Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 118

Commercial release of GM herbicide tolerant (Roundup Ready Flex® MON 88913) pima cotton in Australia

Applicant: Monsanto Australia Ltd

August 2013

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 118

Decision

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

The application

Application number	DIR 118
Applicant	Monsanto Australia Ltd (Monsanto)
Project title	Commercial release of GM herbicide tolerant (Roundup Ready Flex® MON 88913) pima cotton in Australia
Parent organism	*Gossypium barbadense* (pima cotton)
Introduced genes and modified trait	Two *cp4 epsps* genes conferring herbicide tolerance
Proposed location	All cotton growing areas in Australia
Primary purpose	Commercial release of the GM herbicide tolerant cotton

This commercial release follows field trial work conducted under licence DIR 074/2007.

Risk assessment

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

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

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

The principal reasons for the conclusion of negligible risks are: the GM cotton has been produced by conventional breeding from a GM cotton line that has previously been assessed and authorised for commercial release in Australia, and which has been grown on a commercial scale in Australia since 2006 without any evidence of adverse effects on human health or environment as a result of gene technology; the widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity or evidence of harm from them; and the limited capacity of the GM cotton to spread and persist in undisturbed natural habitats.

Risk management

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

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 proposed licence conditions.

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

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Abbreviations

AGSWG	Australian Glyphosate Sustainability Working Group
APVMA	Australian Pesticides and Veterinary Medicines Authority
CaMV	Cauliflower mosaic virus
CFIA	Canadian Food Inspection Agency
cp4 epsps	*epsps* gene from *Agrobacterium* sp. strain CP4
CP4 EPSPS	EPSPS protein from *Agrobacterium* sp. strain CP4
ctp	Chloroplast transit peptide
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FDA	United States Food and Drug Administration
FMV	Figwort mosaic virus
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
g	Gram
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
µg	Microgram
µm	Micrometre
OGTR	Office of the Gene Technology Regulator
PEP	Phosphoenol pyruvate
PRR	Post release review
TIMS committee	Transgenic and Insect Management Strategy committee
USDA-APHIS	Animal and Plant Health Inspection Service of the United States Department of Agriculture

Chapter 1 Risk assessment context

Section 1 Background

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

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

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.

RISK ASSESSMENT CONTEXT

LEGISLATIVE REQUIREMENTS
(including Gene Technology Act and Regulations)

RISK ANALYSIS FRAMEWORK

OGTR OPERATIONAL POLICIES AND GUIDELINES

PROPOSED DEALINGS
Proposed activities involving the GMO
Proposed limits of the release
Proposed control measures

GMO
Introduced genes (genotype)
Novel traits (phenotype)

PREVIOUS RELEASES

PARENT ORGANISM
Origin and taxonomy
Cultivation and use
Biological characterisation
Ecology

RECEIVING ENVIRONMENT
Environmental conditions
Agronomic practices
Presence of related species
Presence of similar genes

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

Section 2 Regulatory framework

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

4. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. This means that, under section 50(3) of the Act, the Regulator is required to consult with prescribed experts, agencies

and authorities to seek advice on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, local council that the Regulator considered appropriate (being those in which commercial cotton crops may be grown) and the Minister for the Environment. A summary of issues contained in submissions received is given in Appendix A.

5. 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. Summaries of submissions received are provided in Appendix B (prescribed experts, agencies and authorities) and Appendix C (public).

6. The Risk Analysis Framework (OGTR 2009) 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.

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

Section 3 The proposed release

8. Monsanto Australia Ltd (Monsanto) proposes to release into the environment GM cotton that has been genetically modified for herbicide tolerance. The GM cotton proposed for release, designated Roundup Ready Flex® pima cotton (MON 88913 *G. barbadense*), has been produced by the conventional breeding of Roundup Ready Flex® *G. hirsutum* (MON 88913 *G. hirsutum)* with unmodified *G. barbadense*.

9. The applicant proposes the release to occur in all commercial cotton growing areas of Australia. No controls are proposed to restrict the release. However, as *G. barbadense* is most suited to western NSW, it is likely that the GM plants will be grown in this region. In the future, expansion could occur into areas in southern Queensland. GM cotton and GM cotton-derived products from the GM plants would enter general commerce, including use in human food and animal feed.

10. The dealings involved in the proposed intentional release would include:

- conducting experiments with the GMO
- making, developing, producing or manufacturing the GMO
- breeding the GMO with Australian cotton cultivars
- propagating the GMO
- using the GMO in the course of manufacture of a thing that is not the GMO
- growing, raising or culturing the GMO
- transporting the GMO
- disposing of the GMO
- importing the GMO

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

11. The applicant states that as *G. barbadense* is only planted on a small scale in Australia, it is unlikely that the lint from the GM plants will be harvested and ginned along with that of other cottons, but in fact will be kept segregated.

Section 4 The parent organism

12. The parent organism is the cultivated cotton species *Gossypium barbadense* L., which is exotic to Australia and is grown as an agricultural crop in NSW. More detailed information on cotton can be found in the document, *The Biology of* Gossypium hirsutum L. *and* Gossypium barbadense L. *(cotton)* (OGTR 2013), which was produced to inform the risk assessment process for licence applications involving GM cotton plants. This document is available from the Risk Assessment References page of the OGTR website.

4.1 Toxicity of non-GM cotton

13. Cotton possesses several natural toxicants. These are gossypol and the cyclopropenoid fatty acids (malvalic acid, sterculic acid and dihydrosterculic acid), all of which are found in seeds and the tissues of certain other organs (Bell 1986). Gossypol is an antioxidant and inhibitor of the polymerisation of tubulin into microtubules, the general symptoms of its toxicity being constipation and depressed appetite, in severe cases death occurring from circulatory failure (Makkar et al. 2007; Medrano & Andreu 1986). However, it has been investigated both as a male contraceptive (Coutinho 2002) and as an anti-cancer drug (Wong et al. 2012). The cyclopropenoid fatty acids reduce the activity of fatty acid desaturases (Raju & Reiser 1967; Yang et al. 1999). The known toxic properties of both gossypol and these fatty acids compounds limit the use of cotton seed derived meal in human food and animal feed. Inactivation or removal of these components during processing enables the use of some cotton seed meal for farmed fish, poultry and swine.

4.2 Weediness of non-GM cotton

4.2.1 Nature of weediness

14. Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens and give rise to negative impacts for people or the environment.

15. Negative impacts from weeds may be associated with competitiveness, rambling or climbing growth, toxicity, production of spines, thorns or burrs, or parasitism. The spread and persistence of weeds is a measure of their potential invasiveness, which may give rise to negative impacts such as reduced establishment of desired organisms, reduced quality of products or services obtained from the land use, reduced access to land, toxicity or increased ill-health of people or other desired organisms and increased degradation of the landscape or ecosystems (National Weed Prioritisation Working Group 2006).

16. Invasiveness is determined by complex interactions between a plant and its environment (including availability of water, nutrients and light). A number of measurable properties of plants that may influence spread and persistence include the ability to establish among existing plants, reproductive ability such as time to seeding, amount of seed set and ability for vegetative spread, mode of dispersal, likelihood of long-distance dispersal and tolerance to existing weed management practices (National Weed Prioritisation Working Group 2006).

4.2.2 Weed risk status of cotton

17. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM cotton plants in particular, is found in the document, *The Biology of* Gossypium hirsutum L. *and* Gossypium barbadense L. *(cotton)* (OGTR 2013).

18. Cotton can be considered a domesticated crop that grows best under agricultural conditions. It prefers soils with high fertility and responds well to irrigation.

19. Although cotton volunteer populations can be found in most agricultural areas where cotton is grown, they are not considered to produce notable harms (OGTR 2013). Naturalised populations of both *G. hirsutum* and *G. barbadense* have been found in a few relatively natural areas in the north of Australia, indicating that it is possible for these species to establish outside agricultural cultivation, but cotton seems to have a limited ability to become invasive in undisturbed nature conservation areas. As such, in most areas of Australia cotton is not considered a weed, and even in those areas where it may be classified as a weed, it would not be regarded as of serious concern to health, safety or the environment.

20. More data is available on the potential weediness of *G. hirsutum* than on that of *G. barbadense*. As both are cultivated cotton species that share a similar biology, there is a tendency to apply conclusions from the former species to the latter. Nonetheless, *G. barbadense* has never been classified as a weed of concern (problematic weed) in Australia.

21. The Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol rates the weed risk potential of plants according to properties that strongly correlate with weediness (Virtue et al. 2008). These properties relate to invasiveness, impacts and potential distribution. The weed risk potential of cotton has been assessed using methodology based on the National Post-Border Weed Risk Management Protocol (OGTR 2013). In summary, as a volunteer (rather than as a crop), cotton is considered to:

- have a low ability to establish amongst existing plants
- have a low tolerance to average weed management practices in cropping and intensive land uses, but a high tolerance in nature conservation areas
- have a short time to seeding (less than one year)
- have a low annual seed production and a low ability for volunteers to establish in any land use
- not reproduce by vegetative means
- unlikely to undergo long distance spread by natural means
- be commonly spread long distance by people from dryland and irrigated cropping areas, as well as from intensive land uses, but unlikely from nature conservation areas
- have a limited ability to reduce the establishment or yield of desired plants
- have a low ability to reduce the quality of products or services obtained from all land use areas
- have a low potential to restrict the physical movement of people, animals, vehicles, machinery and/or water
- have a low potential to negatively affect the health of animals and/or people
- can act as a reservoir for a range of pests and pathogens
- have a low effect upon soil salinity and the water table

22. This provides a baseline for the assessment of GM cotton.

Section 5 The parental GM cotton line

23. Roundup Ready Flex® pima (MON 88913 *G. barbadense*) has been produced by the conventional breeding of Roundup Ready Flex® *G. hirsutum* (MON 88913 *G. hirsutum)* with unmodified *G. barbadense,* the latter being also known as pima cotton. Roundup Ready Flex® *G. hirsutum* has two copies of the *cp4 epsps* gene. It does not contain any antibiotic resistance marker(s). This GM plant has been approved for commercial release under DIR 066/2006 (see Chapter 1, Section 8, for further details).

5.1 The introduced genes, their encoded proteins and their associated effects

5.1.1 The cp4 epsps gene and its encoded protein

24. The *epsps* gene encodes 5-enol-pyruvylshikimate-3-phosphate synthase, EPSPS, which catalyses the conversion of phosphoenol pyruvate (PEP) and shikimate 3-phosphate (SHKP) to 5-enol-pyruvylshikimate-3-phosphate (EPSP). This reaction, part of the shikimic acid pathway, is essential for the biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Herrmann & Weaver 1999). These amino acids, besides being constituents of proteins, are used in plant specific metabolic pathways. For example, phenylalanine is a precursor of flavonoids and lignin, while tryptophan is a precursor of indole acetic acid.

25. The EPSPS enzyme is found in plants and microorganisms, but is absent from mammals, birds, reptiles and fish (Gasser et al. 1988). On the basis of their amino acid sequences and catalytic efficiencies in the presence of glyphosate, EPSPS enzymes have been divided into two classes. Those from plants and *E. coli*, which are largely sensitive to glyphosate, are designated as class I, while those from some species of bacteria, such as *Agrobacterium* strain CP4 (the *cp4 epsps* gene) and *Achromobacter* strain LBAA, which have tolerance to this herbicide, are designated as class II (Funke et al. 2006).

26. The insertion of two copies of the *cp4 epsps* gene is designed to produce a high and prolonged expression of the EPSPS protein in cotton plants, resulting in tolerance to the herbicide glyphosate for a long period during the growing season. The first glyphosate tolerant cotton, MON 1445, containing only one *cp4 epsps* gene, was found to have low expression of the introduced gene in male reproductive tissue, in-turn limiting application of glyphosate to the four-leaf stage. The two gene insert in Roundup Ready Flex® (MON 88913) plants has been found to give constitutive expression of the gene in both vegetative and reproductive tissues of *G. hirsutum* (Cerny et al. 2010). Hence, the window in which glyphosate can be applied for weed control is longer, giving growers increased flexibility in timing herbicide applications for integrated weed management.

