



APPLICATION FOR LICENCE FOR INTENTIONAL RELEASE OF GMOs INTO THE ENVIRONMENT: Application No. DIR 055/2004

SUMMARY INFORMATION

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

Applicant: Monsanto Australia Ltd
PO Box 6051
Melbourne, VIC 8008

Common name of the parent organism: Cotton

Scientific name of the parent organism: *Gossypium hirsutum* L.

Modified trait(s): Enhanced herbicide tolerance, insecticidal action, antibiotic resistance, reporter gene expression

Identity of the gene(s) responsible for the modified trait(s):

- *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 (herbicide tolerance)
- *cry1Ac* and *cry2Ab* genes from the bacterium *Bacillus thuringiensis* (insecticidal)
- *nptII* gene from the bacterial Tn5 transposon (antibiotic resistance)
- *uidA* gene from *Escherichia coli* (reporter gene)

Proposed Location(s) New South Wales (NSW), Queensland (Qld), northern Western Australia (WA), Northern Territory (NT)

Proposed release sizes and dates: September 2005 – November 2006

Season	Number of sites	Maximum total area (hectares)
Summer 2005/06	86	1769
Winter 2006	5	42
Total	91	1811

Introduction

The *Gene Technology Act 2000* (the Act) took effect on 21 June 2001. The Act, supported by the *Gene Technology Regulations 2001*, an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, underpins Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene

technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs).

The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), an Australian Government regulatory agency located within the Health and Ageing portfolio.

The legislation sets out the requirements for considering applications for licences for dealings with GMOs, which includes the preparation of a risk assessment and risk management plan (RARMP) for each proposed intentional release of a GMO into the environment, and the matters that the Regulator must take into account before deciding whether, or not, to issue a licence.

The application and the proposed dealings

The OGTR has received an application from Monsanto Australia Limited (Monsanto) for a licence for the intentional release of genetically modified (GM) herbicide tolerant cotton (Roundup Ready[®] Flex MON 88913) and herbicide tolerant/insect resistant cotton (Roundup Ready[®] Flex MON 88913/Bollgard II[®]) into the environment, under limited and controlled conditions.

Monsanto proposes to carry out field trials on up to 91 sites covering a total area of up to 1811 hectares over two planting seasons, the southern summer growing season and the northern winter growing season, between September 2005 and November 2006. The summer trials (maximum of 1769 hectares, including four large sites comprising 100 hectares each) would be conducted in the cotton growing regions of NSW and southern Qld, and the winter trials (maximum of 42 hectares) in northern WA, the NT and northern Qld.

GM Roundup Ready[®] Flex MON 88913 cotton (abbreviated here as RR Flex cotton) contains two copies of the *cp4 epsps* gene that provides tolerance to glyphosate, the active ingredient in the herbicide Roundup[®]. Conventional cotton is susceptible to glyphosate damage. The use of RR Flex cotton allows the application of the herbicide Roundup[®] for the control of weeds that emerge in the crop, without damaging the crop itself.

RR Flex cotton differs from the previous commercially released Roundup Ready[®] cotton in that tolerance to Roundup[®] herbicide is prolonged. Currently, yield loss occurs if Roundup[®] is applied to Roundup Ready[®] cotton beyond the four leaf growth stage. RR Flex cottons are able to tolerate application of Roundup[®] at later stages of plant growth without yield loss, allowing a wider window to apply herbicide during growth of the cotton crop. This is intended to give growers increased flexibility in timing of herbicide application for integrated weed management and is not expected to increase the overall amount of herbicide use.

Roundup Ready[®] Flex MON 88913/Bollgard II[®] (RR Flex/Bollgard II[®]) cotton was produced by conventional breeding of Roundup Ready[®] Flex MON 88913 cotton with Bollgard II[®] cotton which contains two insecticidal genes (*cryIAc* and *cry2Ab*) that provide resistance to the major caterpillar pests of cotton.

The aims of the proposed release are to:

- incorporate the RR Flex trait into elite cotton varieties suitable for use under Australian conditions;
- test agronomic performance including disease resistance (bacterial blight, fusarium and verticillium wilt);
- produce seed for future release;

- set up demonstration sites for industry, government, researchers and the wider community; and
- collect data required for future applications to the OGTR and other regulators for commercial release such as levels of novel protein expression and seed composition (required by the OGTR and Food Standards Australia New Zealand (FSANZ)) and data on the GM cottons' tolerance to glyphosate, weed control and glyphosate residue levels (required by the Australian Pesticides and Veterinary Medicines Authority (APVMA)).

