an Insecticidal Gene *(vip3A)*	
036/2003	L&C	Breeding and pre-commercial evaluation of transgenic cotton expressing a vegetative insecticidal protein (VIP) and a herbicide tolerance gene	HT[c]: *bar*
058/2005	L&C	Limited and controlled release of insect resistant (VIP) GM cotton	
065/2006	L&C	Limited and controlled release of GM insect resistant (VIP3A and/or modified Cry1Ab) cotton	IR[d]: *cry1Ab*
073/2007	L&C	Limited and controlled release of GM insect resistant and insect resistant/herbicide tolerant cotton	IR: *cry1Ab*; HT: *cp4 epsps*
101	L&C	COT102 alone and in combination with the *cry1Ab* insect resistance gene.	IR: *cry1Ac, cry2Ab*; HT: *cp4 epsps*
120	L&C	Limited and controlled release of cotton genetically modified for insect resistance and herbicide tolerance	IR: *cry1Ac, cry2Ab*; HT: *dmo*, *bar*, *cp4 epsps*
124	C[b]	Commercial release of cotton genetically modified for insect resistance and herbicide tolerance (Bollgard® III and Bollgard® III x Roundup Ready Flex®)	IR: *cry1Ac, cry2Ab*; HT: *cp4 epsps*
133	L&C	Limited and controlled release of cotton genetically modified for insect resistance and herbicide tolerance	IR: *cry1Ab, cry2Ae*; HT: *bar*, *2mepsps*
143	C	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))	IR: *cry1Ab, cry2Ae*; HT: *bar*, *2mepsps*
145	C	Commercial release of cotton genetically modified for insect resistance and herbicide tolerance (Bollgard® 3 XtendFlex™ (SYN-IR102-7 x MON 15985-7 x MON-88913-8 x MON 88701-3) and XtendFlex™ (MON-88913-8 x MON 88701-3) cotton)	IR: *cry1Ac, cry2Ab*; HT: *dmo*, *bar*, *cp4 epsps*
147	L&C	Limited and controlled release of cotton genetically modified for insect resistance and herbicide tolerance	IR: *cry1Ac, cry2Ab, mCry51Aa2*; HT: *dmo*, *bar*, *cp4 epsps*

[a] L&C, limited and controlled release; [b] C, commercial release; [c] HT, herbicide tolerance; [d] IR, insect resistance

7.2 Approvals by other Australian agencies

108. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. However, 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.

109. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved food derived from the oil and linters of COT102 as safe for human consumption (FSANZ, 2004, 2006).

110. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. COT102 meets the definition of an agricultural chemical product under the Agricultural and Veterinary Chemicals Code Act 1994, due to its production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA. The APVMA has approved *Bacillus thuringiensis* strain AB88 exotoxin, VIP3A, as an insecticide for use in Bollgard® 3 cotton (APVMA, 2016).

7.3 International authorisations and experience

111. A number of countries have approved COT102 for commercial cultivation, as well as food and feed use (Table 7).

Table 7 International approvals of COT102

Country	Food - direct use or processing	Feed - direct use or processing	Cultivation - domestic or non-domestic use
Brazil	2016[a]	2016[a]	2016[a]
Canada	2011	2011	
China	2016	2016	
Colombia	2016		
Costa Rica			2009[a]
Japan	2012	2012	2012
Mexico	2010	2010	2010[b]
New Zealand	2005		
Philippines	2015	2015	
South Korea	2014	2015[a]	2014[b]
Taiwan	2015		
USA	2005	2005	2011

Source: ISAAA GM approval database; accessed July 2017; [a] in combination with other events; [b] for processing only.

112. In addition to the countries listed in Table 7, COT102 has been released for the purpose of regulatory trials, efficacy testing, yield testing, product development, and/or demonstration in Argentina, South Africa, Burkina Faso, Zimbabwe, India and Vietnam (data supplied by applicant). Field trials of COT102, in combination with other events, were also approved in Spain (Biosafety Clearing House, accessed 21 August 2017).

113. There have been no reports in the international literature of harm to human health and safety, or the environment, resulting from field trials or commercial release.

Chapter 2 Risk assessment

Section 1 Introduction

114. 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.