5.1.2 Toxicity and allergenicity of the CP4 EPSPS protein

27. There is no evidence that the EPSPS protein is toxic or allergenic. Toxicity experiments with animals (mainly mice and rats), often involving the feeding of exaggerated doses of the protein, have failed to establish any deleterious effects upon the subjects (Hammond et al. 2004; Harrison et al. 1996; Teshima et al. 2001; Zhu et al. 2004). The widespread occurrence of homologues of the *cp4 epsps* gene in plants and microorganisms implies that both vertebrates and invertebrates are unlikely to have any adverse toxic or allergenic effects from the EPSPS protein. Further, as the habitat of *Agrobacterium tumefaciens* is the soil and roots of plants, it is expected that soil microorganisms are regularly exposed to EPSPS proteins or their degradative peptide products, thus minimising the risk that the GM cotton will have to such microorganisms. In this context, one study has found that 90% of the CP4 EPSPS protein is degraded in the soil within 9 days (Dubelman et al. 2005). Discussion of the toxicity of the

CP4 EPSPS protein can also be found in the RARMPS for DIRs 055/2004, 059/2005 and 066/2006.

28. Analysis of the amino acid sequence has failed to demonstrate any significant homology with any known toxin or allergen. Further, the protein is rapidly denatured by heat, enzymatic digestion and acid in simulated mammalian digestive fluid, indicating it is unlikely to have any toxic or allergenic property (Harrison et al. 1996).

29. The applicant has received approval from FSANZ for the use of oil and linters derived from Roundup Ready Flex® *G. hirsutum* and Roundup Ready Flex® pima cotton in food (FSANZ 2005). In Australia, FSANZ has also approved for consumption material derived from GM plants (lucerne and soybean) expressing the CP4 EPSPS protein (FSANZ 2006; FSANZ 2007). The assessments by FSANZ note that there is no evidence of toxic and allergenic properties associated with these proteins.

5.1.3 Glyphosate tolerance

30. Glyphosate is the active ingredient in a number of broad-spectrum systemic herbicides that have been approved for use in Australia, and was first marketed as the propriety herbicide Roundup®.

31. The action of glyphosate is due to its structural resemblance to PEP. In plants, glyphosate binds preferentially and stably to the active site of endogenous (plant) EPSPS proteins (Steinrucken & Amrhein 1980). However, the CP4 EPSPS enzyme has a greater affinity for PEP than glyphosate, this difference in substrate affinity being sufficient for GM plants carrying the gene coding for this bacterial enzyme to be tolerant to the herbicide.

5.1.4 Toxicity of herbicide metabolites

32. The potential toxicity of the herbicide metabolites is considered by the APVMA in its registration of herbicides. There is not expected to be any difference in the metabolic fate of glyphosate in non-GM cotton and in GM cotton expressing the *cp4 epsps* gene. In the case of CP4 EPSPS, no new metabolic products are formed as the only difference from the native enzyme is the reduced affinity for glyphosate (OECD 1999).

5.2 The regulatory sequences

33. The two copies of the *cp4 epsps* gene are under the control of different plant viral derived promoters, although both induce constitutive expression of the genes. Each gene is preceded by a chloroplast transit peptide, *ctp2* from the *epsps* gene of thale cress *Arabidopsis thaliana* (Klee et al. 1987), to enable targeting of the protein to the chloroplast, where it is active in plant cells.

34. One *cp4 epsps* gene is driven by a chimeric promoter (P-35S/Act8), consisting of sequences from the cauliflower mosaic virus 35S gene (CaMV 35S) promoter and the promoter of the *Arabidopsis ACT8* gene (An et al. 1996; Kay et al. 1987). Between the chimeric promoter and *ctp2/cp4 epsps* coding sequence are non-translated leader and intron/exon sequences from the *ACT8* gene (An et al. 1996).

35. The other *cp4 epsps* gene is driven by a chimeric promoter (p-FMV/Tsf1) consisting of sequences from the figwort mosaic virus 35S gene (FMV 35S) promoter and the promoter of the *Arabidopsis TSF1* gene (Axelos et al. 1989; Richins et al. 1987). Between the chimeric promoter and *ctp2/cp4 epsps* coding sequence is a non-translated leader sequence from the *TSF1* gene (Axelos et al. 1989).

36. Each *epsps* gene is linked at its 3' end to a termination sequence, T-E9, derived from the *Pisum sativum* (pea) ribulose-1,5-bisphosphate carboxylase (rbc) small subunit E9 gene (Coruzzi et al. 1984).

37. Although some of these regulatory sequences are derived from organisms (viruses) that are plant pathogens, by themselves they do not cause disease.

38. Recently it has been suggested that protein P6, encoded by gene VI of the Caulimovirus and Soymovirus families, could result in harm to humans if expressed in GM plants, and perhaps interfere with the anti-pathogen defences of GM plants (Latham & Wilson 2013). Both the cauliflower mosaic and figwort mosaic viruses belong to the Caulimovirus family, and the CaMV 35S and FMV 35S promoters overlap sequences of gene VI. However, bioinformatics searches, experience from the consumption of food infected with these viruses, and the experience with commercial release of other GM plants with these promoters, indicate that the P6 protein does not possesses any allergenic or toxic properties. Likewise, there is no evidence of any environmental harms associated with use of these promoters in GM plants on a commercial scale.

5.3 Method of genetic modification

39. The two *cp4 epsps* genes in Roundup Ready Flex® *G. hirsutum* were introduced into this species by *Agrobacterium tumefaciens* mediated transformation, a method that has been widely used in Australia and overseas for introducing new genes into plants. Information about this transformation method can be found in the document *Methods of plant genetic modification* available from the Risk Assessment References page of the OGTR website. In the United States, the parental *G. hirsutum* germplasm is the cultivar Coker 312, which was released in 1974 by the Coker Pedigree Seed Company. For Australian conditions, the insert was bred into other cultivars of *G. hirsutum* (such as Sicot 71, Sicot 75, Sicot 80, Sicala 43). In both the United States and Australia, conventional breeding (initial hybridisation followed by backcrossing (introgression)) was used to transfer the genetic modification from Roundup Ready Flex® *G. hirsutum* to non-GM *G. barbadense*, in each case using parental cultivars specific to those countries. In Australia, a Roundup Ready Flex® *G. hirsutum* cultivar related to Sicot 80 was crossed with the non-GM *G. barbadense* cultivar Sipima 280, a cultivar that has been developed for resistance to bacterial blight. The transfer of the genetic modification to *G. barbadense* in the United States involved the use of the *G. barbadense* cultivar W2490.

5.4 Toxicity/allergenicity of the parental GM cotton line

40. The toxicity/allergenicity of the parental GM cotton to people and other organisms was considered in the RARMPs for DIRs 059/2005 and 066/2006, which also refer to material in earlier RARMPs. The Regulator concluded that the parental GM cotton was as safe as non-GM cotton. These assessments, plus new or updated information, are summarised below.

5.4.1 Toxicity/allergenicity to humans

41. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved the use of oil and linters derived from Roundup Ready Flex® *G. hirsutum* in food (FSANZ report A553). This approval also applies to Roundup Ready Flex® pima cotton proposed for release.

5.4.2 Toxicity to animals, including livestock

42. Mammals generally avoid feeding on cotton plants. The presence of gossypol and cyclopropenoid fatty acids in cotton seed limits the use of whole cotton seed as a protein supplement in animal feed, except for cattle which are less affected by these components. Inactivation or removal of these components during processing enables the use of some cotton

seed meal for farmed fish, poultry and swine. The meal and hulls of cotton seed can also be used for cattle feed. Its use as stockfeed is limited, nonetheless, to a relatively small proportion of the diet and it must be introduced gradually, to avoid potential toxic effects.

43. As noted above (Chapter 1, Section 5.1) the CP4 EPSPS protein is readily degraded in simulated mammalian digestive fluid, implying that it is unlikely to have any toxic or allergenic property. Cotton seed from GM cotton varieties (Bollgard®, Bollgard II®, and Roundup Ready®) has been fed to dairy cows, measurements then being made of characteristics such as dry matter intake, milk yield, milk composition and body weight (Castillo et al. 2001). No significant differences have been measured between the treatments in each experiment.

44. The feeding of Roundup Ready canola (a GM crop expressing the CP4 EPSPS protein) to broiler chickens (Stanisiewski et al. 2002; Taylor et al. 2004), had no observable effects upon the animals. These animals were used to compare diets containing Roundup Ready® canola GT73, the parental non-GM canola line, and six commercially available canola lines. Values obtained for a range of parameters (*eg* carcass yields, breast meat, thighs, legs, wings, protein levels, moisture) were similar across the diets demonstrating that Roundup Ready® canola GT73 is as nutritious as non-GM canola.

45. Similarly, feeding studies in bobwhite quail chicks (Campbell & Beavers 1994; Campbell et al. 1993), trout (Brown et al. 2003), lambs (Stanford et al. 2003; Stanford et al. 2002) and pigs (Aalhus et al. 2003; Caine et al. 2007) found no significant differences between animals fed Roundup Ready® canola GT73 containing diets and control diets. Factors that were examined between the trials differed, but amongst the land animals these included carcass composition, meat tenderness and quality, fat content, and colour. Trout were examined for weight gain, feed efficiency, protein efficiency ratio, and protein retention. These studies support the conclusion that Roundup Ready® canola meal is nutritionally equivalent to non-GM canola meal (EFSA 2009).

46. Three one-month feeding studies were also conducted on rats (Naylor 1994; Nickson & Hammond 2002). These animals were examined for factors such as body weight, food consumption, and at the end their livers and kidneys removed and weighed. No changes attributable to the genetic modification were observed. FSANZ thoroughly considered these studies in its assessment of Roundup Ready® canola GT73 before reaching the conclusion that ‘oil derived from glyphosate-tolerant canola GT73 is as safe for human consumption as oil from other commercial canola varieties’ (ANZFA 2000). As noted above, FSANZ has approved the food the use of oil and linters derived from Roundup Ready Flex® pima cotton.

5.4.3 Toxicity to honey bees

47. Cotton is primarily self-pollinating, but cross pollination does occur, which is likely facilitated by honeybees. Regulatory assessments of GM cotton and GM canola plants that express the CP4 EPSPS protein have concluded that those plants would not harm arthropods. In its examination of Roundup Ready Flex® *G. hirsutum* cotton and Roundup Ready® canola (GT73), the USDA-APHIS determined that these GM plants would not harm threatened or endangered species, or other organisms, such as bees, that are beneficial to agriculture (USDA-APHIS 1999a; USDA-APHIS 1999b; USDA-APHIS 2004a; USDA-APHIS 2004b). One of these assessments notes that there are no reports of the CP4 EPSPS protein possessing any toxic properties, and exposure of a range of arthropods (*eg* bees, springtails, greenbugs, aphids) to tissues from a number of Roundup Ready® crops has not resulted in negative consequences (USDA-APHIS 2004b). The Canadian Food Inspection Agency (CFIA) concluded that the unconfined release of Roundup Ready® canola (GT73) would not result in altered impacts on interacting organisms, and that their potential impact on biodiversity is

equivalent to that of currently commercialised canola varieties (Canadian Food Inspection Agency 1995).

48. The level of the CP4 EPSPS protein in pollen from the GM cotton has been measured at 4μg/g fresh weight (Chapter 1, Section 2, RARMP for DIR 059/2005).

5.4.4 Toxicity to soil microbes

49. As the *cp4 epsps* gene is derived from an organism, *Agrobacterium tumefaciens*, that is found in the soil, it is possible that many soil organisms have been exposed to the derived protein. In addition, homologues of the *cp4 epsps* gene are widespread in plants and microorganisms. However, the CP4 EPSPS protein is likely rapidly degraded in soils, one study showing that 90% of the protein is degraded in the soil within 9 days (Dubelman et al. 2005).

50. Recent studies have confirmed the lack of permanent effects on soil biota by GM glyphosate tolerant crops. For example, no permanent effects on soil biota were observed in a series of experiments designed to estimate the effect of glyphosate tolerant soybean and maize, and their management, on the abundance of detritivorous soil biota and crop litter decomposition (Powell et al. 2009). While statistically significant effects were observed in a few of the measured groups, in most cases the effects were only observed in the first year of the study and were not consistent across sample dates or across the four study years. The most frequent effect of the glyphosate tolerant herbicide system was a transient shift toward more fungal biomass relative to bacterial. The genetic modification in the soybean and maize had little effect on litter decomposition, however the use of glyphosate did reduce decomposition of surface (but not buried) litter.

