



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

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EXECUTIVE SUMMARY
of
THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN
for
APPLICATION NO. DIR 052/2004
from
CSIRO

INTRODUCTION

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving intentional release of GMOs into the environment, in respect of application DIR 052/2004 from the Commonwealth Scientific and Industrial Research Organisation (CSIRO).

The *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001* (the Regulations) set out requirements which 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, and if so, what conditions to impose.

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 the development and use of gene technology.

THE APPLICATION

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) applied for a licence (application number DIR 052/2004) for the intentional release, under limited and controlled conditions, of approximately 1 500 GM rice lines¹. CSIRO proposes to conduct a small scale field trial over three summer growing seasons (between 2005 and 2008) on one site covering a total area

¹ The term 'line' has been used throughout this RARMP to denote a group of rice plants containing a particular set of genetic modifications that are descendants of a single, original rice plant that was transformed (i.e. has new genetic material) using gene technology.

of 0.04 hectares in each season in New South Wales (NSW). A second site of 0.1 hectares would be used for approximately five weeks in the second growing season for the purposes of assessing gene flow (see below).

This trial is a basic research project that forms part of collaborative international research efforts to identify rice genes and their regulatory elements and to gather information on the functions of all the genes in the rice genome. The International Rice Genome Sequencing Project has identified many of the genes in the rice genome. However, the functions of the genes can often only be determined through experimental methods such as gene ‘knockouts’². The applicant proposes to release the GM rice lines for two main purposes:

- to identify rice genes that influence traits of biological or agronomic interest, by observing alterations in the visible characteristics (phenotypes³) of modified rice lines that were generated by earlier work in certified contained facilities (laboratory and glasshouse); and
- to determine the extent of gene flow between rice plants under Australian field conditions.

The rice cultivar to be used in this trial, Nipponbare, originated and is grown commercially in Japan but is not cultivated in Australia.

The GM rice lines proposed for release contain randomly located insertions of genetic materials (T-DNA or *Ds* elements, see details below), each carrying a combination of selectable marker genes (i.e. genes encoding herbicide tolerance or antibiotic resistance) and visual marker genes (i.e. reporter genes enabling detection of gene expression via staining or fluorescence). These random insertions will disrupt the expression of native⁴ rice genes, resulting in loss of a functional gene product, i.e. gene ‘knockout’. Individual gene knockout lines may exhibit altered physical characteristics, which would give clues to the normal function of the disrupted genes in each line.

In addition, some of the inserted gene constructs are designed to express a visual marker protein only if they are inserted near a native promoter⁵ or within a rice gene. The location of expression of the marker protein within these GM rice plants will reflect the normal expression pattern of the tagged rice gene. This is expected to provide valuable information on the usual control of expression of rice genes. As the rice genes into which these constructs have been inserted are ‘tagged’ by both these elements and their associated marker genes, this facilitates the later identification and isolation of the altered genes in the laboratory for further study.

This methodology is effectively the biological equivalent of ‘reverse engineering’ where a piece of equipment is carefully deconstructed in order to understand how it works and to enable the mechanism to be copied.

It is well known that plants grow differently under glasshouse conditions than in the open. The field trial will allow a large number of GM rice lines to be studied simultaneously under Australian field conditions and enable their phenotypes to be assessed and compared. Up to 500 lines will be grown each season. Thirty seeds from each line will be planted. These GM rice lines will be studied for phenotypic changes such as altered growth habit, flowering time, or any other characteristics of agronomic or biological interest. Following hand harvesting, seed from potentially interesting lines

² The term ‘knockout’ refers to techniques that disrupt the sequence of a gene, resulting in loss of function i.e. the protein normally encoded by the gene is no longer expressed.

³ The term ‘phenotype’ refers to the physical characteristics of a plant e.g. size, growth rate, number of flowers etc.

⁴ In the context of genes and other genetic material, the term ‘native’ refers to genes and regulatory sequences that are naturally present in the indicated organism.

⁵ The term ‘promoter’ refers to a genetic sequence that controls the expression of the gene that is linked to it.

will be selected for further study in the laboratory to identify the genes responsible for these traits. All unharvested GM rice material will be destroyed at the end of each season.