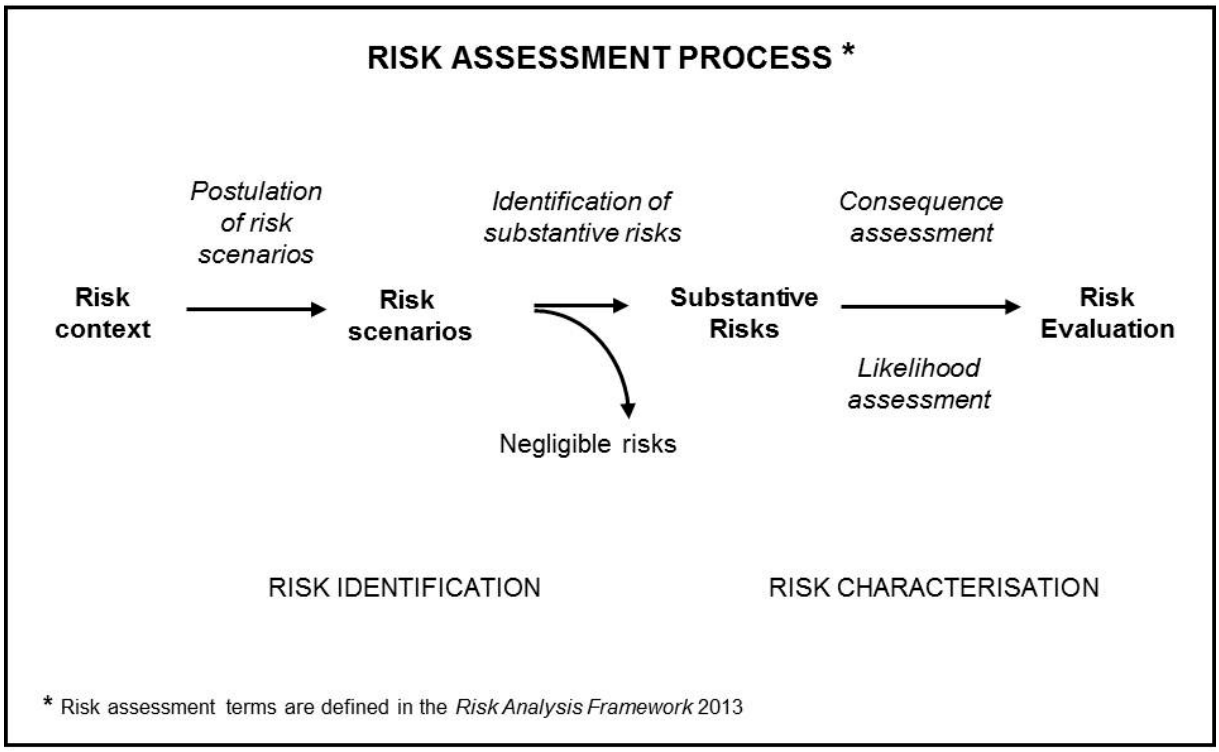

Figure 2 The risk assessment process

115. 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 in the short and long term. These are called risk scenarios.

116. 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, as this approach addresses the full range of potential adverse outcomes associated with 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., 2014). Risk scenarios postulated in previous RARMPs prepared for licence applications for the same or similar GMOs are also considered.

117. Postulated risk scenarios are screened to identify those that are 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.

118. 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). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

119. Postulated risk scenarios are comprised of three components:

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

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

iii. Potential harm to people or the environment.

120. 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
- any proposed limits including the extent and scale of the proposed dealings
- any proposed controls to restrict the spread and persistence of the GMOs
- the characteristics of the parent organism(s).

2.1 Risk source

121. The sources 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.

122. As discussed in Chapter 1, Section 5.1.1, the GM cotton proposed for release has been modified by the introduction of an insect resistance gene. This introduced gene and its encoded protein are considered further as a potential source of risk.

123. COT102 also contains the *aph4* antibiotic resistance selectable marker gene. The HPT protein encoded by *aph4* inactivates the antibiotic hygromycin B, and could therefore potentially interfere with hygromycin feed additive treatment of livestock eating GM cottonseed. However, hygromycin is not registered for use as a veterinary medicine in Australia (Section 6.3.4), and feed safety of exported GM cottonseed is assessed by the importing country. Furthermore, HPT is expressed in all COT102 cotton plant tissues at extremely low concentrations (Table 4). The *aph4* 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.

124. The introduced genes are controlled by introduced regulatory sequences. These regulatory sequences are derived from thale cress and a common soil bacterium (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 in GM cotton lines grown commercially in Australia and overseas without reports of adverse effects. Hence, potential risks from the regulatory elements will not be considered further.

125. The genetic modification has 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 protein, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously in plants generated by conventional breeding. Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al., 2015). Plants generated by conventional breeding have a long history of safe use, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013). No biologically significant differences were found in the biochemistry, physiology or ecology of COT102, when compared with non-GM cotton (Chapter 1, Section 5.3), and the introduced genes are stable (Chapter 1, Section 5.3.1).Therefore, unintended effects resulting from the process of genetic modification will not be considered further.

2.2 Causal pathway

126. 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 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 of the GM plants (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. pests, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer
- unauthorised activities.

127. Although all of these factors are taken into account, some are not included in risk scenarios because they are regulated by other agencies, have been considered in previous RARMPs or are not expected to give rise to substantive risks (see Sections 2.2.1 to 2.2.4, below).

2.2.1 Insects developing resistance to a single-gene GM line

128. Since the release of the first insect resistance cotton variety, Ingard®, the Australian cotton industry has developed resistance management plans (RMP) to reduce the risk of *Helicoverpa armigera* developing resistance to *Bt* proteins (Wilson et al., 2013). The Transgenic and Insecticide Management Strategy (TIMS) Committee is involved in the development and revision of RMPs for insect resistant cotton varieties (Downes and Mahon, 2012). These RMPs have resulted in Australian populations of *H. armigera* remaining susceptible to the Cry1Ac and Cry2Ab insect resistance toxins since the first introduction of GM cotton producing Cry1Ac in 1996 (Tabashnik and Carriere, 2017).

129. The RMP for Ingard®, which contains a single Cry1Ac insect resistance gene, included a cap of 30% total cotton planting area, along with refuge crops, a defined sowing period and “pupae busting” cultivation following harvest. Single gene varieties are at higher risk of resistance development, and the Vip3A protein already has higher than expected baseline resistance in Australian *Helicoverpa*

populations (Mahon et al., 2012). As insect resistance management to Vip3A would be regulated by TIMS and APVMA, this potential risk will not be further considered for this application.

2.2.2 Tolerance to abiotic factors

130. 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, 2016). The introduced gene is unlikely to make the GM cotton plants more tolerant to abiotic stresses that are naturally encountered in the environment and is therefore unlikely to alter the potential distribution of the GM cotton plants. Also, as discussed in Chapter 1, Section 5.3, there was no consistent significant difference between COT102 and non-GM cotton varieties in response to abiotic factors. Therefore, tolerance to abiotic stresses will not be assessed further.