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

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

5.5 Weediness of the parental GM line

53. The weediness of the parental GM cotton was considered in the RARMPs for DIRs 059/2005 and 066/2006, which also refer to material in earlier RARMPs.

54. The two copies of the *cp4 epsps* gene in Roundup Ready Flex® *G. hirsutum* provide tolerance to glyphosate throughout the growing season. Although there is no evidence that expression of these genes by themselves increase the potential weediness of the plants, their expression could confer a selective advantage on cotton plants in non-agricultural areas where glyphosate is used to control weeds, leading to increased spread and persistence of plants. However, glyphosate is not generally used to control established cotton as it usually fails to kill the plants, this being the case even if the plants are non-GM. The control of cotton

volunteers can be achieved by the use of mechanical means or a range of other herbicides, including bromoxynil, carfentrazone and a combination of paraquat and diquat (Roberts et al. 2002).

55. As part of the commercial release of Roundup Ready® and Roundup Ready®/Ingard® cotton in 2000, Monsanto was required to conduct an environmental monitoring program (Monsanto Australia 2004). In two surveys (one conducted in the autumn of a single year, and the other conducted in two successive autumns), sites were sampled for cotton volunteers on roadsides in both Queensland (Atherton Tablelands or Darling Downs) and New South Wales (Lower Namoi Valley), and on dairy farms where cottonseed was used as a stockfeed supplement in the Atherton Tablelands. On roadsides, only a minority of sites had cotton volunteers, and only a minority of volunteers were GM. It was concluded that the germination of roadside volunteer cotton is highly variable between seasons, that most volunteers result from new germinations (rather than the survival of plants from previous seasons) and that the Roundup Ready® trait (conferred by the *cp4 epsps* gene) did not influence the ability of roadside volunteers to establish and persist (Farrell & Roberts 2002; Monsanto Australia 2004). In dairy farms using cottonseed as stock feed, 260 volunteers were found on seven out of nine farms that were visited, 46% of which were GM; the authors concluded that while volunteer cotton had established on some farms, none had completed a reproductive cycle to produce new seed, and that it is unlikely that use of cotton seed (GM or non-GM) as stock feed on dairy farms in the Atherton Tablelands will lead to self-perpetuating cotton populations.

56. It is possible that the GM *G. hirsutum* plants may spread and persist in the environment outside the sites where they are planted and harvested. However, the expression of the *cp4 epsps* gene construct is not expected to alter susceptibility to the environmental conditions that limit and spread the persistence of cotton in Australia.

5.6 Potential for gene transfer from the parental GM cotton line

57. The potential for gene transfer from the parental GM cotton line, Roundup Ready Flex® *G. hirsutum*, to other sexually compatible plants was assessed in the RARMPs for DIRs 059/2005 and 066/2006, which also refer to material in earlier RARMPs. Cotton is largely self-pollinating and no self-incompatibility mechanisms exist. Whatever cross-pollination that does occur is likely facilitated by honeybees.

58. The absence of any known or predictable toxic/allergenic properties associated with the CP4 EPSPS protein in Roundup Ready Flex® *G. hirsutum* (or any other GM plant) implies it is very unlikely to have such properties in any plant to which it is transferred.

5.6.1 Gene transfer to other cotton crops

59. Roundup Ready Flex® *G. hirsutum* is sexually compatible with *G. barbadense* as well as (obviously) GM and non-GM *G. hirsutum* plants. Despite the usual self-pollination characteristic of *G. hirsutum*, the commercial release of Roundup Ready Flex® *G. hirsutum* means that if other *G. barbadense* and *G. hirsutum* plants are sufficiently close, some cross-hybridisation is inevitable.

60. Transfer of the *cp4 epsps* gene to non-glyphosate tolerant *G. hirsutum* and *G. barbadense* cotton plants (whether GM or non-GM) could confer glyphosate tolerance upon these plants. Such plants would have a fitness advantage in environments where glyphosate is used to control weeds.

61. However, as noted in Section 5.5, glyphosate is not generally used to control established cotton as it usually fails to kill even non-GM cotton plants. The control of cotton volunteers

can be achieved by mechanical means or a range of other herbicides, including bromoxynil, carfentrazone and a combination of paraquat and diquat (Roberts et al. 2002).

62. The GM cottons Liberty Link® and Widestrike® have introduced genes for tolerance to the herbicide glufosinate ammonium (phosphinothricin). Hybridisation between Roundup Ready Flex® *G. hirsutum* and these plants would produce a plant with tolerance to both glyphosate and glufosinate ammonium. Neither of these herbicides is effective in controlling adult cotton plants. As above, such dual herbicide tolerant plants could be controlled by mechanical means or a range of other herbicides, including bromoxynil, carfentrazone and a combination of paraquat and diquat.

5.6.2 Gene transfer to native cotton species

63. There are 17 native species of *Gossypium* in Australia, most of which are found in the Northern Territory and the north of Western Australia (OGTR 2013). 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.

64. In the natural environment, for successful hybridisation to occur, the parent plants would have to occur in close proximity, flower at the same time, the pollen from one deposited on the stigma of the other, fertilisation occur, survival of the progeny to sexual maturity, and any progeny seed would have to be viable. If any such hybrid was fertile, it may well backcross with one or both of the parents as opposed to forming a new population (species).

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

Section 6 The GMO, nature and effect of the genetic modification

6.1 Introduction to the GMO

66. Roundup Ready Flex® pima cotton (MON 88913 *G. barbadense*) has been produced by the conventional breeding of Roundup Ready Flex® *G. hirsutum* (MON 88913 *G. hirsutum)* with unmodified *G. barbadense*, the latter being also known as pima cotton. The particular cultivar of pima cotton used for the initial crossing was Sipima 280. As outlined above (Chapter 1, Section 5.3) Roundup Ready Flex® *G. hirsutum* was transformed with two copies of the *cp4 epsps* gene from the soil bacterium *Agrobacterium tumefaciens*. The present application is the first in Australia for a commercial release of a GM *G. barbadense* line.

67. *G. hirsutum* and *G. barbadense* are the two major cultivated cotton species worldwide. They share a fundamental core of biological characteristics (*eg* general morphology, toxic substances produced, responses to abiotic and biotic stresses), but have minor differences that reflect their classification as separate species. Neither species is recognised as a problematic weed (either agriculturally or environmentally) in Australia. The biology of these species is

further discussed in Chapter 1, Section 7.1 and the document, *The Biology of* Gossypium hirsutum L. *and* Gossypium barbadense L. *(cotton)* (OGTR 2013).

6.2 Characterisation of the GMO

6.2.1 Molecular characterisation

68. Characterisation of the insert and the expressed EPSPS protein are provided by the applicant for the United States bred line of Roundup Ready Flex® pima cotton, in the cultivar W2490 background. No such data are provided for the Australian cultivar Sipima 280. However, the introduced *cp4 epsps* genes are expected to behave in the same manner during crosses in both US and Australian cultivars of *G. barbadense*, and indeed in the same manner as endogenous genes during any breeding. Ultimately, presence of the introduced gene and activity of the protein in each cultivar is demonstrated by glyphosate tolerance.

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

70. Southern blot analysis of DNA from three generations of introgressed Roundup Ready Flex® pima cotton (W2490 background) verified the stability of the insert in *G. barbadense* (Groat et al. 2009). Hybridisation of a *cp4-epsps* probe to DNA samples from these generations, each digested with two restriction enzymes (*Spe*I/*Sal*I), produced a pattern identical to that seen with Roundup Ready Flex® *G. hirsutum*.

71. The presence of the insert, together with the stability of the protein product (EPSPS) in Roundup Ready Flex® pima cotton (W2490 background), was further assessed by western blot analysis. Isolation of proteins from three generations of these plants showed that the EPSPS protein, of predicted molecular weight, was present in all generations (Mozaffar & Silvanovich 2009).

72. Monsanto has provided data pertaining to the expression of the CP4 EPSPS protein in leaf and seed tissue of Roundup Ready Flex® pima cotton. The mean protein levels across five sites in the United States where the plants were trialled were 2400±390 and 410±87 µg/g for dry and fresh weight leaf samples, respectively, and 360±63 and 340±58 µg/g for dry and fresh weight seed samples, respectively (Mozaffar & Silvanovich 2009). The data for seeds is similar to that recorded for Roundup Ready Flex® *G. hirsutum* (means of 310±30 and 280±30 µg/g for dry and fresh weight seed samples, respectively), but in leaf the mean level in the Roundup Ready Flex® pima cotton is up to approximately twice that recorded in Roundup Ready Flex® *G. hirsutum* (Mozaffar & Silvanovich 2009; OGTR 2006). These differences in the levels of the CP4 EPSPS protein may reflect the genetic variation between the two species affecting *cp4 epsps* gene expression and the associated biochemical reactions. Other factors that affect gene expression are the climate and environment (the GM *G. hirsutum* and pima cottons were not trialled at the same locations), and/or variation in the timing of the collection of leaf samples (Jamal et al. 2009; Maheshri & O'Shea 2007; Raser & O'Shea 2005).

6.2.2 Phenotypic and agronomic characterisation

73. To evaluate basic phenotypic characteristics, field trials of Roundup Ready Flex® pima cotton (W2490 background), the non-GM *G. barbadense* cultivar W2490 and four other unmodified pima cotton cultivars were conducted at five sites in the United States in 2007 (Phillips et al. 2009). A total of 42 characteristics, such as height, boll number and seed per

boll, were evaluated. Only three significantly different characteristics were detected: the GM plants were shorter than controls at the second measurement only of height during growth, had a lower seed index and a lower fibre micronaire (measurement of fibre fineness). Such variant data would be expected in any program of conventional plant breeding (Acquaah 2007; Bradford et al. 2005).

74. The agronomic characteristics of the Roundup Ready Flex® pima cotton plants that may possibly be related to weediness are essentially identical to non-GM *G. barbadense*. The germination characteristics (percent seed germinated, percent viable hard seed, percent viable firm swollen seed, and percent dead seed) of Roundup Ready Flex® pima cotton (W2490 background) were examined against unmodified (conventional) W2490 cotton and seven other unmodified control varieties, seed for all plants being produced at three sites in the United States. Minimum and maximum mean values for each characteristic (reference ranges) were determined by reference to the data from these control varieties (Giovannini & Kendrick 2009). Combined analysis of results for all seed batches demonstrated no statistically significant differences between the GM pima cotton and control varieties for percent viable hard seed at any temperature, percent viable firm swollen seed at 20°C or 20°C/30°C, percent abnormal germinated seed at 20°C/30°C, or percent dead seed at 10°C. However, six statistically significant differences were detected. Compared to the controls, the GM pima cotton had higher percent germinated seed at 10°C, lower percent germinated seed at 20°C, lower percent germinated seed at 20°C/30°C, lower percent viable firm swollen seed at 10°C, and higher percent dead seed at 20°C and 20°C/30°C. Nevertheless, these differences were within the reference ranges for the characteristics of percent germinated, percent viable firm swollen and percent dead seeds.

75. The viability and morphology of pollen was examined for Roundup Ready Flex® pima cotton (W2490 background), unmodified (conventional) W2490 cotton, and four reference varieties (Laufer et al. 2009b). Although no statistically significant differences were detected between the GM pima cotton and the controls for percent viable pollen, the pollen grain diameter was larger for the GM pima cotton (105.5 vs 102.6 μm). However, both average values are lower than those reported for unmodified *G. barbadense*, and there is no reason to believe that these variations in size are likely to impact on the weediness of any plants (unmodified or not) of this species. No other differences were detected in general pollen morphology between the pollen from the GM pima cotton, unmodified W2490, and the reference varieties.

76. The response of Roundup Ready Flex® pima cotton (W2490 background), unmodified (conventional) W2490 cotton and four other unmodified pima cotton cultivars to a range of abiotic and biotic stresses was determined at four sites in the United States in 2007 (Phillips et al. 2009). This included response to cold, drought, heat, soil moisture, and a number of pathogenic microorganisms (*Alternaria*, boll rot, cotton rust, *Fusarium*, leaf crumple, *Pythium*, *Rhizoctonia*, *Thielaviopsis*, and *Verticillium*) and arthropods (aphid, armyworm, boll weevil, bollworm, *Lygus*, mites, whitefly, pink bollworm, thrips). Minimum and maximum mean values for each stress (reference ranges) were determined by reference to the data from the control varieties. Only on one occasion, a single observation at one site for soil moisture (an abiotic stress) was a difference observed ('moderate' versus 'none-slight'), a finding that was not repeated during the other nine recording observations. Further, this difference was within the reference range. Overall, it can be concluded that the genetic modification in Roundup Ready Flex® pima cotton did not alter the responses of the plants to abiotic or biotic stresses.