The applicant has used a total of eight different gene constructs to generate the 1 500 GM rice lines. The gene constructs contain various combinations of marker genes (*bar*, *nptII*, *hph*, *uidA*, *gfp* and/or *eyfp*). Two types of genetic elements were used to insert the marker genes into the GM rice lines:

- transfer DNA (T-DNA) border sequences from *Agrobacterium tumefaciens* (a common soil bacterium), required for the generation of GM plants by *Agrobacterium*-mediated transformation; and
- *Ds* transposable element border sequences from maize, which, once present in the rice genome, can be induced to move to new genomic locations along with the associated introduced marker genes. In the GM plants proposed for release, the *Ds* transposable element is immobile due to the absence of the enzyme that facilitates its movement.

The net effects of these rice gene knockouts are analogous to those achieved in conventional breeding programs utilising chemical mutation or radiation to disrupt gene function. However with this technique the location and number of genetic changes can be precisely determined, whereas with the latter techniques the extent of the genetic changes is unknown.

As part of the trial, in the first growing season, a small plot will be planted with approximately 500 GM rice plants from a single line, containing two reporter genes, an antibiotic resistance gene and a gene that confers tolerance to glufosinate ammonium (the active ingredient in herbicides such as Basta[®] and Liberty[®]). These will be surrounded by approximately 5 000 non-GM rice plants. Seeds will be hand harvested from the non-GM plants for assessment of gene flow from the GM rice and both the GM and non-GM plants will be destroyed. The seeds will be planted in a second site, at the same location, of 0.1 hectares at the start of the second growing season. At an early stage of seedling growth, well before the start of flowering, glufosinate ammonium will be applied in order to test for the presence of the herbicide tolerance gene. Any herbicide tolerant seedlings will be removed to the laboratory for further analysis.

The GM rice lines are for research only and are not intended to be developed for commercial purposes. Seeds will be collected from selected lines for potential future research in Australia (subject to additional approval) and will be shared with overseas laboratories that are participating in this international research project.

The applicant proposed the following containment measures for the release of the GM rice plants:

- surrounding the trial site with a fenced area with a lockable gate;
- covering the site with a chicken wire cage;
- surrounding the trial site with an isolation zone of 150 m in which no other rice plants are to be grown;
- none of the rice plants from the release, nor their by-products, to be used for human food or animal feed;
- seed that is not required for research or possible future trials (subject to approval) to be destroyed;
- following hand harvesting of selected GM material for research purposes, plant material remaining at the site to be incorporated into the soil through cultivation;
- after harvest in one season, and prior to planting in the following season, the rice bays to be irrigated on at least two occasions to encourage germination of any seeds in the soil. Any volunteer seedlings to be destroyed before any further planting;

- following completion of the trial, continue to monitor the area for volunteer rice seedlings for a period of at least 12 months, and destroy any volunteer seedlings before flowering; and
- the GMOs and material from the GMOs to be transported and stored in accordance with the OGTR guidelines.

The selected trial site is also at least 1 km away from natural waterways. The rice growing bays used for the trial will have water-impermeable linings below the soil surface and no drainage system, minimising the potential for drainage into the water table. The banks surrounding each bay will be 40 cm high, while water in the bays would be maintained at a maximum height of 25 cm during the growing season. This will limit the risk of water from the GM rice growing bays overflowing during any rainfall. The trial site is not in a high flood risk area. The selected trial site is also at least 2 km away from any rice breeding programs and the nearest commercial rice growing areas of NSW are approximately 85 km away (information supplied by the applicant).

There have been no previous releases of any GM rice lines in Australia. However, licences for the intentional release of other GMOs containing various combinations of the introduced genes (*bar*, *hph*, *nptII*, *uidA* and *gfp*) have been issued under the current regulatory system (see Chapter 1, Table 1 of the RARMP). There have been no reports of adverse effects on human health or the environment resulting from these releases. The GM rice lines were originally generated by CSIRO in the laboratory as a Notifiable Low Risk Dealing (NLRD 640/2003) under the Act.