2.2.3 Gene transfer to sexually compatible relatives

131. 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. Gene transfer to Australian native cotton species is not expected due to genetic incompatibility.

132. The potential for adverse effects resulting from the transfer of the *vip3Aa19* gene into non-GM cotton or currently licenced GM cotton varieties (see Table 5) was considered in detail in the RARMPs for DIR 124 and DIR 143. The risk of gene transfer was not considered substantive as resulting hybrids would be transient, and would not lead to increased toxicity to people or other desirable organisms.

133. In 2016/17, over 90% of the Australian cotton crop was sown to Bollgard® 3 varieties, which contain the *vip3Aa19* gene (Monsanto Company website, accessed 13 July 2017), with no reports of adverse effects. Therefore, gene transfer to sexually compatible relatives will not be assessed further.

2.2.4 Horizontal gene transfer

134. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in the literature (Keese, 2008) and assessed in previous RARMPs. No risk greater than negligible was identified, due to the rarity of HGT events and because the gene sequences (or sequences which are homologous to those in the current application) are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, HGT will not be assessed further.

2.2.5 Unauthorised activities

135. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome. 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

136. Potential harms from GM plants include:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity for nature conservation
- reduced establishment or yield of desirable plants
- 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).

137. These harms are based on those used to assess risk from weeds (Standards Australia et al., 2006; Keese et al., 2014). Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. For example, a plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

138. Three risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 8 and discussed in depth in Sections 2.4.1 to 2.4.4. 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 commercial use or the spread and persistence of plant material.

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

Table 8 Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced gene for insect resistance.	Commercial cultivation of GM cotton expressing the insect resistance gene. ⬇ Exposure of people and organisms other than insects to the introduced protein by contact, ingestion, or inhalation.	• Increased toxicity or allergenicity to people. • Increased toxicity to desirable organisms.	No	• Limited exposure of humans to the Vip3A protein. • Lack of toxicity or allergenicity of Vip3A protein to people • Lack of toxicity of Vip3A protein to organisms other than insects.
2	Introduced gene for insect resistance.	Commercial cultivation of GM cotton expressing the insect resistance gene. ⬇ Exposure of non-target insects to GM plant material through contact or ingestion.	• Increased toxicity to non-target insects.	No	• Lack of toxicity of Vip3A protein to non-lepidopteran insects. • Insect control methods for non-GM cotton affect a greater range of insects than the Vip3A protein.
3	Introduced gene for insect resistance.	Dispersal of GM cottonseed outside intended cropping areas. ⬇ Establishment of populations of volunteer GM plants. ⬇ Reduced insect herbivory of GM plants, leading to increased spread and persistence.	• Reduced establishment of desirable vegetation. • Reduced quality of the biotic environment. • Toxicity or allergenicity in people or toxicity to desirable organisms.	No	• Cotton has limited ability to establish outside of cultivation. • Spread and persistence of cotton is restricted by factors other than lepidopteran herbivory. • The Vip3A protein is not toxic to humans or desirable organisms.

2.4.1 Risk scenario 1

Risk source	Introduced gene for insect resistance.
Causal pathway	⬇ Commercial cultivation of GM cotton expressing the insect resistance gene. ⬇ Exposure of people and organisms other than insects to the introduced protein by contact, ingestion or inhalation. ⬇
Potential harm	Increased toxicity or allergenicity to people. OR Increased toxicity to desirable organisms.

Risk source

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

Causal pathway

141. The insect resistance gene *vip3Aa19* is expressed in the vegetative parts, pollen and seed of the GM cotton plants (Chapter 1, Section 5.2.3). Therefore, people may be exposed to the Vip3A protein through contact with plant parts, consumption of plant parts, or inhalation of pollen. However, the introduced gene and expressed protein is not present in cotton products such as cottonseed oil, fibres and linters (FSANZ, 2006; US EPA, 2008). Therefore, the majority of people that would be exposed to the introduced gene and its product would be workers involved with breeding, cultivating, harvesting, transporting and processing the GM cotton. The public, who consume cottonseed oil and cottonseed linters, or have contact with cotton fabrics, would not be exposed to the introduced gene and its product.

142. Expression of the insect resistance gene in cultivated GM cotton plants, or in volunteer GM cottons, may expose other organisms, including livestock, to the GM protein through contact or ingestion. Apart from presence in all parts of the GM cotton plants, the insecticidal protein may also occur at low levels in the soil from plant material left after harvesting and exudates from roots. However, the Vip3A protein degrades rapidly in soil (Chapter 1, Section 5.2.3).

143. Livestock are exposed to cotton in the form of white cottonseed and cottonseed meal in feed rations, or through limited grazing of stubble. However, the amount of cotton plant material (both GM and non-GM) that is consumed by livestock is, by necessity, limited due to the presence of endogenous toxins such as gossypol. Other organisms, including wild mammals, birds, soil microbes and non-insect invertebrates would also be exposed to GM cotton material in agricultural areas under GM cotton cultivation. These organisms may be exposed to the introduced insecticidal protein through contact, ingestion or indirectly by feeding on herbivores that have ingested the GM cotton.

Potential harm

144. People exposed to the Vip3A protein expressed by the introduced insect resistance gene are not expected to suffer toxic effects or allergic reactions. As discussed in Chapter 1, Section 5.2.3, the Vip3A protein is unlikely to pose any toxicity hazard to humans, other vertebrates, or the great majority of non-target invertebrates that lack the receptors to which Vip3A binds.