The APVMA is responsible for the use and safety of herbicides in Australia. Glyphosate is currently not registered for use on cotton beyond the four leaf stage of growth. A research permit from the APVMA for use of glyphosate after this stage on RR Flex (and RR Flex/Bollgard II) cotton will be required for the proposed field trials. If the trials were successful and a new pattern of glyphosate use were proposed in relation to commercial release of RR Flex cotton, this would require registration by the APVMA. Further information about the APVMA can be obtained from www.apvma.gov.au.

None of the cotton plants from the release, or their by-products, would be used for animal and human food. An approval from FSANZ would be required before oil from RR Flex cotton could be used for human consumption. However, the applicant proposes to sell lint from the release for use as fibre in the textile industry. Lint does not contain genetic material or protein.

Transport and storage of the GM material would be conducted in accordance with the guidelines issued by the Regulator. Following harvest, plant material remaining at the site will be removed and/or destroyed. Any cottonseed not kept for research purposes or possible future releases (which would require separate applications) would also be destroyed.

Details of the gene construct, including the plasmid map and some of the regulatory sequences have previously been declared as Confidential Commercial Information (CCI) under section 185 of the Act, in connection with licence application DIR 35/2003. However, the CCI will be made available to the various prescribed expert groups that will be consulted on the preparation of the risk assessment and risk management plan for this application.

Previous releases of the GMOs

RR Flex and RR Flex/Bollgard II[®] cotton were previously approved for limited and controlled releases under licence DIR 35/2003. These field trials (total area of 946 hectares) are being conducted from 2003 to 2005 in NSW, Qld, NT and northern WA. The DIR 35/2003 licence conditions contain a requirement to conduct a research program that would assist the evaluation of future applications. Data gathered from the 2003/04 summer trials are provided in the current application and data from the 2004 winter trials will be available prior to the finalisation of the RARMP for this application.

Roundup Ready[®] cotton, containing one copy of the *cp4 epsps* herbicide tolerance gene, was approved for general (commercial) release in 2000 (GR-9), by the Minister for Health and Aged Care, on the basis of advice from GMAC (Genetic Manipulation Advisory Committee). After commencement of the Act, on 21 June 2001, Roundup Ready[®] cotton was re-assessed in accordance with the requirements of the regulatory system and the Regulator issued a licence for commercial release in June 2003 (DIR 023/2003). Roundup Ready[®] cotton is tolerant to the application of the herbicide Roundup[®] only up to the four leaf stage of growth.

Bollgard II[®] and Bollgard II[®]/Roundup Ready[®] cotton were approved for commercial release by the Regulator in 2002 (licence DIR 012/2002).

All of these commercial releases were restricted to the cotton growing regions of NSW and Qld south of latitude 22° South because of concerns about the potential weediness of the GM cottons in the northern tropical areas. The licence also authorised some field trials under limited and controlled conditions north of latitude 22° South to gather data to further evaluate this issue.

Prior to obtaining approval for commercial release, numerous limited and controlled releases of Roundup Ready[®], Bollgard II[®] and Roundup Ready[®]/Bollgard II[®] cotton were conducted under the voluntary system overseen by GMAC, and four licences for limited and controlled releases of Bollgard II[®] and Roundup Ready[®]/Bollgard II[®] cotton were approved by the Regulator, as listed below:

Roundup Ready[®] cotton - 23 limited and controlled releases undertaken by:

- CSIRO Division of Plant Industry (PR-55, PR-55X, PR-55X2, PR-55X3 and PR-55X5 and PR-55X6);
- Deltapine Australia Pty Ltd (PR-32, PR-52, PR-52X, PR-52X2, PR-52X3, PR-71, PR-71X, PR-71X2, PR-83, PR-83X, PR-83X3, PR-140, PR-140X and PR-143);
- Monsanto (PR-83X2 and PR-83X4); and
- Cotton Seed Distributors Pty Ltd (PR 55-X4).

Bollgard II[®] and Roundup Ready[®]/Bollgard II[®] cotton - 20 limited and controlled releases undertaken by:

- CSIRO Division of Plant Industry (PR-123, PR-123X, PR-123X2, PR-131, PR-131X, PR-131X2, PR-131X3);
- Deltapine Australia Pty Ltd (PR-51X4, PR-112, PR-112X, PR-112X2, PR-118, PR-118X, PR-118X2);
- Cotton Seed Distributors (Bollgard II[®] and Bollgard II[®]/Roundup Ready[®] cotton in Qld; DIR 005/2001);
- CSIRO (INGARD[®], Bollgard II[®] and Bollgard II[®]/Roundup Ready[®] cotton in WA and NT; DIR 006/2001);
- Department of Agriculture (WA) (Bollgard II[®] cotton in WA; DIR 009/2002); and
- Monsanto (Bollgard II[®] and Roundup Ready[®]/Bollgard II[®] cotton in northern WA, NT and northern Qld; DIR 012/2002).