THE EVALUATION PROCESS

A risk assessment and risk management plan (RARMP) has been prepared in relation to licence application DIR 052/2004 from CSIRO 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. The framework is currently under review to more fully explain the OGTR's risk analysis process. A copy of the current version 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 6 of the RARMP. The complete RARMP, along with a review document 'The Biology and Ecology of Rice (*Oryza sativa* L.) in Australia' produced to inform the risk analysis, can be obtained from the OGTR by contacting the Office on 1800 181 030 or from the OGTR's website at www.ogtr.gov.au.

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

Through this process, potential hazards to human health and safety or the environment that may be posed by the proposed release of the GM rice lines were identified. These have been carefully evaluated to determine 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 and other organisms:** could these GM rice plants be more toxic or allergenic than non-GM rice plants to humans or harmful to other organisms as a result of the novel gene products or because of unintended effects resulting from the introduced genetic elements or gene knockouts?
- **weediness:** could the genetic modifications be harmful to the environment by increasing the potential for these rice plants to establish as problematic weeds? and
- **transfer of introduced genes to other organisms:** could there be adverse consequences from potential transfer of the introduced genes/genetic elements to non-GM rice plants, wild or weedy rice plants, or to other organisms?

CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator has concluded that the proposed limited and controlled release, of up to 1 500 GM rice lines in 0.04 hectares over 3 seasons, and the short-term planting of seed from the gene flow study in a site of 0.1 hectares for less than 2 months, does not pose significant risks to human health and safety and the environment as a result of the genetic modification. As noted above, the applicant proposed a number of containment measures to minimise the spread and persistence of the GMOs and introduced genetic materials. In addition to these, the Regulator has imposed other licence conditions to minimise further the potential exposure of humans and other organisms and to limit the likelihood of spread and persistence of the GMOs or the introduced genetic materials in the environment. The risk assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans and other organisms

Although it is unknown if the gene knockouts in individual GM rice lines will potentially lead to increased toxicity or allergenicity to humans, exposure to each line will be limited because of the small scale of the release and the low number of plants of each line to be released. Furthermore, gene knockouts are not unique to the GM rice lines proposed for release but can also occur as a result of conventional breeding (involving mutagenesis by radiation or chemical treatment) or natural mutation events.

Allergies to non-GM rice grains and pollen are rare in the general population and rice grains are not toxic. Some toxicity may occur if livestock consume large quantities of non-GM rice straw. The level of occupational exposure to the introduced proteins through working with the GM rice is likely to be low, although the expression levels and locations of the proteins have not been determined. Most of these proteins (or similar proteins) are already present in the environment and/or the human intestinal tract, as common soil or enteric bacteria naturally produce them. Two of the introduced visual marker proteins (Green Fluorescent Protein and Enhanced Yellow Fluorescent Protein) are derived from a jellyfish protein and are not naturally present in the terrestrial environment. However, none of the introduced proteins are considered to be toxins or allergens and there have been no reports of toxic or allergenic effects from previous releases of other GMOs containing the same or similar introduced genes. The proposed release is limited in scale and licence conditions have been imposed to limit unintended exposure to the GMOs (refer to key licence conditions, below).

Exposure of other organisms, including aquatic organisms, will be limited by conditions proposed by the applicant, namely to surround the sites with fencing, cover the sites with chicken wire cages and locate the sites at least 1 km away from natural waterways.

The applicant does not intend to use any rice material produced in the release in human food or stockfeed, 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 GM rice could be used in human food.

Weediness

Climatic conditions limit the unirrigated growth of rice plants to the tropical areas of northern Australia. It is unlikely that the genetic modifications would increase the ability of rice to grow in unirrigated pastures or non-agricultural environments and, thereby, increase the weediness of the GM rice plants in southern Australia. However this is the first proposed field trial of these GM rice plants and there may be unintended or secondary effects resulting from the introduced genetic materials or gene knockouts that could alter their potential for weediness under field conditions. The applicant intends to closely monitor the growth characteristics of the GM rice plants as part of the trial in order to identify any phenotypic changes due to disruption of rice genes that might affect weediness.

The risk of the GM rice lines establishing as weeds in the area of the release is considered to be very low due to the very small scale of the release and because the region is not suited to the growth of rice plants without irrigation. The applicant has proposed further measures to minimise the spread and persistence of the GMOs in the environment, including the use of an isolation zone, distance from natural waterways and post-harvest monitoring of the sites. These and additional containment measures have been imposed to minimise the spread and persistence of these GM rice lines in the environment (refer to key licence conditions, below).