77. Although *G. barbadense* is a perennial plant, it is cultivated in Australia as an annual. Part of the reason for this is that from a climatic perspective, the locations where it is grown have winters that are too cold for its survival. To evaluate whether the genetic modification would increase the tolerance of *G. barbadense* to cold, Roundup Ready Flex® pima cotton plants (W2490 background), non-GM W2490 cotton and six other unmodified pima cotton cultivars were subjected to growth at "cold" temperatures (21 days at 15°C day/10°C night) in the United States. No statistically significant differences were observed between the control and GM plants in reference to vigour, height, fresh weight or dry weight (Laufer et al. 2009a).

78. A set of phenotypic and agronomic data was collected for Roundup Ready Flex® pima cotton (Sipima background) trialled in Australia under DIR 074/2007 (Constable 2012). This data was for emergence, seedling vigour, plant height, main stem node counts, fruit positions, yield and fibre quality. No significant differences were recorded between the GM plants and controls with respect to agronomic performance, but there was a significant trend for longer fibre length and decrease in micronaire, as well as a slight yield improvement in the GM plants. This may be the result of selection for the best performing individuals during the backcrossing program, as would be expected in any program of conventional plant breeding (Acquaah 2007; Bradford et al. 2005).

79. Compositional analysis was performed for Roundup Ready Flex® pima cotton (W2490 background) and the non-GM W2490 cotton grown at five sites in the United States. Tolerance intervals[1] were calculated by the isolation of components from eight different lines of non-GM *G. barbadense*, different sets of four of which were grown at the five sites where both Roundup Ready Flex® pima cotton and non-GM W2490 were trialled. In a combined site analysis, measured compositional components were not statistically different for 38 (73.1%) of 52 comparisons (Alba et al. 2009). However, none of these differences were consistent across the five sites, and the component mean values all fell within the tolerance intervals for non-GM *G. barbadense* (Table 1.1).

Table 1.1. Summary of significant compositional differences between cotton seed of Roundup Ready Flex® pima cotton (W2490 background) and non-GM *G. barbadense* in the United States.

General component category	Specific component	Roundup Ready Flex® pima mean	W2490 (non-GM *G. barbadense*) mean	Mean difference (% of W2490)	Significance (p-value <0.05)	Commercial tolerance interval[1]
Amino acids	Arginine	2.89	2.73	5.87	0.038	[1.09, 4.80]
Fatty acids	16:0 Palmitic acid (% total fat)	21.51	21.85	-1.55	0.030	[19.04, 25.30]
	16:1 Palmitoleic acid (% total fat)	0.72	0.74	-2.64	0.049	[0.53, 0.92]
	18:3 Linolenic acid (% total fat)	0.18	0.15	19.40	0.035	[0.010, 0.23]
	Dihydrosterulic acid (% total fat)	0.084	0.064	30.51	0.011	[0.013, 0.13]
	Malvalic acid (% total fat)	0.30	0.23	32.18	0.020	[0.027, 0.50]
	Sterculic acid (% total fat)	0.19	0.14	41.46	0.005	[0, 0.38]

[1] Tolerance intervals in this experiment are those where, with 95% confidence, 99% of the values are located.

Fiber	Acid detergent fiber (% DW)	29.98	29.96	7.50	0.035	[18.93, 37.69]
Mineral	Calcium (% DW)	0.20	0.18	11.63	0.030	[0.084, 0.30]
	Magnesium (% DW)	0.40	0.38	6.12	0.003	[0.34, 0.48]
	Potassium (% DW)	1.22	1.16	4.64	0.008	[1.01, 1.40]
Proximate	Calories (Kcal/100g DW)	488.78	497.55	-1.76	0.030	[437.03, 548.33]
	Protein (% DW)	25.65	24.48	4.76	0.004	[14.18, 38.95]
	Total fat (% DW)	21.59	23.20	-6.92	0.045	[11.46, 33.29]

Section 7 The receiving environment

80. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the size, duration and regions of the dealings, any relevant biotic/abiotic properties of the regions where the release would occur; intended agronomic practices, including those that may be altered in relation to normal practices; other relevant GMOs already approved for commercial release; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2009).

81. The applicant has proposed to release Roundup Ready Flex® pima cotton in all commercial cotton growing areas of Australia. Therefore, for this particular licence application, it is considered that the receiving environment would be wherever it is suitable to cultivate cotton.

82. Cotton has been commercially cultivated in Australia since the 1860s (OGTR 2013). Areas where cotton can be grown in Australia are mainly limited by water availability (*ie* the right amounts at optimal times of the growth cycle via irrigation or rainfall), the suitability of the soil (good water retention qualities are required), temperature and the length of the growing season. The risk that the genetic modification will produce a plant that is capable of growth beyond these areas will be discussed in Chapter 2 (Risk Assessment).

7.1 Relevant agricultural practices

Cultivation of cotton in Australia

83. Cotton is a perennial plant that is cultivated as an annual. Detailed information on the cultivation of cotton in Australia can be found in the document, *The Biology of* Gossypium hirsutum L. *and* Gossypium barbadense L. *(cotton)* (OGTR 2013), and is summarised below.

84. Temperature is the dominant environmental factor affecting cotton development and yield. Cotton is planted when the minimum soil temperature at 10 cm depth is 14°C for at least three successive days. Seedlings may be killed by frost and a minimum of 180–200 frost-free days of uniformly high temperatures (averaging 21-22°C) is required after planting for *G. hirsutum* and 200–250 days for *G. barbadense*. Growth and development of cotton plants below 12°C is minimal and a long, hot growing season is crucial for achieving good yields.

85. The majority of Australia's *G. hirsutum* cotton crop is grown in the Murray-Darling Basin, in the states of NSW and Queensland, under irrigation. Typically in Australia, more than 80% of the cotton is grown as a furrow irrigated crop and fields are commonly irrigated

five or six times during the growing season between flowering and peak boll development. The remaining cotton production occurs on dryland farms. Cultivation of *G. barbadense* is confined to western NSW. This is mainly due to the need for a longer drier growing season with low humidity being necessary to ensure high fibre quality in that species.

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

87. The agronomic management of the GM cotton lines containing the *cp4 epsps* gene (herbicide tolerance trait) would differ from the management of non-GM cotton in that glyphosate herbicide could be applied over the top of the cotton crop to control weeds. However, application of glyphosate herbicide is standard practice, as almost 99.5% of the Australian cotton crop is GM (see the website of the Department of Agriculture and food, WA) and Roundup Ready® and Roundup Ready Flex® cottons currently occupy about 97 per cent of total cotton area, most of it stacked with the Bollgard® II insect resistant trait (CSIRO 2010).

88. Cotton is primarily grown as a fibre crop. It is harvested as ‘seed cotton’ which is packed into large bales or modules and transported to ginning facilities, where the seed cotton is ‘ginned’ to separate the seed and lint. The long ‘lint’ fibres are further processed by spinning to produce yarn that is knitted or woven into fabrics. Ginned *G. hirsutum* seed is covered in short, fuzzy fibres, known as ‘linters’, which must be removed before the seed can be used for planting or crushed for oil. The linters are used as a cellulose base in high fibre dietary products as well as a viscosity enhancer (thickener) in ice cream, salad dressings and toothpaste. *G. barbadense* cotton seed does not produce linters and therefore is only processed into oil, meal and hulls.

89. De-linted cotton seed (ie. seed with no lint or linters) is processed into oil, meal and hulls. Cotton seed meal is the product remaining once the oil has been removed by crushing and can contain up to contain 41% protein. Cotton seed, or meal, flour or hulls derived from it, is used for animal feed, but this is limited by the presence of natural toxicants in the seeds.

Herbicide Resistance

90. Integrated weed management practices help to avoid selection of resistant weed biotypes. The Australian cotton industry has embraced such weed management practices to decrease the possibility that herbicide tolerant weeds will become a problem (http://cottonaustralia.com.au/). This is coordinated through the Transgenic and Insect Management Strategy (TIMS) committee, which among other things helps coordinate Roundup Crop Management Plans.

91. Monsanto has prepared a crop management plan for Roundup Ready Flex® cotton (available on their website). This plan states the necessity that stakeholders using Roundup Ready® technology should complete a particular training program that explains the sustainable use of glyphosate resistant plants and the associated herbicide, and is part of a Technology User Agreement.

92. The Australian Glyphosate Sustainability Working Group (AGSWG) is a collaborative initiative of Australian academics and industry members that is dedicated to improving the management and sustainability of glyphosate use in Australian agriculture (http://www.glyphosateresistance.org.au). Monsanto is a member of the group. In overview,

the group has concluded that the main factors that contribute to the evolution of glyphosate resistance are intensive use of the herbicide and the failure to use other alternate measures of weed control. In relation to Australian cotton growing areas, the group has recorded that glyphosate resistant barnyard, liverseed and windmill grasses may be a problem. To reduce the problem of such resistance it recommends the use of strategies such as tillage, alternate herbicides, and "double knock" (glyphosate followed by tillage or another herbicide). Further, the group has produced a document entitled *Integrated Weed Management in Australian Cropping Systems* for use by farmers and other interest groups.

93. CropLife Australia, an organisation that represents agricultural chemical and plant biotechnology interests in Australia, publishes a *Herbicide Resistance Management Strategies* guide (CropLife Australia 2012b). Monsanto is a member of the organisation. At a general level, this document emphasises the need to resist the temptation to rely upon a single strategy to prevent the development of herbicide resistances. Specifically in relation to glyphosate (a Group M herbicide), the document records that weeds resistant to this herbicide are associated with its intensive use, the lack of rotation of with strategies, and the failure to till/cultivate after its application.

94. In respect to the control of cotton itself, glyphosate is generally not used against adult plants, as it usually fails to kill them. Adult cotton plants can be controlled by other herbicides and mechanical means. It should also be noted that the OGTR does not regulate issues of efficacy or resistance management as these issues most appropriately fall under the Agricultural and Veterinary Chemicals Code Act 1994, and as such are the responsibility of the APVMA. The APVMA assesses all herbicides used in Australia and sets their conditions of use, including for resistance management.

7.2 Relevant biotic factors

95. *G. barbadense* can hybridise with *G. hirsutum*, the latter constituting approximately 99% of the commercial cotton crop grown in Australia. Generally such hybrids do not form stable populations, but tend to segregate towards either parental phenotype over a number of generations (OGTR 2013).

96. As noted above (Section 5.6.2), Australia contains 17 native species of *Gossypium*, all of which are diploid (C, G or K genomes), while the cultivated cotton species are tetraploid (AD genomes). Most of these native species are found in the Northern Territory and the north of Western Australia.

97. Gene transfer from Roundup Ready Flex® *G. barbadense* to native cotton plants is prevented by both genetic incompatibility (see section 5.6.2, above) and geographic constraints.

98. *G. barbadense* is susceptible to a number of insect pests, most prominently caterpillars of the moths *Helicoverpa armigera* (cotton bollworm) and *Helicoverpa punctigera* (native budworm), the two-spotted spider mite *Tetranychus urticae,* as well as a range of bacterial and fungal pathogens (OGTR 2013). *Xanthomonas campestris* is a major disease (bacterial blight) of *G. barbadense* in Australia, and efforts are being made to breed (non-GM) resistant cultivars (CCCCRC 2012). It should also be noted that *G. barbadense* has a longer growing season than *G. hirsutum*, which potentially exposes it to both greater predation and a greater array of pests.

7.3 Relevant abiotic factors

99. Like all plants, the growth of cotton is dependent upon a number of abiotic factors, such as soil nutrients and climate (OGTR 2013). The areas where cotton can be grown in Australia

are mainly limited by water availability (*ie* the right amounts at optimal times of the growth cycle via irrigation or rainfall), the suitability of the soil (good water retention qualities are required), temperature and the length of the growing season (see also Section 7.1).

100. In Australia *G. barbadense* is cultivated in western NSW (OGTR 2013). However, climatic modelling has shown that permanent populations could be expected to establish anywhere in the coastal regions from the tip of Cape York to Yamba in NSW, an area that is essentially identical to that predicted for *G. hirsutum* (Rogers 2007). By contrast, western NSW is too cold for populations to survive over winter, even in the presence of adequate irrigation or natural precipitation.