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

Parent organism

The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia and is grown as an agricultural crop in NSW and southern Qld and on a trial basis in northern Qld, WA and the NT.

Genetic modification and its effect

RR Flex cotton contains two copies of a herbicide tolerance gene, *cp4 epsps*, derived from a common soil bacterium, *Agrobacterium* sp. strain CP4. The native plant *cp4 epsps* gene encodes an enzyme that is critical for the synthesis of aromatic amino acids (essential building blocks for proteins). Glyphosate, the active ingredient in Roundup[®] herbicide inhibits this enzyme and as a result kills conventional cotton. The bacterial gene encodes an enzyme that is not as sensitive to glyphosate as the plant's own equivalent enzyme.

Roundup Ready[®] cotton contains only one copy of the *cp4 epsps* gene and has little tolerance to glyphosate in reproductive tissues. This means that glyphosate can currently only be applied up to the four leaf stage of growth (ie prior to flowering) to control weeds, as after this stage, application of the herbicide can lead to yield loss. The applicant has indicated that, as RR Flex cotton has prolonged expression of the *cp4 epsps* gene and is tolerant to glyphosate at later stages of growth, 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.

RR Flex/Bollgard II[®] cotton was obtained by conventional crossing of the two GM varieties. The plants contain, in addition to the herbicide tolerance genes, two insecticidal genes *cryIAc* and *cry2Ab*, derived from a common soil bacterium, *Bacillus thuringiensis* (Bt). The insecticidal genes encode proteins that are toxic to lepidopteran caterpillars, including the two key *Helicoverpa* pests of cotton.

No antibiotic resistance marker genes are present in RR Flex cotton. However, RR Flex/Bollgard II[®] plants contain bacterial genes, as a result of the crossing with Bollgard II[®], conferring resistance to the antibiotics kanamycin and neomycin (*nptII* gene), and streptomycin and spectinomycin (*aad* gene). The *aad* gene is not expressed in the plants because the bacterial regulatory sequence that controls its expression is not active in plants. This gene was used in the laboratory prior to the production of the genetically modified plants to select for bacteria containing the modified DNA.

A reporter gene (*uidA*) from the bacterium *Escherichia coli* is also present in the RR Flex/Bollgard II[®] cotton plants. This gene encodes the enzyme β -glucuronidase (GUS) that enables visual identification of plant tissues in which it is being expressed.

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

Method of genetic modification

The two copies of the *cp4 epsps* gene and associated regulatory sequences were introduced into Roundup Ready[®] MON 88913 cotton on a plasmid vector carried by *Agrobacterium tumefaciens*. The vector is 'disarmed' since it lacks the genes that encode the tumour-inducing functions of *A. tumefaciens*.

Bollgard II[®] cotton was derived by particle bombardment of the *cry2Ab* and *uidA* genes into the GM INGARD[®] cotton containing the *cryIAc*, *nptII* and *aad* genes. This technique involves coating the DNA containing the *cry2Ab* and *uidA* genes onto very small particles which were 'shot' into the cotton tissue, followed by selection of plants that contained single, functional copies of the genes.

RR Flex/Bollgard II[®] cotton was generated through conventional breeding of GM RR Flex and GM Bollgard II[®] cotton.

Consultation on preparation of the Risk Assessment and Risk Management Plan

The Regulator has made an initial assessment as to whether the proposed release may pose significant risks to human health and safety or the environment, in accordance with section 49 of the Act. Due to the low risk potential of the GMOs, the control measures that will be imposed, and the limited scope of the dealings, **the Regulator has decided that the proposed release does not pose a significant risk to human health and safety or the environment.**

This means that the Regulator is **not required to seek public comment** on the assessment of this proposal until after a risk assessment and risk management plan (RARMP) has been prepared. In the interim, copies of the application are available on request from the OGTR. Please quote application number DIR 055/2004.

In preparing the RARMP, the Regulator will seek input from a wide range of key stakeholders and expert groups comprising State and Territory Governments, relevant Australian Government agencies, the Minister for the Environment and Heritage, the Gene Technology Technical Advisory Committee and appropriate local councils, as required by section 50 of the Act. In accordance with section 52 of the Act, the Regulator will again consult with these prescribed agencies and authorities and the public in finalising the RARMP.

At this stage, the consultation version of the RARMP is expected to be issued for a six week consultation period in **mid January 2005**. The public will be invited to provide submissions on the RARMP via advertisements in the media and direct mail to anyone registered on the OGTR mailing list. Summaries and copies of the RARMP will be available from the OGTR, or on the OGTR website.

If you have any questions about the application or the assessment process, please contact the OGTR at:

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