Transfer of introduced genes to other organisms

Gene transfer from the GM rice lines to other rice plants (including cultivated rice, wild rice species or weedy rice varieties) is unlikely to occur, due to isolation from other rice plants. Rice is primarily self-pollinating with rates of out-crossing to other cultivated rice plants generally reported to be less than 1%. Out-crossing to other non-cultivated rice species is limited further by various forms of genetic incompatibility.

The applicant intends to perform research on the occurrence of gene flow from GM to non-GM rice under Australian field conditions as part of the trial. Licence conditions have been imposed, including the use of an isolation zone as proposed by the applicant, to minimise the risk of transfer of the introduced genes to plants outside the release site (refer to key licence conditions, below).

The risk of transfer of the introduced genes to other organisms is negligible because of well-established genetic incompatibility. Even if such transfer occurred, the consequences for human health and safety and the environment are likely to be negligible.

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 Conclusion of the Risk Assessment, above). This plan is given effect by the licence conditions imposed. The key licence conditions are outlined below.

Toxicity or allergenicity to humans and other organisms

Licence conditions have been imposed which require the applicant to:

- prevent the GMOs and products derived from the GMOs entering the human food supply;
- prevent GM rice products being used as stockfeed;

- limit the scale and duration of the release;
- limit exposure to humans and other animals by covering the trial sites with a chicken wire cage and surrounding them with a lockable fence;
- destroy all GM rice material not required for possible future trials or research;
- securely transport and store the GM rice materials and seeds; and
- report any adverse effects to the Regulator.

Weediness

Licence conditions have been imposed which require the applicant to:

- limit the scale and duration of the release;
- surround the 0.04 hectare site with a 150 m isolation zone in which no other rice is planted;
- locate the release site at least 1 km from natural waterways;
- limit access to the sites by birds and animals by covering the trial sites with a chicken wire cage and surrounding them with a fence;
- minimise the risk of transport of GM rice material away from the sites via water movement by lining the 0.04 ha site with water impermeable material and surrounding both sites with earth banks 40 cm high and 100 cm wide;
- implement control measures for rodents at the site;
- securely transport and store the GM rice material and seeds;
- clean the release site after harvest and equipment used at the site; and
- monitor the isolation zone and the release sites after harvest and destroy volunteers.

Transfer of introduced genes to other organisms

Licence conditions have been imposed which require the applicant to:

- locate the trial sites away from commercial rice growing areas;
- limit the scale and duration of the release;
- surround the 0.04 hectare site with a 150 m isolation zone in which no other rice is planted;
- the seedlings planted on the 0.1 ha site must be harvested or destroyed within 2 months of being planted (i.e. before flowering);
- securely transport and store the GM rice material and seeds;
- clean the release site after harvest and equipment used at the site; and
- monitor the isolation zone and the release sites after harvest and destroy volunteers.

General conditions

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

- identification of the persons or classes of persons covered by the licence;
- a requirement that the applicant allows access to the release site 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 or safety or to the environment.

Chapter 2 of the RARMP provides a tabulated summary of assessment conclusions and corresponding management conditions. Full details of the proposed licence conditions are provided in Appendix 5.

Identification of issues to be addressed for future releases

The proposed release of the GM rice lines is a small, basic research trial, contributing to a collaborative international project that aims to identify and functionally characterise rice genes and gene regulatory sequences. There is no intention by the applicant to conduct further releases of any of these lines. Thus no research conditions are imposed in relation to the characterisation of these lines.

During the field trial, the applicant will conduct research on pollen mediated gene flow between closely situated GM and non-GM rice plants. Licence conditions require the applicant to consult with the OGTR in planning this gene flow research. Data from this trial, measuring rates of gene flow under Australian conditions, would be required for evaluation of any future applications for releases of GM rice into the environment.

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 unforeseen problems.

FURTHER INFORMATION

Detailed information on the evaluation of the application, including the licence conditions, is available in the RARMP document for this application. This can be obtained from the website of the Office of the Gene Technology Regulator (www.ogtr.gov.au), or by calling 1800 181 030 (please quote application number DIR 052/2004).