145. The Vip3A protein has no sequence similarity to known protein allergens, and is degraded in simulated gastric fluid, whereas oral allergens are typically resistant to degradation (Hill et al., 2003). Therefore, the insect resistance gene product is not considered toxic or allergenic to workers involved in breeding, cultivating, harvesting, transporting and processing the GM cotton.

146. FSANZ assessed the safety of human food derived from linters and cottonseed oil of COT102, and concluded that it is safe for human consumption (FSANZ, 2004, 2006).

147. The introduced insecticidal gene product is not expected to be toxic to animals other than insects. In dietary exposure studies, the insecticidal properties of the Vip3A protein did not result in adverse effects on springtails, earthworms, bobwhite quails, mice, water fleas and channel catfish (Raybould and Vlachos, 2011; Raybould et al., 2014). Strains of *Bt* carrying *vip3A* gene homologues can be isolated from the alimentary tracts of small mammals, including voles, mice and shrews, and apparently cause no harm (Swiecicka et al., 2011).

148. *Bt* bacteria are ubiquitous in the environment (CERA, 2012), with a high proportion of strains carrying *vip3A* genes (Beard et al., 2008). Therefore, it is expected that microorganisms, especially soil microorganisms, are regularly exposed to the Vip3A protein. There is no evidence from currently available literature to suggest that the Vip3A protein or similar proteins are toxic to microorganisms including various species of protozoa, bacteria, fungi, algae and diatoms.

Conclusion

149. Risk scenario 1 is not identified as a substantive risk, due to limited exposure of humans to the expressed Vip3A protein, the lack of toxicity or allergenicity of Vip3A to humans, and the lack of toxicity of Vip3A to organisms other than insects. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	Introduced gene for insect resistance.
Causal pathway	⬇ Commercial cultivation of GM cotton expressing the insect resistance gene. ⬇ Exposure of non-target insects to GM plant material through contact or ingestion. ⬇
Potential harm	Increased toxicity to non-target insects.

Risk source

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

Causal pathway

151. Expression of the insect resistance gene in pollen, seed and vegetative material of cultivated or volunteer GM cotton could directly expose non-target insects to the Vip3A protein through contact or ingestion, or indirectly expose them to Vip3A via feeding on herbivores that feed on the GM material. Non-target insects with exposure to Vip3A could include pollinators such as bees, non-pest insect species that consume the GM crop, and desirable insects such as parasitoids and other natural insect predators of pest organisms. Pollinators would be exposed to nectar and pollen from the GM cotton. Soilborne insects such as springtails would contact root exudates or decomposing plant material after harvest.

Potential harm

152. Exposure of non-target insects to the Vip3A protein expressed by the introduced *Bt* insect resistance gene may result in adverse effects such as death, slowed growth rate or reduced fecundity if the protein is toxic to exposed organisms. Arthropods that depend on lepidopteran insects in the food web could be adversely affected due to the loss of a food source.

153. As discussed in Chapter 1, Section 5.3.6, Vip3A protein has been assessed for potential toxicity to non-target invertebrates through testing of a range of representative species including bees, bugs, beetles, springtails, water fleas, lacewings and monarch butterflies. From such testing it was concluded that plants expressing Vip3A have only a narrow range of target specificity within lepidopteran species and would not harm non-lepidopterans.

154. Within the Lepidoptera, most toxicity studies have focussed on species that are pests of cotton or other crops (Table 3). The only non-target lepidopteran challenged with Vip3A is the North American monarch butterfly (*Danaus plexippus*), which is not susceptible and whose range extends to Australia. It is not known whether native Australian non-target lepidopteran species are susceptible to Vip3A. Native lepidopterans would only be exposed to Vip3A if they consume cotton, which is not a native Australian species.

155. Australian field experiments with COT102 did not reveal any major effects on non-target insects, although there were some shifts in the abundance of different arthropods caused by the effective control of target pests (Whitehouse et al., 2007). In the 2016/17 season over 90% of cotton production in Australia was planted to Bollgard® 3 varieties containing three *Bt* toxin genes including *vip3Aa19* (Chapter 1, Section 6.3.1), with no reported adverse effect on non-target insects.

156. Cotton pests susceptible to Vip3A, in particular *H. armigera* and *H. punctigera*, are controlled in non-GM crops by spraying with broad spectrum insecticides (CRDC and CottonInfo, 2017). These sprays kill lepidopteran insects, as well as any non-target insect species present in the crop.

157. Vip3A or very similar proteins are present in microbial formulations in commercial *Bt* insecticide preparations used on organic crops (Hill et al., 2003). It is expected that GM cotton containing only one *Bt* insecticidal gene would affect a narrower range of insects than whole *Bt* preparations containing multiple insecticidal genes expressing proteins that bind to different insect gut receptors.

Conclusion

158. Risk scenario 2 is not identified as a substantive risk due to the lack of toxicity of Vip3A protein to non-lepidopteran insects, and because insect control methods for non-GM cotton affect a greater range of insects than the Vip3A protein. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk source	Introduced gene for insect resistance.
Causal pathway	⬇ Dispersal of GM cottonseed outside intended cropping areas. ⬇ Establishment of populations of volunteer GM plants. ⬇ Reduced insect herbivory of GM plants, leading to increased spread and persistence. ⬇
Potential harm	Reduced establishment of desirable vegetation. OR Reduced quality of the biotic environment. OR Toxicity or allergenicity in people or toxicity to desirable organisms.

