



30 JULY 2004

EXECUTIVE SUMMARY
of
THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN
for
APPLICATION No. DIR 048/2003

(Field trial to assess transgenic insecticidal cotton expressing natural plant genes)

INTRODUCTION

The *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001* (the Regulations) set out requirements which the Gene Technology Regulator (the Regulator) must follow when considering an application for a licence to intentionally release a genetically modified organism (GMO) into the environment.

For a licence to be issued, the Regulator must be satisfied that the release will not pose any risks to human health and safety or the environment that can not be managed. As part of the evaluation process, section 51 of the Act requires the Regulator to prepare a risk assessment and risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and stakeholders.

Under section 52 of the Act, the Regulator is required to seek comment on the RARMP from those consulted in its preparation and to invite submissions from the public. Matters raised relating to the protection of human health and safety or the environment are taken into account in finalising the RARMP, which then forms the basis of the Regulator's decision on whether, or not, to issue a licence.

The Act is designed to operate in a cooperative legislative framework with other regulatory authorities that have complementary responsibilities and specialist expertise. As well as enhancing coordinated decision making, this arrangement avoids duplication. The OGTR liaises closely with other regulators to ensure the identification, evaluation and management of risks that may be associated with development and use of gene technology.

The Regulator has made a decision to issue a licence in respect of application DIR048/2003 from Hexima Limited (Hexima).

THE APPLICATION

Hexima has applied for a licence for the intentional release, under limited and controlled conditions, of four types of genetically modified (GM) cotton expressing serine protease inhibitors (PIs). Hexima proposes to conduct small, "proof of concept" field trials over three summer growing seasons (October 2004 to May 2007) on two sites covering a total area of 0.5 hectares per year in the shires of Wambo, Pittsworth or Jondaryan in south eastern Queensland.

The genetic modifications in the GM Hexima cottons aim to target Lepidopteran (caterpillar) pests of cotton. However, mechanism of action of serine PIs is significantly different to the insecticidal proteins such as the *Bacillus thuringiensis* (Bt) crystal toxin (Cry) and vegetative insecticidal protein (VIP), present in other GM cottons released previously.

Briefly, both Cry and VIP are activated in the Lepidopteran gut and bind to various proteins that act as receptors on the gut lining, subsequently forming lytic pores which interfere with fluid balance. This results in gut paralysis and eventual insect death. Detailed discussion of the mechanism of Cry and VIP can be found in the RARMPs for other GM insecticidal cottons including DIR 044/2003 and DIR 034/2003 respectively.

In contrast, serine PIs bind to the digestive enzymes trypsin and chymotrypsin, inhibiting their activity and thereby preventing ingested protein from being broken down. This slowed digestion of food leads to retardation of insect growth, lethargy, delayed development and possibly death, if ingested early in the insect's lifecycle. In addition, altered insect behaviour may increase the likelihood of being eaten by predators and affect breeding success.

In accordance with the provisions of section 185 of the Act, Hexima sought and received approval for details of the gene constructs, gene sequence information, identification of the transgenic lines and preliminary expression and bioassay data, to be considered as confidential commercial information (CCI). While the Regulator was satisfied that the public interest in the release as proposed did not outweigh the prejudice that disclosure would cause the applicant, the CCI was made available to the various prescribed expert groups that were consulted on the preparation of the RARMP for this application.

Two of the four types of GM cotton that Hexima proposes to release contain genes that code for serine PIs derived from ornamental tobacco (*Nicotiana glauca*). The third type of GM cotton proposed for release contains a serine PI gene derived from potato (*Solanum tuberosum*). The fourth type of GM cotton contains serine PI genes derived from both tobacco and potato (details declared CCI).

All four types of GM cotton also contain the antibiotic resistance gene *nptII* from *Escherichia coli* (a bacterium) as a selectable marker, which confers resistance to neomycin antibiotics such as kanamycin. The marker gene was used in the laboratory during the development of the GMOs for identification and selection of plant tissues in which the serine PI genes were also present.

Short regulatory sequences are also present in the GM cottons that control the expression of the introduced genes. These are derived from the common plant pathogens Cauliflower mosaic virus and *Agrobacterium tumefaciens*. However, they comprise only a small part of the total plant genome and are not by themselves capable of causing disease.

The aims of the proposed release are to test the efficacy of the four types of GM Hexima cottons against lepidopteran pests of cotton and to evaluate their respective agronomic performance. Some material from the GM cottons will be retained for research purposes and some seed for subsequent seasons of the trial.

None of the cotton plants from the release, or their by-products, would be used for animal feed or human food, and seed not required for future plantings would be destroyed. Following hand harvesting of selected seed, the applicant proposes that plant materials remaining at the site will be destroyed on site and incorporated into the soil by shallow cultivation. Seed from the conventional cotton planted as a pollen trap will be destroyed. Any regrowth on the site will be controlled by herbicide application and / or cultivation. The GMO and material from the GMO will be transported in accordance with the OGTR guidelines.