101. *G. barbadense* is more susceptible to both high and low temperatures than *G. hirsutum*. Compared to *G. hirsutum*, the growth of *G. barbadense* is completely inhibited at a lower temperature threshold 1.5°C higher, and a higher temperature threshold 2.0°C lower (Rogers 2007). Due to the need for longer drier growing seasons to achieve maximum fibre quality (Section 7.1), the opportunities for expansion of *G. barbadense* cultivation are limited. It has been suggested that in the future crops of this species could be grown further eastwards in NSW, perhaps as far as a line from Walgett to Cunnamulla (Monsanto 2007). The prospects for expansion are also dependent upon the relative prices of *G. hirsutum* and *G. barbadense* on the international market, and the development of new varieties with reliable yield.

7.4 Presence of the same or similar proteins in the receiving environment

102. The CP4 EPSPS protein is produced naturally by the CP4 strain of the common soil bacterium *Agrobacterium* sp. (Padgette et al. 1996). This bacterium can also be found on plants and fresh plant produce. Genes coding for closely related EPSPS proteins are present in plants, bacteria and fungi (Gasser et al. 1988).

Section 8 Previous releases

8.1 Australian approvals of the GM cotton lines

8.1.1 Approvals of Roundup Ready Flex® pima (G. barbadense) cotton

103. A limited and controlled release of Roundup Ready Flex® pima cotton is currently authorised under licence DIR 074/2007. There have been no other releases of Roundup Ready Flex® pima cotton authorised in Australia.

8.1.2 Approvals of Roundup Ready Flex® G. hirsutum cotton

104. A number of releases of the GM parent, Roundup Ready Flex® *G. hirsutum,* or other GM cottons containing the *cp4 epsps* gene, have been authorised in Australia (Table 1.2)

105. A licence, DIR 059/2005, for the commercial (general) release of Roundup Ready Flex® *G. hirsutum*, and Roundup Ready Flex® *G. hirsutum*/Bollgard II® was issued in 2006 for the growth of these GM plants south of latitude 22° South. Bollgard II® varieties contain the *cry1Ac* and *cry2Ab* genes that have insecticidal properties. The geographical constraint was due to concerns at that time about the potential weediness of the GM cottons in northern tropical regions. This licence has since been surrendered, and another licence, DIR066/2006, issued in 2006, currently authorises the commercial release of Roundup Ready Flex®*,* Roundup Ready®*,* Roundup Ready Flex®/Bollgard II, Roundup Ready®/ Bollgard II® and Bollgard II® cotton throughout Australia. Limited and controlled field trials of Roundup Ready Flex® *G. hirsutum* were previously conducted under the licences DIR 035/2003 and DIR 055/2004.

106. Roundup Ready® *G. hirsutum* and Roundup Ready®/INGARD *G. hirsutum* cotton were issued with a licence by the Regulator for commercial release in June 2003 (DIR 023/2002).

However, these GM cottons were previously approved for commercial release in 2000 by the Minister for Health and Aged Care, on the basis of advice from GMAC (Genetic Manipulation Advisory Committee). Roundup Ready® cotton, containing one copy of the *cp4 epsps* gene, is tolerant to the application of the herbicide Roundup® only up to the four leaf stage of growth. Roundup Ready®/INGARD cotton also possesses the *cry1Ac* gene for insect resistance. Bollgard II®/Roundup Ready® cotton, containing one copy of the *cp4 epsps* gene, together with the *cry1Ac* and *cry2Ab* genes that have insecticidal properties, was first approved for commercial release by the Regulator in 2002 (licence DIR 012/2002). Again, due to concerns about potential weediness in northern regions of Australia, these commercial releases were restricted to south of latitude 22° South. The licences DIR 023/2003 and DIR 012/2002 have been surrendered.

Table 1.2 Commercial releases in Australia of GM *G. hirsutum* cottons containing the *cp4 epsps* gene

DIR	Commercial name of cotton	Year approved	Licence status
012/2002	Roundup Ready®/Bollgard II®	2002	surrendered
023/2002	Roundup Ready®, Roundup Ready®/INGARD	2003	surrendered
059/2005	Roundup Ready Flex® Roundup Ready Flex®/Bollgard II®	2006	surrendered
066/2006	Roundup Ready Flex®, Roundup Ready®, Roundup Ready Flex®/Bollgard II® , Roundup Ready®/Bollgard II®,	2006	current

8.2 Approvals by other Australian agencies

107. 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, including FSANZ and APVMA (see section 2, this chapter).

108. Monsanto has received approval from FSANZ for the use of oil and linters derived from Roundup Ready Flex® *G. hirsutum* in food (FSANZ report A553). This approval also applies to Roundup Ready Flex® pima cotton.

109. The APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. Roundup Ready® herbicide has been registered for use on Roundup Ready® cotton since 2000. Since 2006 Roundup Ready® herbicide has been registered for use on Roundup Ready Flex® cotton, a registration that applies to both *G. hirsutum* and *G. barbadense*.

8.3 International approvals

110. Roundup Ready® and Roundup Ready Flex® cotton, as well as stacks of these with Bollgard II® cotton, have been approved for commercial release in a number of other countries; and products from these cottons are also approved for human food use and/or animal feed. Some of these approvals are listed below.

<u>Roundup Ready Flex® cotton</u>

- United States – the USDA-APHIS approved the commercial release in 2004, and the FDA approved use in human food and animal feed in 2005.

- Canada – Health Canada's Office of Food Biotechnology and the CFIA gave approval for use in human food and animal feed in 2005.

These approvals apply to both Roundup Ready Flex® *G. hirsutum* and Roundup Ready Flex® pima (*G. barbadense*).

<u>Roundup Ready® cotton</u>

- United States – the USDA-APHIS approved the commercial release in 1995, and the FDA approved use in human food and animal feed in 1995.
- Japan – the Japanese Ministries of Health, Labour and Welfare and Agriculture, Forestry and Fisheries approved the commercial release and use in human food in 1997, and in animal feed in 1998.
- Canada – Health Canada's Office of Food Biotechnology and the CFIA approved use in human food in 1996, and in animal feed in 1997.
- The European Union – approved use in human food in 2002 under the Novel Food Regulation (EC 258/97) and the European Commission is considering approval for use as animal feed under the current regulatory system.
- Argentina – the Secretary of Agriculture, Livestock, Fisheries and Food approved the commercial release in 1999, and the use in human food and animal feed in 2000/2001.
- South Africa – commercial release was approved by the Executive Council for Genetically Modified Organisms in 2000.
- China – the Minister for Agriculture gave approval for use in human food and animal feed in 2003.
- Philippines – the Bureau of Plant Industries, Department of Agriculture, gave approval for use in human food and animal feed in 2003.

<u>Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II® cotton</u>

- United States – both stacked varieties are approved for commercial release, as under the current US regulatory system a stacked GMO is automatically approved if it was produced by conventional crossing of two GMOs, containing unrelated traits that have previously been approved in the United States.
- Japan – the Japanese Ministry of Health, Labour and Welfare approved use in human food in 2003 for Roundup Ready®/Bollgard II and in 2005 for Roundup Ready Flex®/Bollgard II®.
- The European Union – Roundup Ready®/Bollgard II® is listed on the Community Register of GM Food and Feed in the European Union (from 2003) and is pending approval by the European Commission under the current regulatory system.
- Philippines - the Bureau of Plant Industries, Department of Agriculture, gave approval for the use of Roundup Ready®/Bollgard II® in human food and animal feed in 2004.

Chapter 2 Risk assessment

Section 1 Introduction

111. 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.

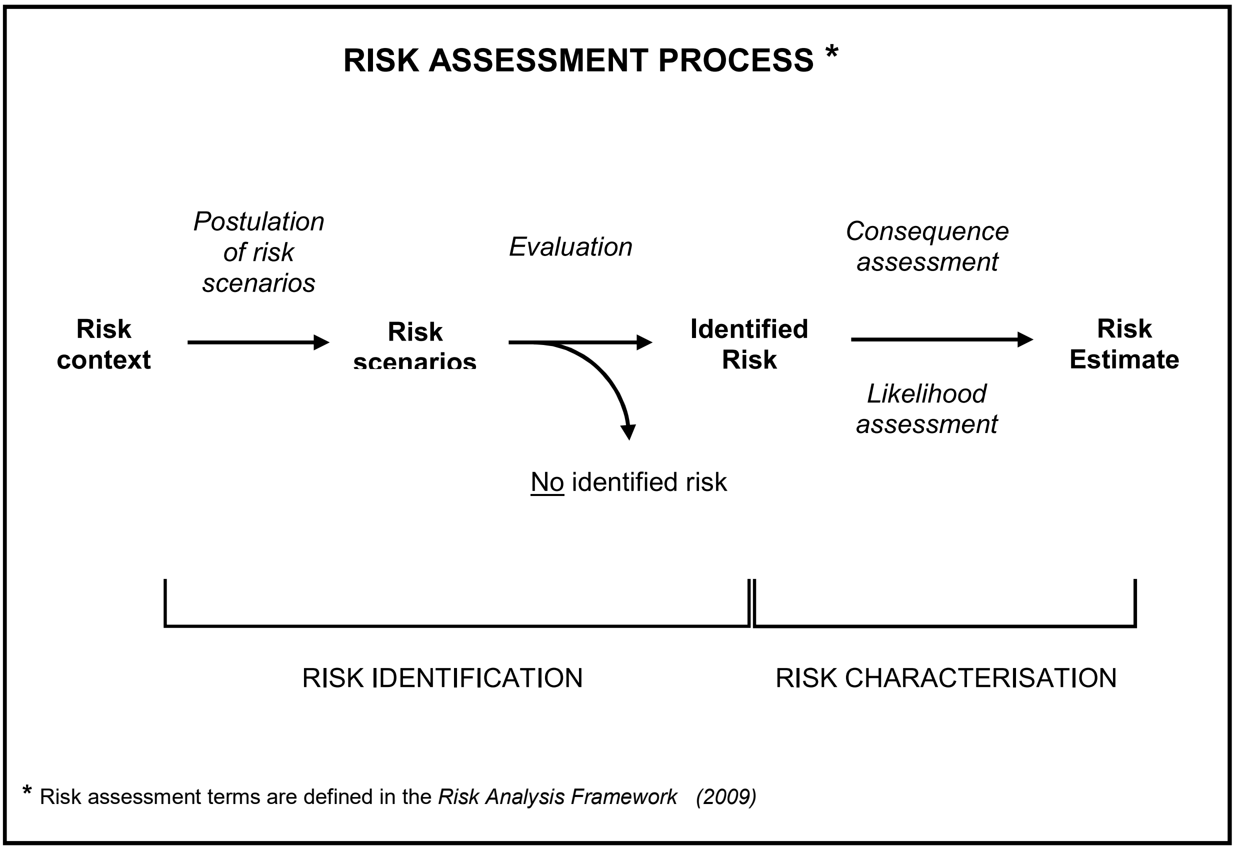

Figure 2 The risk assessment process

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

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

114. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, common sense, reported international experience and consultation (OGTR 2009). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

115. Identified risks (*ie* those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments.

Section 2 Risk Identification

116. The following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- the proposed limits, if any
- the proposed controls, if any
- characteristics of the parent organism(s)
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- 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.

117. The GMO proposed for release in the current application, Roundup Ready Flex® pima cotton, has previously been assessed prior to the Regulator issuing a licence for a limited and controlled trial DIR 074/2007. This licence is still current. Further, the GMO is derived by conventional breeding from Roundup Ready Flex® *G. hirsutum*, which itself was comprehensively assessed by the Regulator before issuing licences for commercial release (DIR 059/2005, DIR 066/2006). There have been no reports of adverse effects on the health and safety of people or the environment resulting from any of these releases.

118. As the GMO is derived by conventional crossing, the risks from unintended changes to the biochemistry (including innate toxic or allergenic compounds), physiology or ecology of the GMOs are not expected to be greater than those for the parental GMO (Roundup Ready Flex® *G. hirsutum*), which were assessed as negligible (see DIR 066/2006 and DIR 059/2005).