Risk source

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

Causal pathway

160. GM insect resistant cottonseed may be transported from farms into nature reserves by humans, animals, water or extreme weather. Cottonseed is primarily spread off-farm within a localised area during transport of modules to gins, and through irrigation and stormwater runoff (Chapter 1, Section 4.2). Cottonseed may also be dispersed during extreme weather events, i.e. via wind during wind storms and water during flooding, to adjacent agricultural areas and natural environments (OGTR, 2016).

161. GM cotton may be introduced into regions that do not grow the crop through the use of whole cottonseed for supplementation feeding of cattle and sheep, particularly during drought when large piles of cottonseed are dumped into a paddock for stock to feed on over the course of several days (QDAF website, accessed 25 August 2017; Business Qld website, accessed 25 August 2017). Cottonseed may also be introduced into environments around cattle feed lots and dairy farms, where it is used as stockfeed (OGTR, 2016). Cotton pickers can transfer seeds between fields and properties if they are not cleaned prior to transport (CRDC and CottonInfo, 2017).

162. Cotton volunteers are most likely to germinate in disturbed habitats, such as areas found on farms, in stockyards and adjacent to waterways (OGTR, 2016). Establishment of cotton in undisturbed natural environments is limited due to a range of abiotic and biotic factors, including lack of soil moisture, soil fertility, competition from other plants and weeds, herbivory by insects and animals, and fire (Eastick and Hearnden, 2006).

163. The GM cotton contains an insect resistance gene, which reduces herbivory by certain lepidopteran insects. Increased boll retention due to reduced insect activity may give GM cotton plants a competitive advantage, compared with non-GM cotton, by producing greater numbers of seeds. In field trials with COT102 near Narrabri, NSW, and Kununurra, WA, the presence of the insect resistance gene allowed COT102 plants to retain more bolls than non-GM comparator plants (Whitehouse et al., 2007). In the Kununurra plots unsprayed COT102 plants grew taller than unsprayed non-GM plants, although this may have been partly due to varietal differences.

164. Boll production and potential invasiveness of GM-cotton containing *Bt* insect resistance genes (*Cry1Ac* or *Cry1Ac* + *Cry2Aa*) has been studied in northern Australia (Eastick and Hearnden, 2006).

Seeds were shallowly sown in four habitats: bush, cattle feedlot, roadside and waterway. GM plants only produced more bolls than non-GM plants at one waterway habitat near Kununurra, WA. Of the seeds that germinated, fewer than 50% of plants survived after one year and fewer than 30% survived after two years at any location. After a further three years survival was below 5%. At these locations, the addition of one or more insect resistance genes did not improve the ability of cotton to survive long-term in non-cropping environments, compared with non-GM cotton.

Potential harm

165. If GM cottonseed 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 could have improved survival and persistence in the environment. The establishment of desirable native plants may be reduced, thereby adversely affecting native plant numbers and organisms reliant on those plants. This may reduce species richness, or cause undesirable changes in species biodiversity.

166. There is only limited evidence of persistence of naturalised cotton populations outside of cultivation in southern Australia. Small naturalised populations of *Gossypium hirsutum* are established in northern Queensland and the Northern Territory, likely as a result of cultivated cotton being grown in those regions in the early 1800s, but there is no evidence that cotton has become an invasive or problematic weed (OGTR, 2016).

167. The GM insect resistance trait is unlikely to improve the fitness of cotton plants, as the effect of lepidopteran herbivory on the persistence of volunteer cotton is minimal, compared with the range of abiotic and biotic factors that limit establishment (Eastick and Hearnden, 2006). Thus, COT102 would not have a greater ability to outcompete native vegetation or reduce biodiversity compared with non-GM cotton.

168. Cottonseed is more likely to germinate and establish in disturbed areas, e.g. agricultural environments (OGTR, 2016). If cotton crop plants or volunteers are not adequately managed, ratoon cotton may regrow from root stock the following season (CRDC and CottonInfo, 2017). Management practices for controlling volunteer and ratoon cotton in agricultural environments are not affected by the COT102 event. Herbicides can be used to control volunteers up to nine nodes, with mechanical removal required for larger plants and ratoons (CRDC and CottonInfo, 2017).

169. Volunteer and ratoon cotton can harbour pests and diseases of cotton, including aphids, mealybugs and cotton bunchy top, which can affect subsequent cotton crops. Differences in the type and number of arthropod species have been recorded between COT102 and non-GM cotton crops (Whitehouse et al., 2007); however there is no evidence that COT102 is a more effective reservoir host for pests and diseases than non-GM cotton.

170. Expression of the introduced insecticidal gene in volunteer cotton could expose people or other desirable organisms, such as livestock or beneficial insects, to the Vip3A protein. However, as discussed in Risk scenarios 1 and 2, the Vip3A protein has no demonstrated toxicity or allergenicity to humans or toxicity to other desirable and non-target organisms.

Conclusion

171. Risk scenario 3 is not identified as a substantive risk because cotton has limited ability to establish outside cultivation. Establishment of cotton populations outside intended cropping areas and competition with desirable vegetation is limited by abiotic factors, rather than lepidopteran herbivory. Furthermore, the Vip3A protein is not toxic or allergenic to humans or desirable organisms. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

172. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis[2]. 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.

173. Uncertainty is addressed by approaches including 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.

174. Uncertainty can arise from a lack of experience with the GMO. The level of uncertainty is low for COT102 given years of experience growing this GMO in Australia and internationally. The Vip3A protein is expressed in Bollgard® 3 cotton, which is currently the predominant variety grown in Australia. None of the previous releases of COT102 has resulted in concerns for human health, safety or the environment.