There have been no previous releases of any of the GM Hexima cottons in Australia or elsewhere. The GM cottons proposed for release were developed from a Notifiable Low Risk Dealing conducted under contained conditions at the University of Melbourne. Some releases of other genes encoding serine PIs introduced into GM plants have been trialed overseas in Europe, UK, USA and China. There have been no reports of adverse effects on human health or the environment resulting from these releases.

THE EVALUATION PROCESS

A RARMP has been prepared in relation to licence application DIR 048/2003 from Hexima in accordance with the Act, the Regulations, and the Risk Analysis Framework. This framework was developed as part of the establishment of the regulatory arrangements in consultation with the public, State, Territory and Australian Government agencies, key stakeholders and the Gene Technology Technical Advisory Committee, and is available at www.ogtr.gov.au/pdf/public/raffinal.pdf.

Details of the process that the Regulator must follow, including the prescribed consultation process on the application, and the matters that she must consider in preparing a RARMP, are set out in Appendix 8 of the RARMP. The complete RARMP can be obtained from the OGTR by contacting the Office on 1800 181 030 or from its web site: www.ogtr.gov.au.

The risk assessment considered information contained in the application (including information required by the Act and the Regulations on the GMO, the parent organism, the proposed dealings and potential impacts on human health and safety and the environment), current scientific knowledge, and submissions received during consultation with expert groups and authorities.

Through this process, potential hazards to human health and safety or the environment that may be posed by the proposed release of the four types of GM Hexima cotton were identified. These have been evaluated to determine whether hazards might arise, based on the likelihood of each hazard occurring and the likely impact of each hazard, were it to be realised.

The identified potential hazards relate to:

- **toxicity and allergenicity to humans:** could these GM cottons be more toxic or allergenic than non-GM cotton, as a result of the introduced gene products or because of unintended effects?
- **toxicity to non-target organisms:** could these GM cottons be harmful to non-target organisms as a result of the introduced gene products or because of unintended effects?
- **weediness:** could the genetic modifications be harmful to the environment by increasing the potential for these GM cottons to establish as problem weeds?
- **transfer of introduced genes to other organisms:** could there be adverse consequences from potential transfer of the introduced genes to non-GM cotton crops, feral or native cottons, or to other organisms? and
- **insecticide resistance:** could target insects develop resistance to the serine PI proteins produced by the introduced serine PI genes in these GM cottons?

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has a complementary regulatory role in respect of this application due to its responsibility for agricultural chemical use in Australia, including insecticides and herbicides, under the *Agricultural and Veterinary Chemicals Code Act 1994*. Further information about the APVMA's assessment and approval process is contained in Chapter 1 and Appendix 6 of the RARMP.

For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits allowing restricted use of an insecticide or herbicide, for example, for a limited period of time or for a limited area. The APVMA can impose conditions if necessary on the use of insecticides and herbicides in registrations and permits.

Hexima has submitted an application to the APVMA for a research permit for the use of the insecticidal serine PI genes in the GM cotton lines for the proposed trial. The APVMA and the OGTR have worked closely to ensure the thorough, coordinated assessment of these parallel proposals.

CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator has concluded that the proposed release of four types of GM cotton will not pose significant risks to human health and safety and the environment as a result of the genetic modification. The Regulator has imposed licence conditions to minimise potential exposure of human and other organisms and to limit the spread and persistence of the GMOs or the introduced genetic materials in the environment. The assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans

The GM Hexima cottons are unlikely to prove more toxic or allergenic to humans via occupational exposure than conventional cotton. None of the introduced genes produce proteins with any similarity to toxins or food allergens.

The applicant does not intend to use any cottonseed produced in the proposed release in human food, or to sell lint or linters for processing, thus limiting potential exposure. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment, and FSANZ approval would be needed before products from these GM cottons could be used in human food.

Toxicity to non-target organisms

The GM Hexima cottons contain serine protease inhibitors that have the potential to affect non-target invertebrates other than Lepidopteran caterpillars. However, as the expression levels in the plant are likely to be low, significant adverse impacts on non-target invertebrates are unlikely. The risk of toxicity to mammals, birds and fish is low as animals avoid feeding on cotton due to the gossypol content and none of the products from this trial will be used for stockfeed. The risks are further reduced by the small size and contained nature of the field trial. Data on the levels of expression of the serine PI proteins in the GM Hexima cottons is required during the first year of the field trial and on toxicity to non-target organisms in the second and third year of the field trial.

Weediness

The risk of the GM Hexima cottons establishing as problematic weeds in the proposed release areas of south east Queensland is low and not likely to be greater than that of non-GM cotton. This is because the germination and persistence of both GM and non-GM cottons in southern Australia are limited by the availability of adequate soil moisture, nutrients, plant competition, fire and/or frosts. It is highly unlikely that the genetic modification has altered the response of cotton to any of these variables. Licence conditions have been imposed to minimise the spread and persistence of these GM cottons in the environment.

Transfer of introduced genes to other organisms

Some gene transfer from the GM Hexima cottons to other cultivated cottons would be likely under uncontrolled conditions, although the overall frequency of out-crossing would be very low, as cotton is primarily self-pollinating. Transfer of introduced genes to other cultivated cotton would pose the same risks as the risks posed by the GM cottons themselves. Licence conditions have been imposed to minimise the risk of transfer of introduced genes to plants outside the release area.