119. Nevertheless, although the GM cotton plants of this application are derived from conventional breeding, three risk scenarios were identified. These are evaluated in the context of the large scale of the release proposed by the applicant and in the absence of proposed limits and controls. These are summarised in Table 2.1, where circumstances that share a number of common features are grouped together in broader risk categories. None of the risk scenarios were identified as a risk that could be greater than negligible. Therefore, they did not warrant further detailed assessment. More detail of the evaluation of these scenarios is provided later in this Section.

120. All of the introduced regulatory sequences are derived from common plants and viruses. Similar regulatory elements are naturally present in cotton, and the introduced elements operate in same way as endogenous ones. Although the transfer of introduced regulatory sequences into new genetic contexts, either in other plants or other organisms, could result in unpredictable effects, the likelihood and impact of transfer of the introduced regulatory

elements will not be different to those from endogenous regulatory elements. Hence these potential effects will not be further assessed for this application.

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

Table 2.1 Summary of risk scenarios from dealings with GM *G. barbadense* cotton genetically modified for herbicide tolerance

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 **Production of a toxic or allergenic substance**	1. Production of a toxic or allergenic compound in the GM plant material and exposure to that compound	Allergenic reactions in people or toxicity in people and other organisms	No	• The GM cotton proposed for release (Roundup Ready Flex® pima cotton) is the product of conventional breeding between a GM cotton line already assessed and approved by the Regulator for commercial release and non-GM *G. barbadense*. The GM parent was assessed by the Regulator to be as safe as non-GM cotton. • The GM cotton proposed for release is not expected to be any more toxic or allergenic than the parental lines. • Products derived from Roundup Ready Flex® pima cotton are approved by FSANZ for use in human food.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.2 **Increased weediness in the environment**	2. Expression of the introduced genes for herbicide tolerance increasing the weediness of the GM cotton	Environmental harms associated with weediness; allergenic reactions in people or toxicity in people and other organisms	No	• The GM cotton proposed for release (Roundup Ready Flex® pima cotton) is the product of conventional breeding between a GM cotton line already assessed and approved by the Regulator for commercial release and non-GM *G. barbadense*. The GM parent was assessed by the Regulator to be as safe as non-GM cotton. • The genetic modifications are not expected to alter the response of GM cotton to biotic and abiotic stresses that naturally limit the geographical distribution of the species. • The genetic modifications are expected to increase the fitness of GM cotton plants in managed environments, but only when the corresponding herbicide is applied. • Cotton plants with tolerance to glyphosate can still be controlled by other herbicides or mechanical means.
Section 2.3 **Vertical transfer of genes to sexually compatible plants**	3. Expression of the introduced genes in other cotton plants	Environmental harms associated with weediness; allergenic reactions in people or toxicity in people and other organisms	No	• Risk scenarios 1 and 2 did not identify risks from the GM cotton itself for people or the environment. • The resulting GMOs will be similar to GM Roundup Ready Flex® *G. hirsutum*, which has been approved by the Regulator for commercial release. • The genetic modifications are not expected to alter the response of GM cotton to biotic and abiotic stresses that naturally limit the geographical distribution of the species. • The genetic modifications are expected to increase the fitness of GM cotton plants in managed environments, but only when the corresponding herbicide is applied. • Cotton plants with tolerance to glyphosate can still be controlled by other herbicides or mechanical means.

2.1 Production of a toxic or allergenic substance

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

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

124. A range of organisms may be exposed directly or indirectly to the protein (and end products) encoded by the introduced gene for herbicide tolerance. Workers cultivating the GM cotton would be exposed to all plant parts. FSANZ has approved the use oil and linters derived from Roundup Ready Flex® *G. hirsutum* in food (FSANZ report A553). This approval also applies to Roundup Ready Flex® pima cotton. Organisms may be exposed directly to the proteins through biotic interactions with GM cotton plants (vertebrates, invertebrates, symbiotic microorganisms and/or pathogenic fungi), or through contact with root exudates or dead plant material (soil biota) or indirectly through the food chain.

Risk scenario 1. Production of a toxic or allergenic compound in the GM plant material, and exposure to that compound.

125. Introduction of the *cp4 epsps* gene into *G. barbadense* could potentially result in the production of toxic or allergenic compounds in the resulting GM Roundup Ready Flex® pima cotton plants. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these people or other organisms.

126. Increased toxicity or allergenicity could be due to direct expression of the introduced *cp4 epsps* genes (discussed below). Alternatively, these properties could also arise unintentionally as a result of the genetic modification process, most particularly by the genomic location of the introduced *cp4 epsps* genes (initially as transformed into *G. hirsutum*) affecting the expression of other genes, or genetic changes during the hybridisation and backcrossing with *G. barbadense* affecting the expression of other genes. These genetic changes could result in either the up- or down-regulation of genes in the genome of *G. barbadense*. Although highly unlikely, it is also possible that novel proteins with unwanted properties could be produced via the fusion of DNA segments during the genetic modification of *G. hirsutum* (to generate Roundup Ready Flex® *G. hirsutum*), or subsequent hybridisation and backcrossing with *G. barbadense*. However, the experience of conventional breeding (a process that continually creates novel junctions between previously separated DNA segments) is that the risk of such unintended events occurring is negligible (Bradford et al. 2005; FAO 2001; Herman & Ladics 2011; Pilacinski et al. 2011). Southern and western blot data provided by the applicant suggests that there are no partial *epsps* sequences in GM *G. hirsutum* or GM pima cotton, and no adverse effects have been associated with the commercial release of Roundup Ready Flex® *G. hirsutum*. The formation of novel junctions as a result of hybridisation and backcrossing of the GM cottons would be no more likely than for breeding with non-GM plants and, as described above, any such sequence would be unlikely to increase toxicity or allergenicity.

127. The *cp4 epsps* gene for herbicide tolerance was isolated from *Agrobacterium tumefaciens*, which is widespread and prevalent in the environment worldwide (Furuya et al. 2004). Homologous genes, and hence EPSPS proteins, are present in plants, and thus are a regular part of human and animal diets. The protein CP4 EPSPS is also present in the GM cotton plants currently approved for commercial release under DIR 066/2006, and commercially released GM Roundup Ready® canola (DIR 020/2002, DIR 108). Additionally, these GM canola plants have been approved for use in animal feed since 1995, thus exposing livestock to the CP4 EPSPS protein without any known ill effects.

128. As noted in Chapter 1, Section 5.1, no published study indicates that the CP4 EPSPS protein has any toxic or allergenic property, and homologous EPSPS proteins that perform the identical biochemical reaction occur in all plants and many other microorganisms.

129. In Australia, FSANZ has approved for human consumption material derived from GM plants (including lucerne and soybean) expressing the CP4 EPSPS protein (FSANZ 2006; FSANZ 2007). The assessments by FSANZ note that there is no evidence of toxic and allergenic properties associated with these proteins.

130. The applicant has received approval from FSANZ for the use of oil and linters derived from Roundup Ready Flex® *G. hirsutum* and Roundup Ready Flex® pima cotton in food (FSANZ 2005). In this report, FSANZ states (pg 17) "EPSPS is expressed at low levels or is undetectable in the cotton and their processed fractions, and therefore exposure to the protein through consumption of food derived from cotton line MON 88913 would be negligible, if at all." Since the approval of Roundup Ready Flex® *G. hirsutum* (2006) there have been no reports of toxicity and/or allergenicity from those GM plants.

131. Outside Australia, the United States Environmental Protection Agency (EPA) has determined that there is no need to establish a maximum permissible (tolerance) level of the CP4 EPSPS protein in plants (EPA 1996). In this ruling, the EPA notes that proteins such as EPSPS have no significant amino acid homology to known mammalian protein toxins, are readily inactivated by heat or mild acidic conditions, and are degraded in a digestibility assay, thus are unlikely to be toxins or allergens.

132. Analysis of the compositional data for Roundup Ready Flex® pima cotton (Chapter 1, Section 6.2.2) indicates that there are no meaningful differences in the levels of analysed compounds between the GM cotton and non-GM *G. barbadense*. Of particular importance are the levels of gossypol and a number of cyclopropenoid fatty acids (malvalic acid, sterculic acid and dihydrosterculic acid), all of which are natural toxins that are produced in cotton. The compositional data indicates that the levels of these compounds were all within the 99% tolerance interval for commercial cotton varieties (Chapter 1, Section 6.2.2). The biosynthesis of these compounds is not related to the shikimic acid pathway, the metabolic pathway in which EPSPS is involved (Alba et al. 2009).

133. As outlined in Chapter 1, Section 5.2, the two *cp4 epsps* genes in Roundup Ready Flex® pima cotton are driven by chimeric promoters, one partially consisting of sequences from the CaMV 35S promoter and the other partially of sequences from the FMV 35S promoter. This section also notes that it has been suggested that the protein P6, of which a truncated form may be expressed from these promoters, could result in harm to humans if expressed in GM plants (Latham & Wilson 2013). However, bioinformatics searches suggest it is extremely unlikely that the P6 protein possesses any allergenic or toxic properties (Podevin & du Jardin 2012). FSANZ has examined the Podevin and du Jardin research paper, as well as subsequent commentary, and is confident there is no scientific grounds for reviewing the approvals for GM foods in which the CaMV 35S promoter has been used (FSANZ 2013). The cauliflower mosaic virus infects Brassicaceae plants that are a regular part of our diet without being associated with any negative effects. A statement to this effect has also been made by EFSA (EFSA 2013). It should also be noted that the use of variants of this promoter in a number of commercially grown GM crops in Australia and other countries, including the Roundup Ready Flex® *G. hirsutum* containing this genetic modification, has not been linked to any harms to human or animal health.

134. ***Conclusion***: The potential for allergenic reactions in people, or toxicity in people and other organisms as a result of exposure to GM plant materials containing the proteins encoded

by the introduced genes is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

2.2 Potential for weediness of the GM cotton in the environment

135. This section addresses the question of whether or not genetic modification of the GM cotton may generate plants that have one or more harms to human health and safety or the environment as a result of increased weediness.

136. All plants have the potential to lead to harm in certain environments. Harms that may arise from a certain plant species in a particular environment include:

- adverse effects on the health of people and/or animals
- reduction in the establishment, yield and/or quality of desired plants
- restriction in the physical movement of people, animals, vehicles, machinery and/or water
- adverse effects on environmental health, such as adverse changes to strata levels, nutrient levels, fire regime, soil salinity, soil stability, or by providing food and/or shelter to pests, pathogens and/or diseases.

137. For the purpose of this document, plant species causing significant levels of one or more of these harms are called ‘weeds’. A plant species may be weedy in one or more land uses, such as dryland cropping or nature conservation.

138. Characteristics that influence the spread (dispersal of the plant or its genetic material) and persistence (establishment, survival and reproduction) of a plant species impact on the degree of its invasiveness. These characteristics include the ability to establish in competition with other plants, to tolerate standard weed management practices, to reproduce quickly, prolifically and asexually as well as sexually, and to be dispersed over long distances by natural and/or human means. The degree of invasiveness of a plant species in a particular environment gives an indication of the likelihood of its weediness in that environment. In addition to local experience, a history of weediness overseas can be used as an indicator for weediness in Australia (Pheloung et al. 1999).

139. Baseline information on the weediness of cotton, including factors limiting the spread and persistence of non-GM cotton plants, is given in in the document, *The Biology of* Gossypium hirsutum L. *and* Gossypium barbadense L. *(cotton)* (OGTR 2013). In summary, cotton seems to have a limited ability to become invasive in undisturbed nature conservation areas. As such, in most areas of Australia cotton is not considered a weed, and even in those areas where it may be classified as a weed, it would not be regarded as of serious concern to human health, safety or the environment. The weediness of the parental GM cotton lines was assessed in the RARMPs prepared for DIR 059/2005 and DIR 066/2006. This information was summarised and updated in Chapter 1, Sections 4.2.2 and 5.5.

Risk scenario 2. Expression of the introduced genes for herbicide tolerance increasing the weediness of the GM cotton

140. If the GM Roundup Ready Flex® pima cotton plants were to become more invasive in the environment (increased ability to spread and persist), one or more of the above discussed potential harms could materialise.

141. For example, if the GM cotton plants possessed a significant selective advantage over commercially released non-GM cotton plants, and if they were able to spread and persist in non-cropped disturbed habitats and undisturbed natural habitats, this may give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species

composition. Further, the exposure of humans and other organisms to the GM plant material could be increased.