175. There is a lack of dietary feeding studies examining the effect of the Vip3A protein on native Australian Lepidoptera. However, as outlined in risk scenario 2, another non-target Lepidopteran, the Monarch butterfly, is not susceptible and native lepidopterans would need to feed on cotton to be exposed.

176. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

177. Post release review (PRR) will be used to address uncertainty regarding future changes to knowledge about the GMO or the receiving environment (Chapter 3, Section 4). PRR is typically required for commercial releases of GMOs, which generally do not have limited duration.

Section 4 Risk evaluation

178. 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.

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

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

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

180. Three 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 8.

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

Chapter 3 Risk management plan

Section 1 Background

182. 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 proposed licence conditions.

183. 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.

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

185. 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

186. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed release of COT102 cotton. These risk scenarios were considered in the context of the large scale of the proposed release and the receiving environment. The risk evaluation concluded that no controls are required to treat these negligible risks.

Section 3 General risk management

187. 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 requirements
- access for the purpose of monitoring for compliance.

3.1 Applicant suitability

188. 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

- 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.

189. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers Syngenta suitable to hold a licence. The licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

190. 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

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

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

192. Any person, including the licence holder, may conduct any permitted dealing with the GMO.

3.4 Reporting requirements

193. The licence requires 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.

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

195. 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

196. 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.

197. 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

198. 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.

199. 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).

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

200. 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), 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 GMOs.

4.2 Requirement to monitor specific indicators of harm

201. 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.

202. 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. The licence holder is required to monitor these specific indicators of harm as mandated by the licence.

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

204. 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 warranted further detailed assessment. Uncertainty is considered to be low. No specific indicators of harm have been identified in this RARMP for application DIR 157. However, specific indicators of harm may also be identified during later stages, e.g. through either of the other components of PRR.

205. 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

206. 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

207. The risk assessment concludes that the proposed commercial release of GM cotton (COT102) poses negligible risks to the health and safety of people or the environment as a result of gene technology and that these negligible risks do not require specific risk treatment measures.

208. 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 how they were addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	No comment relating to this matter.	Noted.
2	The following should be considered in preparation of the RARMP: • Whether hygromycin resistance has been conferred to the genetic characteristics of the GM plant cells. • Draw upon the expertise of the Australian Strategic and Technical Advisory Group (ASTAG) on antimicrobial resistance (AMR), with regard to any AMR risk posed by this cultivation method. This would provide broad, technical coverage within the government framework to support the OGTR assessment. • Consult Australia's antimicrobial national importance ratings, the OIE *List of antimicrobial agents of veterinary importance* and the WHO's *Critically Important Antimicrobials for Human Medicine* list. We have checked these lists and hygromycin is not found in any searches on these lists.	The hygromycin resistance gene is present in all GM plant cells (see Chapter 1, Section 5.3.1). Expression of the hygromycin resistance protein is low in all plant tissues (see Chapter 1, Section 5.3.2). ASTAG will be co-chaired by the Australian Government Chief Medical Officer (Department of Health) and Chief Veterinary Officer (Department of Agriculture and Water Resources). These two departments were invited to provide comments on this application and are invited to provide comments on the consultation RARMP. As hygromycin B is not used in human or veterinary medicine in Australia (Chapter 1, Section 6.3.4), the OGTR has not consulted ASTAG for this application; however, the OGTR will consider consulting ASTAG if future licence applications include resistance traits for antibiotics that are used in human or veterinary medicine in Australia. The use of hygromycin B in agriculture and medicine, with reference to these lists, is discussed in Chapter 1, Section 6.3.4.
3	No issues raised as the region is not a cotton growing region and is quite geographically removed from such areas.	Noted.
4	No comment provided, due to not having a specialist scientific expert to make an assessment.	Noted.
5	Noted that any proposed release should be undertaken in a way that is safe to both the public and the environment.	Risks to public safety and the environment from the proposed release are evaluated in Chapter 2, Sections 2.4.1 to 2.4.3 (Risk Scenarios 1 – 3). The consultation RARMP concludes that the risks are negligible.

Submission	Summary of issues raised	Comment
6	Noted that limited and controlled trials of the GM plants have been conducted under a number of DIR licences, and the genetic modification has been approved for commercial release as a stacked event in DIR licences 124, 143 and 145. Data and conclusions in these DIRs should be directly relevant to the RARMP for DIR 157. The following issues should be taken into consideration in the RARMP:	
	• Potential for the introduced genes to code for proteins with toxic properties and/or for these proteins to catalyse the production of a toxic metabolite in the GM plants.	
	• Address the specificity of the toxic properties of the Vip3Aa19 protein to target insects	Specificity of the Vip3Aa19 protein towards target organisms is discussed in Chapter 1, Sections 5.2.3 and 5.3.6.
	• The toxicity of the introduced protein should be analysed against a number of criteria, such as i) history of safe use, ii) bioinformatics data, iii) mode of action of the protein, and iv) digestibility of the protein (through *in vitro* tests). If the 'weight-of evidence' of an evaluation of such criteria suggests the protein is safe, then data from a 'higher tier' study may not be necessary.	The toxicity of the Vip3A protein, considering all the criteria mentioned, is discussed in Chapter 1, Section 5.2.3.
	• Address the potential of the Vip3Aa19 protein to produce (directly or indirectly) a toxic metabolite. Noted that there is no obvious way to connect the expression of the *vip3Aa19* gene with an increase in metabolites in cotton that have known toxic properties. The Vip3Aa19 protein does not appear to have an enzymatic activity that could affect the levels of these toxic metabolites.	Compositional analysis of the GM cotton, including levels of toxic metabolites, is discussed in Chapter 1, Section 5.3.4.
	• Review the toxicity of the protein product of the selectable marker gene (*aph4*). Noted that *aph4* has been used in a number of commercially released GM plants with no reports of it adversely affecting the health of humans, animals or the environment.	The toxicity of the HPT protein encoded by *aph4* is discussed in Chapter 1, Section 5.2.3.
	Potential for the introduced trait to increase innate weediness of cotton.	
	• Noted that *G. hirsutum* is not recorded in the Australian government's '*Weeds of National Significance*' list, the '*National Environment Alert List*' (a list of plant species in Australia that have been identified as potential weeds), or the '*Noxious Weed List for Australian States and Territories*'. Cotton is regarded (both in Australia and overseas) as a cultivated plant, which although it can be a problem in agricultural systems, is only a minor problem in natural ecosystems.	Noted.
	• Discuss the possibility that insect pressure is a significant factor in preventing the spread and	The weediness of cotton and its ability to spread and persist in natural ecosystems in