The risk of transferring the introduced genes to native cotton is negligible, because of geographic distance and genetic incompatibility. Herbarium records suggest that naturalised/feral cotton populations may occur, or might have occurred in the past, in central and south eastern Queensland, northern Northern Territory and northern Western Australia. As part of the licence conditions for DIR 022/2002, a survey of naturalised cotton populations in Queensland, in locations suggested by herbarium records, is being conducted. The applicant has stated that no populations of feral cotton are known in the vicinity of this field trial.

The likelihood of transfer of the introduced genes to other organisms is negligible because of well established genetic incompatibility.

Insecticide resistance

Development of resistance in insects to the serine PIs in these four types of GM Hexima cotton is unlikely to occur during the course of this field trial. The serine PIs act by inhibiting the digestive enzymes of target insects and retarding their growth. Therefore, as target insects are not killed outright the selection pressure is low and unlikely to result in a build up of resistance at the population level. Given the limited scope of the release in scale and time, the likelihood of this risk arising resulting from the release is negligible.

As these GM cottons fall under the *Agricultural and Veterinary Chemicals Code Act 1994* definition of an agricultural chemical product due to their production of insecticidal substances, they are subject to regulation by the APVMA. The hazard of development of insecticide resistance in pests will also be assessed by the APVMA in considering Hexima's permit application for the use of the serine PI genes as insecticides in the GM cottons.

THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)

As part of the evaluation process for this licence application, a risk management plan has been developed to address the risks identified (refer to conclusions of the risk assessment, above). This plan has been given effect by the licence conditions imposed. The key licence conditions are outlined below.

Toxicity or allergenicity to humans

Licence conditions have been imposed which require the applicant to:

- prevent entry of the GMOs and products derived from the GMOs into the human food supply;
- limit the scale of the release;
- destroy all GM materials not required for future trials;
- securely transport and store the GMOs; and
- report adverse impacts.

Toxicity to non-target organisms

Licence conditions have been imposed which require the applicant to:

- prevent GM cottonseed being used as stockfeed;
- limit the scale of the release;
- destroy all GM materials not required for future trials;
- securely transport and store the GMOs;
- ensure trial sites are at least 3 km away from national parks, state forests and nature reserves; and
- require research on protein expression levels in different parts of the plant and toxicity to representative non-target invertebrates.

Weediness

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release;
- prevent cottonseed being used as stockfeed;
- surround the GM cottons by a 20 m pollen trap of non-GM cotton;
- securely transport and store the GM cottons;
- ensure trial sites are at least 50 m from natural waterways;
- clean the release sites and equipment used at release sites; and
- monitor release sites after harvest and destroy volunteers.

Transfer of introduced genes to other organisms

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release;
- surround the GM cottons by a 20 m pollen trap of non-GM cotton;
- securely transport and store the GM cottons;
- clean the release sites and equipment used at release sites; and
- monitor release sites after harvest and destroy volunteers.

Insecticide resistance

No conditions have been imposed in relation to insecticide resistance management, as these risks are negligible from this field trial. The APVMA has responsibility for regulating these issues. The applicant's obligation to comply with any conditions imposed by the APVMA is noted in the licence.

General conditions

The licence issued by the Regulator also contains a number of general conditions, which are also relevant to risk management. These include, for example:

- identification of the persons or classes of person covered by the licence;

- requirement that the applicant allow access to the release sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing; and
- a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to human health and safety or to the environment.

Chapter 2 of the risk assessment and risk management plan provides a tabulated summary of assessment conclusions and corresponding management conditions. Full details of the licence conditions are provided in Appendix 7.

Research Requirements

Hexima is required to collect data on the levels of expression of the serine PIs and the plant parts in which they are expressed under Australian field conditions (including pollen and nectar) in the first season of the trial. In the second and third growing seasons of the current trial more data is required to be collected on the toxicity of the GM Hexima cottons to representative non-target organisms.

Identification of issues to be addressed for future releases

Additional information would be required to assess future applications before larger scale releases of these GM cottons or reduced containment conditions could be contemplated. Data would need to be provided on:

- details of the molecular characterisation of the insertion site of the introduced gene constructs in the GM Hexima cotton;
- the stability of the insertion in the GM Hexima cotton;
- further studies on non-target toxicity;
- the stability and persistence of introduced proteins in the soil and water and the effects on soil microorganisms;
- agronomic characteristics relating to fitness of the GM Hexima cotton; and
- potential weediness of the GM cottons, including the effect of reduced invertebrate herbivory on the survival characteristics of these GM cottons.

Monitoring and enforcement of compliance by the OGTR

As well as the legislative capacity to enforce compliance with licence conditions, the Regulator has additional options for risk management. The Regulator can direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment. The OGTR also independently monitors releases that the Regulator has authorised. At least 20% of all field trial sites will be inspected each year, in accordance with a monitoring and compliance strategy based on risk profiling (which takes into account biological, seasonal, geographical and ecological risk factors) to determine whether licence holders are complying with the licence conditions, or whether there are any unintended effects.