142. The potential for increased toxicity or allergenicity in people and other organisms as a result of contact with GM plant materials was discussed in Risk scenario 1 and was not considered an identified risk. As such, even if the GM cotton plants were to have increased weediness, the risk of toxicity or allergenicity in all organisms would remain negligible.

143. There is no evidence that the *cp4 epsps* gene produces a protein that is directly related to any trait that would lead to increased weediness of the GM cotton, other than in settings where weed control is achieved primarily by application of glyphosate (CERA 2010). Furthermore, since the approval of Roundup Ready Flex® *G. hirsutum* (2006), there have been no reports of these GM cotton plants (or other plants containing this gene) being classified as weeds on the basis of harms linked to the expression of this gene.

144. In the event of seed dispersal, the introduced trait would only provide a selective advantage as a consequence of application of the herbicide (glyphosate), which is not generally used to control established cotton plants (Chapter 1, Section 5.5). In the absence of the herbicide, the GM cotton plants would be subjected to the same selective pressures as unmodified cotton.

145. If the GM plants were nevertheless to become established in non-cropped disturbed habitats (*eg* along roadsides), undisturbed natural habitats and/or cultivated areas, other herbicides, such as bromoxynil, carfentrazone and a combination of paraquat and diquat have been shown to be effective in eradication of cotton (Roberts et al. 2002). As opposed to glyphosate, these latter herbicides target different plant molecules. Glyphosate, a Group M herbicide, targets EPSPS, whereas bromoxynil (Group C), carfentrazone (Group G), paraquat and diquat (Group L) target photosystem II, protoporphyrinogen oxidase, and photosystem I, respectively (CropLife Australia 2012a). Non-chemical means, such as slashing, grading or cultivation, can be used for volunteer Roundup Ready Flex® pima cotton.

146. As the genetic modification is not associated with pest or pathogen resistance, there is no reason to expect that the GM plants will have a significantly different interaction with such organisms when compared to unmodified plants.

147. A set of agronomic and phenotypic characteristics of the Roundup Ready Flex® pima cotton plants that possibly may be related to weediness were examined in the United States and Australia (Constable 2012; Phillips et al. 2009). These characteristics, including germination, seedling vigour, yield and response to cold, were found to be essentially identical to those found in non-GM *G. barbadense.* A small number of these characteristics were found to be statistically different. However, the level of difference was within the range of the reference non-GM pima cottons included in the studies and of a level that would be expected in any conventional breeding program. In the Australian trials (Constable 2012), no significant differences is agronomic performance were recorded but there was a trend for longer fibres and decreased micronaire, as well as a slight yield improvement, in the GM plants. Such variation in characteristics would be expected in any program of conventional plant breeding (Acquaah 2007; Bradford et al. 2005). These fibre characteristics are not expected to impact on weediness. This data is further discussed in Chapter 1, Section 6.2.2.

148. Increased weediness could also arise unintentionally as a result of the genetic modification process, most particularly by the genomic location of the *cp4 epsps* genes (initially as transformed into *G. hirsutum*) affecting the expression of other genes, or genetic changes during the hybridisation and backcrossing with *G. barbadense* affecting the expression of other genes. No adverse effects have been associated with the commercial

release of Roundup Ready Flex® *G. hirsutum* containing this genetic modification. Potential pathways which could result in unintentional changes were discussed in risk scenario 1, which concluded that the risk of unintended changes producing a harm is negligible.

149. As outlined in Chapter 1, Section 5.2, the two *cp4 epsps* genes in Roundup Ready Flex® pima cotton are driven by chimeric promoters, one partially consisting of sequences from the CaMV 35S promoter and the other partially of sequences from the FMV 35S promoter. It has been suggested that the protein P6, of which a truncated form may be expressed from these promoters, could interfere with the anti-pathogen defences of GM plants in which it is expressed (Latham & Wilson 2013). However, no adverse effects have been associated with the commercial release of Roundup Ready Flex® *G. hirsutum* containing this genetic modification, or with other GM crops containing variants of this promoter. GM crops displaying increase susceptibility to disease they would be unlikely to pass through a breeding program and be selected for commercialisation.

150. ***Conclusion***: The potential for improved survival of the GM cotton through the expression of the introduced genes leading to weediness is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

2.3 Vertical transfer of genes to sexually compatible plants

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

152. It should be noted that vertical gene flow *per se* is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome. For an increased potential for adverse effects to arise as a result of gene flow of the introduced genetic elements from the GM cotton to sexually compatible plants, both of the following steps must occur:

- transfer of the introduced genetic elements to sexually compatible plants
- increased potential for adverse effects, such as toxicity, allergenicity or spread and persistence of the recipient plants, due to expression of the introduced gene.

153. Baseline information on vertical gene transfer associated with non-GM cotton plants can be found in *The Biology of* Gossypium hirsutum L. *and* Gossypium barbadense L. *(cotton)* document (OGTR 2013). In summary, cotton is predominantly self-pollinating and no self-incompatibility mechanisms exist. As pollen is large, sticky and heavy it is not easily dispersed by wind, any cross-pollination likely being conducted by honeybees. It is does not reproduce by asexual mechanisms, although root cuttings can be propagated under laboratory conditions.

Risk scenario 3. Expression of the introduced genes in other cotton plants

154. Transfer and expression of the introduced genes for herbicide tolerance to other cultivated or naturalised cotton plants (*G. hirsutum* or *G. barbadense*) could increase the toxicity, allergenicity and/or weediness of the resulting hybrid plants. Such plants could either establish their own population (via self-fertilisation), or backcross with one of both parental types, in either case passing on the acquired characteristic(s).

155. As discussed in Risk Scenarios 1 and 2, the genetic modification is not expected to change the toxicity, allergenicity and weediness of the GM *G. barbadense* plants. Hence, any cross-hybridisation of these GM plants to other cotton plants, whether GM *G. hirsutum* plants

(Roundup Ready Flex®, Roundup Ready®, Roundup Ready Flex®/ Bollgard II®, Roundup Ready®/ Bollgard II®, Bollgard II®, Liberty Link®, or Widestrike®) or non-GM cottons, is not likely to generate a plant that that would cause an adverse outcome with respect to toxicity, allergenicity or weediness. It should of course be appreciated that Roundup Ready Flex®, Roundup Ready®, Roundup Ready Flex®/ Bollgard II®, and Roundup Ready®/ Bollgard II® are already engineered for glyphosate tolerance, containing at least one *cp4 epsps* gene.

156. The GM cottons Liberty Link® and Widestrike® contain an introduced *bar* or *pat* gene, respectively, which confer tolerance to the herbicide glufosinate ammonium (phosphinothricin). Hybridisation between Roundup Ready Flex® pima cotton and either Liberty Link® or Widestrike® cotton would produce a plant with tolerance to both glyphosate and glufosinate ammonium. Widestrike® cotton, although approved by the Regulator for commercial release in Australia, has never been commercially grown. It should also be noted that this combination of herbicide tolerances is also possible through hybridisation between the GM cotton (*G. hirsutum*) varieties currently authorised for commercial release listed above. The possibility of stacking of these GM herbicide tolerance traits was considered in detail in the RARMPs for DIRs 062/2005, 066/2006 and 091 and was not found to represent a risk greater than negligible. These hybrid plants would still be susceptible to mechanical methods or eradication and other unrelated herbicides such as bromoxynil, carfentrazone and a combination of paraquat and diquat (Roberts et al. 2002).

157. The introduced trait in Roundup Ready Flex® pima cotton, herbicide tolerance, will only provide a selective advantage when plants are exposed to the appropriate herbicide (glyphosate). In the absence of the herbicide, any plants that receive the *cp4 epsps* gene via hybridisation will be subjected to the same selective pressures as unmodified plants.

158. There are a number of native *Gossypium* species in Australia (Chapter 1, Section 5.6.2), three of which occur in regions where cotton is cultivated. However, due to genetic differences between cultivated cottons and these native species (cultivated cottons are tetraploids with the AD genomes, whereas the native species are diploids with either the C, G or K genomes), gene transfer from any cultivated cotton to these species is extremely unlikely (OGTR 2013). 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. Attempts to hybridise cultivated cottons (mainly *G. hirsutum*) with other native species under optimal artificial conditions, including use of plant hormones, have produced some hybrid seed, but in nearly all cases this seed has not been viable, and it is highly unlikely that such hybridisations would occur in the natural environment.

159. A number of insect resistant cottons have been commercially released in Australia. These are the *G. hirsutum* varieties Bollgard II®, Roundup Ready®/Bollgard II®, Roundup Ready Flex®/Bollgard II®, and Widestrike®. Potentially these could breed with Roundup Ready Flex® pima cotton, and the insect resistant traits introgressed into the Roundup Ready Flex® pima cotton background. Although fertile F_1 hybrids between *G. hirsutum* and pima can be readily formed, hybridisation is restricted by both genetic and physical isolating mechanisms. F_2 and later generations show evidence of lethal gene combinations, and different timings of flowering during the day limit the transfer of pollen between the species (OGTR 2013). Hybrids also have a lower capacity to produce cotton bolls, hence reducing the amount of seed that they can produce. Furthermore, from an agricultural perspective, as the purity of cotton varieties is of importance to cotton farmers (especially for certified seed production (OGTR 2013)), the separation of different varieties from a single species, and those from different species, is standard commercial practice. Therefore, it is unlikely that the

GM insect resistant traits present in the *G. hirsutum* plants would be transferred to any pima plant (GM or non-GM), and lead to a stable introgressed *G. barbadense* population. The formation of *G. hirsutum* and pima hybrids with herbicide tolerant and insect resistance traits has also been considered in the RARMPs for DIRs 059/2005, 062/2005, and 066/2006, with *G. hirsutum* as the pollen donor. The risk of such plants to human health, safety or the environment was assessed as negligible.

160. ***Conclusion***: The potential for allergenicity in people, or toxicity in people and other organisms, or increased weediness due to expression of the introduced genes in other cotton plants as a result of gene transfer is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

Section 3 Risk estimate process

161. The risk assessment begins with postulation of credible pathways that might lead to harm to the health and safety of people or the environment as a result of the proposed release of GMOs and due to gene technology, and how it could happen. This is considered in comparison to the parent organism and within the context of the receiving environment.

162. Three risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms, or alter characteristics that may impact on the spread and persistence of the GM plants. The opportunity for gene flow to other organisms and its effects if it occurred were also assessed. These risk scenarios were considered over both the short and long term.

163. A risk is only identified when a risk scenario is considered to have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

164. The characterisation of the three risk scenarios in relation to both the seriousness and likelihood of harm did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- the GM pima cotton has been produced by conventional breeding of a GM cotton line that has previously been assessed and authorised for commercial release in Australia
- widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity or evidence of harm from them
- limited capacity of the GM pima cotton to spread and persist in undisturbed natural habitats.

165. Therefore, any risks to the health and safety of people, or the environment, from the proposed release of the GM cotton plants into the environment are considered to be negligible**.** Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment[2].

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

Section 4 Uncertainty

166. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (*ie* consequence and likelihood) are always uncertain to some degree.

167. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability[3]. For commercial/general releases, where there may not be limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, uncertainty may be addressed through post release review (Chapter 3, Section 4).

168. Roundup Ready Flex® pima has been approved by the Regulator for limited and controlled release (field trials) under licence DIR 074/2007. Further, Roundup Ready Flex® *G. hirsutum* has been approved for commercial release in DIR 066/2006. The latter licence also authorises the release of several other GM cotton lines that possess the same introduced gene.

169. The RARMP for DIR 074/2007 identified additional information that may be required for a large scale or commercial release of Roundup Ready Flex® pima. The relevant information can be summarised as:

- confirmation that the proteins produced by the introduced genes in the GM Roundup Ready Flex® pima cotton do not differ from those produced in GM Roundup Ready Flex® (*G. hirsutum*) cotton
- expression levels of the introduced proteins in various parts of the cotton plant
- effect of lepidopteran herbivory on *G. barbadense* (this relates to insect resistant GM pima cotton also assessed in DIR074/2007, not to Roundup Ready Flex® pima cotton)
- comparison of establishment potential, including germination potential, or *G. barbadense* and *G. hirsutum*
- data on agronomic characteristics indicative of weediness of the GM *G. barbadense* lines compared to *G. hirsutum*

170. In preparing the application for DIR 118 Monsanto provided information in relation to these points. These have been discussed in relevant areas in this RARMP (Chapter 1, Sections 6.2.1 and 6.2.2) and are summarised here. Much of this data comes from crossing Roundup Ready Flex® *G. hirsutum* with non-GM *G. barbadense* in the United States, but it is unlikely that the results would be significantly different in regards to the Roundup Ready Flex® pima cotton plants produced in Australia.