Submission	Summary of issues raised	Comment
	persistence of cotton in natural environments. Evaluate accumulated experience of dealing with both commercialised insect resistant GM cotton varieties and non-GM plants that have been conventionally bred for insect resistance. It is worth noting that release from insect herbivory does not appear to be a universal characteristic that can be associated with invasive plants.	Australia are discussed in Chapter 1, Section 4.2.
	• It is recommended that the RARMP thoroughly cover both the general factors that restrict the ability of cotton (unmodified and currently commercially released GM lines) to spread and persist in natural ecosystems, and the potential for the genetic modification to increase the ability of the GM plants to spread and persist.	The potential for the introduced insect resistance trait to increase the ability of cotton to spread and persist is addressed in Chapter 2, Section 2.4.3 (Risk Scenario 3).
	Potential for the genetic modification to transfer to another species and generate a plant with increased toxicity or weediness.	
	• Noted that there are indigenous *Gossypium* species in Australia. Studies have confirmed that due to different genome compositions, hybridisation between these Australian species and *Gossypium hirsutum* is unlikely. Although this topic has been considered in previous cotton RARMPs, it should be summarised in the RARMP for this application.	The presence of sexually compatible plant species and potential for hybridisation in Australia is discussed in Chapter 1, Section 6.3.1. The potential for the genetic modification to transfer to a sexually compatible plant species is discussed in Chapter 2, Section 2.2.3.
	The applicability of current methods used to manage the GM cotton.	
	• Cotton is a domesticated plant that is cultivated in Australia. There is extensive experience in the general management of this plant in agricultural settings, including other GM cotton varieties (some stacked) that have been engineered for insect resistance and the management of volunteers in natural ecosystems. This experience should be directly applicable to the management of the GM plants in this application, and therefore it is recommended that it is discussed in the RARMP.	Cultivation practices for cotton crops in Australia are discussed in Chapter 1, Section 6.1. Weed management practices for cotton volunteers are discussed in Chapter 1, Section 4.1.2 and their effectiveness is discussed in Chapter 1, Section 4.2.2.
7	Opposes the cultivation of GM crops in the environment, and considers that the local region should be a GMO free zone. Encourages elimination of GMOs from the food chain in the region. Requests that all foods containing GMOs should be clearly labelled and all sites where GMOs are grown should be mapped and publicly released.	The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. Declaration of areas to be GM free for marketing purposes is a power of State governments, not the Regulator. Food safety and labelling, including GM foods, is the responsibility of FSANZ. Labelling of GM status is legally required for GM foods that contain novel DNA or protein or have altered characteristics. Sites where GMOs are grown in field trials are mapped and publicly released on the OGTR website. Sites where GMOs are grown commercially are not mapped, because

Submission	Summary of issues raised	Comment
		GMOs are authorised for commercial release only if they are considered as safe as non-GM crops.
8	Objects to the release of GM cotton (DIR 157). States that insect resistance and antibiotic resistance are temporary achievements leading to further insect and antibiotic problems. States that too much cotton is grown and wasted, which in turn is a waste of land, water and climate resources. Instead of supporting this industry with regulatory approvals, the government should instead be regulating over-consumption of cotton, water and energy.	The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. Regulation of resource use or societal consumption related to the cotton industry is outside the scope of assessments conducted by the Regulator. The potential for the introduced insect resistance gene to lose efficacy over time is discussed in Chapter 1, Section 4.1.1 and Chapter 2, Section 2.2.1. It is noted that insect resistance management is a matter for the APVMA. The introduced antibiotic resistance gene was used during product development and is not intended to have any function in the field. The potential for risks from this gene is discussed in Chapter 2, Section 2.1.
9	Noted that the Shire is not aware of any public health concerns regarding this matter.	Noted.
10	Noted that the licence application did not include a thorough assessment of the effect of the GM cotton on non-mammalian organisms and immediate or long term environmental impacts and whether or not any harm is reversible. More detail was expected in the application. Advised that more information on the effect of the GM cotton on non-mammalian organisms be included in the RARMP.	The toxicity of the insect resistance protein to non-mammalian organisms and the effects on biodiversity are discussed in Chapter 1, Sections 5.2.3 and 5.3.6. The RARMP considers a range of publications from the scientific literature in addition to data provided in the licence application.
11	Noted that, overall, the application has negligible risks to the health and safety of people and the environment. The following matters should be considered in the RARMP: • Cotton can occasionally grow wild, but the proposed genetic modification under DIR 157 is not expected to increase the species' weed risk. • The application summary for DIR 157 states that the parent organism is cotton (*Gossypium hirsutum* L.), which is exotic to Australia and grown as an agricultural crop throughout Australia. *Gossypium hirsutum* L. is now naturalised in central and southern Queensland.	The weed risk potential of cotton in Australia is discussed in Chapter 1, Section 4.2. Information on the current distribution of naturalised or volunteer cotton is included.
12	The committee agrees with issues identified by OGTR for consideration in the RARMP. No further matters were identified by the committee for consideration in the RARMP. A committee member commented that there appear to be some concerns in the literature around the impact of Bt toxin release from roots on the soil biota	Noted. Toxicity of the Vip3A protein towards representative soil organisms and the rate of environmental degradation of the Vip3A