171. Molecular data demonstrated that the size and structure of the introduced gene sequence is as predicted, and is as found in Roundup Ready Flex® *G. hirsutum*, indicating that the *cp4 epsps* genes are intact, while western data confirms that the expressed EPSPS protein is of the expected size (Groat et al. 2009; Mozaffar & Silvanovich 2009). The presence of a functionally active EPSPS protein is demonstrated by the glyphosate tolerance of the GM plants.

172. Data pertaining to the expression of the CP4 EPSPS protein in leaf and seed tissue of Roundup Ready Flex® pima has been provided by the applicant. This data demonstrates that the mean protein levels in seed is similar to that recorded for Roundup Ready Flex® *G. hirsutum*, but in leaf the level in the Roundup Ready Flex® pima cotton can be up to twice

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

that in Roundup Ready Flex® *G. hirsutum* (Mozaffar & Silvanovich 2009; OGTR 2006). This difference may reflect genetic variation or environmental factors. Regardless, as noted above (Chapter 2, Section 2.1), there is negligible risk of any toxicity or allergenicity associated with the expression of the CP4 EPSPS protein.

173. A set of agronomic characteristics (percent seed germinated, viable hard seed, viable firm swollen seed, dead seed and response to cold) of the Roundup Ready Flex® pima cotton plants that possibly may be related to weediness were examined. These were found to be essentially identical to those found in non-GM *G. barbadense*.

174. A small number of phenotypic characteristics were found to be statistically different between Roundup Ready Flex® pima cotton and non-GM pima cotton cultivars in the United States and in Australia (Constable 2012; Phillips et al. 2009). However, this level of difference would be expected in any conventional breeding program.

175. The effect of lepidopteran herbivory on Roundup Ready Flex® pima is not considered, as this request is only relevant to the insect resistant lines trialled under DIR 074/2007.

176. Uncertainty can also arise from a lack of experience with the GMO itself. In regards to Roundup Ready Flex® pima cotton, the level of uncertainty is low given the several years of growing this GMO in the United States, and many years of growing Roundup Ready Flex® *G. hirsutum* in Australia and the United States. None of these releases have resulted in concerns for human health, safety or the environment.

Chapter 3 Risk management

Section 1 Background

177. 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, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

178. 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.

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

180. 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. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Risk treatment measures for identified risks

181. 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 GM cotton. These risk scenarios were considered in the context of the large scale of the proposed release and the receiving environment. The *Risk Analysis* Framework (OGTR 2009) which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. Therefore, no conditions are imposed to treat these negligible risks.

Section 3 General risk management

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

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

3.1 Applicant suitability

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

- any relevant convictions of the applicant (both individuals and the body corporate)
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

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

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

186. 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

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

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

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

3.4 Reporting requirements

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

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

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

191. 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

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

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

Section 4 Post release review

194. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator does not fix durations, but takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

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

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

196. 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

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

4.2 Requirement to monitor specific indicators of harm

198. 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.

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

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

201. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks that could be greater than negligible. Therefore, no specific indicators of harm have been identified in this RARMP for application DIR 118.

4.3 Review of the RARMP

202. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would be desktop-based and take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components

of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s), or identified new risks to people or to the environment that needed managing, this could lead to review of the risk management plan and changes to the licence conditions.

Section 5 Conclusions of the RARMP

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

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

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Appendix A Summary of advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the consultation RARMP for DIR 118[4]

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

Summary of issues raised	Comment
Weediness of the GM cotton, including potential to impact on weed control operations.	This issue is considered in Chapter 2 of the RARMP.
In preparing the RARMP, the Regulator should consider the differences between *G. barbadense* and *G. hirsutum* where relevant.	Differences between these cotton species are highlighted in Chapter 1, section 7 of the RARMP and taken into account in the risk assessment where appropriate. In assessing potential risks, including increased weediness, the main comparator for the GM pima cotton is non-GM pima cotton.
New non-GM blight resistant varieties of pima cotton being developed may increase the area where this species of cotton is cultivated, perhaps expanding to areas near existing naturalised cotton populations, and where cold is not a limiting factor. Unintended consequences from the genetic modification, such as increased germination rate in cold conditions, may impact on weediness, either directly or in any future blight resistant varieties. The risk management plan should include provisions to monitor for any unintended effects on its weediness.	If blight resistance does result in an increase in the areas where pima cotton is cultivated, and is combined with the engineered herbicide tolerance, then it is possible that the genetic modifications will spread into naturalised cotton populations. However, the genetic modification will only provide a selective advantage when plants are exposed to the corresponding herbicide (glyphosate). In the absence of the herbicide, any plants that receive the *cp4 epsps* gene via hybridisation will be subjected to the same selective pressures as unmodified plants (Risk scenario 3, Chapter2). A small increase in the germination rate for some GM pima seed compared to the non-GM pima control was identified in the data provided by the applicant (section 6.2.2, Chapter 1). However, the difference was within the range of seven other non-GM pima cottons used as references and such differences would be expected in any conventional breeding program (Risk scenario 2, Chapter 2). The risk management plan has not identified specific provisions to monitor for any unintended effects on the weediness of the GM pima cotton. However, proposed licence conditions would require reporting of any new information about risks or unintended effects of the authorised dealings.
Consequences for human health or the environment of any unintended effects should be assessed. Suggests that feeding studies be conducted with a range of organisms that represent those found in cotton growing field conditions.	Unintentional changes were considered in risk scenario 1, Chapter 2, which concluded that the risk of unintended changes producing harm is negligible. No adverse effects have been associated with the commercial release of Roundup Ready Flex® *G. hirsutum*, which carries the same genetic modification. The introduced genes have been assessed (including numerous feeding studies) in GM cotton and many other GM crops which have been approved for human consumption in Australia and other countries (see summaries and references in Chapter 1).
Considers that the GM pima cotton does not pose any additional risk than Roundup Ready *G. hirsutum.*	Noted.
Supportive of this application.	Noted.

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

Appendix B Summary of advice from prescribed experts, agencies and authorities on the consultation RARMP for DIR 118[5]

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

Summary of issues raised	Comment
FSANZ has already assessed and approved the use of food derived from the GM parent (Roundup Ready Flex® G. hirsutum). This approval covers food produced from any offspring resulting from conventional breeding.	Noted.
No concerns with the proposed release given that Victoria is not a commercial cotton growing area, and genetic modification is not expected to alter the response of the GM pima cotton to the environmental stressors that naturally limit the geographical distribution of this species.	Noted.
Supportive of the assessment that the proposed dealing poses negligible risk of harm to human health and the environment.	Noted.
No comments on the RARMP.	Noted.
Supportive of the assessment that the proposed dealing poses negligible risk of harm to human health and the environment.	Noted.
The shire voted in 2009 to be a "GM-free cropping zone".	Due to the geographical location of this shire, and associated climatic factors, cotton is unlikely to be grown in this shire. 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. Marketing issues are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. However areas may be designated under State or Territory law for the purpose of preserving the identity of GM or non-GM crops (or both) for marketing purposes. The licence contains a preamble and condition 3 which indicate that dealings with GMOs are not authorised if otherwise prohibited as a result of such State legislation.
Agrees with the overall conclusions of the RARMP. The risk assessment identified relevant risk scenarios. There is no additional relevant information that should be considered.	Noted.
Clarification of the growth of Widestrike® cotton in Australia should be provided in the RARMP.	The RARMP has been amended to note that although the Regulator has approved Widestrike® cotton for commercial release in Australia, it has never been grown commercially.

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

Summary of issues raised	Comment
The RARMP should note when applying data from *G. hirsutum* (upland cotton) to the assessment of weediness of *G. barbadense* (pima cotton).	The RARMP has been amended to make the plant species to which data relate clear. Discussion of the application of data from *G. hirsutum* to *G. barbadense* has been inserted in Chapter 1 Section 4.2.2.
In Chapter 1 Risk Assessment Context, it is not clear whether the information provided is relevant to pima cotton (*G. barbadense*), upland cotton (*G. hirsutum*) or both. Hence, request that early in the RARMP the word 'cotton' be defined as referring either to pima cotton or both pima and upland cotton. Differences between these two cottons should be explicitly discussed in this chapter, one example being the factors that contribute to the limited growing region of pima cotton. Although the main comparator for the GM pima cotton is non-GM pima cotton, it is noted that some parts of the RARMP rely upon references to upland cotton. These include a report from FSANZ on the use of oil and linters from the GM upland parent, there being no adverse effects arising from the release of upland cotton containing the genetic modification, and hybridisation with native Australian cottons (paragraphs 128, 146, and 156, respectively of the consultation RARMP). In this context, it is suggested that a comparison between pima and upland cotton should be included, thus giving a foundation for using data from the latter species. Information on the differences between the two species in relation to topics such as resistance to pests and pathogens, centres of origin, long-distance dispersal by water and uneven transfer of chromosomal segments to hybrid progeny, may be relevant. [A number of scientific articles were cited.]	'Cotton' as generally used in agriculture is understood to refer to either pima or upland cotton. The RARMP has been amended to make the plant species to which data relate clear. Differences between pima and upland cottons are discussed in the OGTR cotton biology document. Sections 4.2.2, 7.1 and 7.3 of Chapter 1 of the RARMP have been amended to emphasise differences relevant to establishing the risk assessment context. Chapter 1 Section 6.1 has also been amended to indicate that although these species have differences, they have a fundamental core of common biological characteristics. As the GM pima cotton is the product of conventional breeding, one parent being upland cotton, information from this parent has been included as deemed appropriate. In specific reference to the limited growing region of pima cotton in Australia (as compared to that for upland cotton), applicable information has been inserted in Chapter 1 Section 7.1. Abiotic and biotic factors which impact the spread and persistence of pima cotton are provided in Chapter 1 Sections 7. 2 and 7.3. The articles cited with regard to dispersal of cotton seed by water have been considered in the OGTR cotton biology document. Although one of these suggests a potential for increased buoyancy and dispersal of *G. barbadense* cotton seed in salt water, the study centred on older races or forms of the species, with hard seed coats. Modern cotton varieties have been bred for softer seed coats, which minimises dormancy and allows for uniform germination. The softer seed coated varieties are expected to have little tolerance to extended exposure to fresh or salt water. Gene transfer to native cottons is discussed in Chapter 1 Section 5.6.2. Although most attempts at hybridisation between cultivated cottons and native cottons have involved upland cotton, as both upland and pima cotton have the same genomic constitution (AADD), it is a reasonable assumption that the results from upland cotton are applicable to pima cotton. It is not expected that traits such as pest and pathogen resistance or dispersal will be affected by the genetic modification. As the genetic modification has been transferred from upland to pima cotton by conventional cross-breeding, differences in transfer of chromosomal segments are not considered useful data in assessing this GM cotton.
The RARMP should include a discussion of the changes to the cropping areas for pima cotton in the future, in particular into regions near existing naturalised populations of this cotton.	Information has been added to Section 7.3 on areas of potential expansion of cultivated pima cotton in the future.
The RARMP has been examined and no additional risks to human health, safety and the environment have been identified.	Noted.
Supports approval of the licence on the terms indicated in the RARMP.	Noted.
Supportive of application as the evidence supplied indicates that the commercial release would pose negligible risks.	Noted.

Appendix C Summary of submissions from the public on the consultation RARMP for DIR 118

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

Abbreviations:

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

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

Other abbreviations: **APVMA**: Australian Pesticides and Veterinary Medicines Authority; **GM**: Genetically Modified; **RARMP**: Risk Assessment and Risk Management Plan.

View	Issue	Summary of issues raised	Comment
x	E,H, hu	Considers that release of the GM cotton will lead to increased use of glyphosate, which in turn could be a threat to the environment and human health.	The RARMP for this release considered information provided by the applicant and the currently available scientific information in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible. The APVMA has regulatory responsibility for the registration of agricultural chemicals, including herbicides, in Australia. The APVMA considers a range of issues in assessing agricultural chemicals for registration, including efficacy, resistance management and human health and environmental impacts. The APVMA will not register a chemical product unless satisfied that its approved use would not be likely to have an effect that is harmful to people or the environment.