Submission	Summary of issues raised	Comment
	and diversity, and questioned whether the release of the Vip3Aa19 toxin from plant roots and the subsequent environmental impact has been fully considered or addressed by the applicant and the current regulatory approvals.	protein in soil are discussed in Chapter 1, Section 5.2.6. The risk of harm to soil organisms is evaluated in Chapter 2, Sections 2.4.1 and 2.4.2 (Risk Scenarios 1 and 2).

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

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

Submission	Summary of issues raised	Comment
1	No comment provided, as does not have a specialist scientific expert to make an assessment.	Noted.
2	No official policy on GM cotton, but would like this proposed release to be undertaken in a way that is safe to both the public and the environment.	Risks to public safety and the environment from the proposed release are evaluated in Chapter 2, Sections 2.4.1 to 2.4.3 (Risk Scenarios 1 – 3) of the RARMP. The RARMP concludes that the risks are negligible.
3	Do not have any concerns about the cotton application under DIR 157.	Noted.
4	Agrees with the overall conclusions of the RARMP.	Noted.
5	Agrees with the conclusions of the consultation RARMP and has no comments.	Noted.
6	Application has negligible risks to the health and safety of people and the environment, and therefore has no concerns about commercial release of COT102.	Noted.
7	Supported the OGTR's conclusion that the proposed dealing poses negligible risk to human health and safety and the environment.	Noted.

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

The Regulator received four submissions from the public on the consultation RARMP. The issues raised in these submissions are summarised in the table below. All issues 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	Comment
1	Disapproves of granting a licence.	
	States that cotton should not be grown in Australia due to its dry climate. Concerned regarding cotton grower corruption and disregard for the laws governing water rights. Growing GM cotton would exacerbate social problems and environmental degradation resulting from cotton farming practices.	The Act requires the Gene Technology Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. Regulation of the cotton industry in general is outside the scope of the Regulator's powers.
		The potential for the introduced insect resistance gene to cause increased environmental impact by the GM cotton, compared with non-GM cotton, is addressed in Chapter 2, Section 2.4.3 (Risk Scenario 3).
	Is concerned that Australia develops GM crops that would not be allowed to be grown in other parts of the world, such as GM wheat, and that the public is deliberately kept in ignorance.	The OGTR website lists all applications for Dealings involving Intentional Release of GMOs (DIRs) into the environment. The full list includes detailed descriptions of applications for field trial as well as commercial release.
	Is concerned that the applicant (Syngenta) carried out testing of the GM cotton. Questions whether the OGTR carried tests of its own, and whether the OGTR is fulfilling its role as a gene technology 'watchdog'.	Before the Regulator decides whether to issue a licence for release of GMOs, a risk assessment and risk management plan (RARMP) is prepared. The RARMP for the GM cotton includes a thorough and critical assessment of data supplied by the applicant, together with a comprehensive review of other relevant national and international scientific literature. The RARMP was finalised following an extensive consultation process involving expert scientists, Australian Government authorities and regulatory agencies, State and Territory Governments, relevant local councils, the Minister for the Environment and the public. The RARMP is supported by a previous assessment by FSANZ which found that food derived from the GM cotton is safe for human consumption. This is a transparent process, in which the licence application and all risk assessment documents and references are available to the public. The RARMP concluded that the commercial release of this GM cotton poses negligible risks to the health and safety of people or to the environment.

Submission	Summary of issues raised	Comment
2	Has serious concerns about the proposed release of GM cotton. Is concerned about climate change, soils and carbon dioxide sequestration. Is concerned about pesticide and herbicide use, and growing dependence on 'inorganic' additives. Questions the need for genetic modification and who benefits from the technology.	Noted. Issues relating to pesticide and herbicide use are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for agricultural chemicals, including pesticides and herbicides, in Australia. The Regulator is required to assess the risks of GMOs and cannot consider the benefits of gene technology when deciding whether or not to issue a licence. Therefore, no claims of benefits from GMOs have been taken into account when preparing the RARMP.
3	Supports the licence application as commodity cottonseed is important to the economics of cotton production at the field, processing and trade level. This licence would assist in sustaining the viability of cotton production in Australia.	Noted.
4	Supports use of transgenic insecticidal (Bt) technology to deliver productivity and sustainability gains to cotton growers. Supports the findings of the RARMP that the COT102 technology poses negligible risk to human health and environmental safety. Ongoing stewardship is required for single-gene Bt products to mitigate risks associated with field-evolved *Helicoverpa* resistance. These resistance risks require consideration by the APVMA and the cotton industry's Transgenic and Insecticides Management Strategies (TIMS) Committee. The development of a robust resistance management plan to accompany commercial release of single-gene Bt products in Australia is essential for avoiding the field-evolved resistance which is currently threatening some overseas cotton systems.	Noted. Issues relating to insect resistance management are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for this area in Australia. Section 2.2.1 of the RARMP notes the potential risk of insects developing resistance to a single-gene GM line, and states that insect resistance management would be regulated by APVMA and the TIMS committee.