



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

21 April 2005

## **EXECUTIVE SUMMARY OF THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN FOR APPLICATION DIR 053/2004**

### **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 053/2004 from the Grain Biotech Australia Pty Ltd (GBA).

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 and 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 development and use of gene technology.

### **THE APPLICATION**

Grain Biotech Australia Pty Ltd (GBA) licence application number DIR 053/2004 requested approval for the intentional release, under limited and controlled conditions, of two genetically modified (GM) wheat lines<sup>1</sup>. The aim of the trial is to evaluate the salt tolerance and agronomic performance of the GM salt tolerant wheat on a site affected by different levels of salinity.

The release will take place during the winter growing season in Corrigin shire in Western Australia (WA) on a single site of 0.45 ha from April 2005 to January 2006. Part of the release site includes a salt scald, ie an area affected by high salt levels.

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<sup>1</sup> The term 'line' has been used throughout this RARMP to denote wheat containing a specific genetic modification derived from a single transformation event.

During the trial, the agronomic performance and salinity tolerance of the GM wheat will be compared with non-GM bread wheat, and non-GM salt adapted bread wheat.

The genetic modification consists of the introduction of two genes, the ornithine aminotransferase gene (*oat*) derived from the common plant species, *Arabidopsis thaliana* and the cyanamide hydratase gene (*cah*), from the soil fungus *Myrothecium verrucaria*. The *oat* gene encodes the enzyme<sup>2</sup> ornithine aminotransferase (OAT) which is part of a metabolic pathway that can lead to the production of the amino acid proline. Over-expression of OAT can increase the levels of the amino acid proline<sup>3</sup> in the plant. Proline is an unreactive compound that can serve as an osmoprotectant and enable plants to grow in the presence of elevated salt levels in soil.

The *cah* gene encodes the enzyme cyanamide hydratase (CAH) which confers tolerance to the herbicidal compound cyanamide. The *cah* gene was used as a selective marker in the selection of transformed plants in the laboratory.

GBA proposed a range of containment and inspection measures (detailed in Chapter 1), in part to limit the possible spread and persistence of the GM wheat lines, but also to maintain the integrity of the trial. The proposed measures were considered during the risk assessment of the application and in the preparation of the risk management plan.

None of the GM wheat plants from the release, or their by-products, would be used for animal feed or human food, and seed not required for possible future trials (subject to approval) or research would be destroyed. This GM wheat would require approval by Food Standards Australia New Zealand (FSANZ) before use for human food.

There have been no previous releases of GM wheat lines containing either the *cah* or *oat* genes in Australia. The GM wheat lines were originally developed in contained laboratories and glasshouses within the provisions of the Act, under NLRD 239/2002.

However, on 13 April 2005 the Regulator approved a limited and controlled field trial of a wheat genetically modified for altered grain starch (DIR054/2004).

In addition, under the former voluntary system that was overseen by the Genetic Manipulation Advisory Committee (GMAC) five field releases of other types of GM wheat were approved (see Chapter 1 of the risk assessment and risk management plan). There have been no reports of adverse effects on human health or the environment resulting from these releases.

## **THE EVALUATION PROCESS**

A risk assessment and risk management plan (RARMP) has been prepared in relation to licence application DIR 053/2004 from GBA 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<sup>4</sup>.

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, a set of Questions and Answers on the decision on this application and a review

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<sup>2</sup> An enzyme is a protein that catalyses a specific biochemical reaction.

<sup>3</sup> Proline is one of the 20 amino acids that are the building blocks of all proteins.

<sup>4</sup> The *Risk Analysis Framework* has been recently revised (refer 'What's New?' at [www.ogtr.gov.au](http://www.ogtr.gov.au)) but was not applied to this RARMP as the consultation version was completed prior to the review's finalisation.

document 'The Biology and Ecology of Bread Wheat (*Triticum aestivum* L. em Thell.) in Australia' (produced to further 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](http://www.ogtr.gov.au).

The risk assessment considered information contained in the application (comprising: information required by the Act and the Regulations on the GMO; on the parent organism, the proposed dealings, including proposed containment conditions; and potential impacts on human health and safety and the environment), current scientific knowledge, and submissions received during consultation with expert groups and authorities and the public (issues raised in submissions are summarised in Chapter 2 and Appendix 7 of the RARMP).

Through this process, potential hazards were identified to human health and safety or the environment that may be posed by the release of the two GM wheat lines. These have been carefully evaluated to determine whether risks might arise, based on the likelihood of each hazard occurring and the likely impact of each hazard were they to be realised.

The identified potential hazards relate to:

- **toxicity and allergenicity** to humans and other organisms: could these GM wheat plants be more toxic or allergenic to humans than non-GM wheat, or harmful to other organisms as a result of the novel gene products, altered proline content or because of unintended effects?
- **weediness**: could the genetic modifications be harmful to the environment by increasing the potential for these wheat plants 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 wheat crops, naturalised wheats, or to other organisms?

## CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator has concluded that the limited and controlled release of the two GM wheat lines over one season will not pose significant risks to human health and safety and the environment as a result of the genetic modification. The risk assessment of each potential hazard identified above is summarised under a separate heading below.

### **Toxicity or allergenicity to humans and other organisms**

The two GM wheat lines are unlikely to prove more toxic or allergenic to humans or other organisms than conventional wheat.

Neither the OAT protein nor the CAH protein are known to be toxic or allergenic, nor are they structurally similar to known protein toxins or allergens. Humans and other organisms are already exposed to both proteins.

The OAT enzyme forms part of a pathway for the metabolism of proline in many microorganisms, plants and animals, including humans, and it is therefore widespread in the environment. The introduced OAT protein from *A. thaliana* is very similar to the OAT proteins of other plants, animals and microorganisms.

The *cah* gene was derived from the soil fungus *Myrothecium verrucaria* and genes thought to encode CAH enzymes have been identified in a number of other fungi and bacteria. As cyanamide is rapidly broken down by microbial activity in soil, CAH enzymes are likely to be widespread in the environment.

The effect of the introduction of the *oat* gene in the GM wheat lines is to increase levels of proline expression within the plants' cells. Under glasshouse conditions, the concentration of free proline in the GM wheat plants is increased approximately 3-fold. Proline is present in all organisms and is therefore ubiquitous in the environment and in food. It is not considered toxic even at high doses. Elevated levels of proline occur naturally in salt tolerant plant species, and proline levels can be increased in many plants in response to other environmental stresses. The level of free proline expected in the GM wheat plants is therefore highly unlikely to pose a risk of toxicity.

The level of occupational exposure to the proteins produced by the action of the introduced genes through working with the GM wheat is likely to be very low. Furthermore, exposure to the GM wheat would be limited as the release is limited in scale and licence conditions have been imposed to limit unintended exposure to the GMOs (refer to key licence conditions below).

The applicant does not intend to use any material produced in the release in human food or animal feed, 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 wheats could be used in human food.

### **Weediness**

The domestication of *Triticum aestivum* L. into what we now know as bread wheat resulted in the loss of most of the characteristics that contribute to successful weediness, such as competitive ability and seed heads that shatter at maturity. Wheat is not considered a problematic weed in Australia. The germination and persistence of non-GM wheats in Australia are limited by the availability of adequate soil moisture and nutrients, herbivory (vertebrate and invertebrate), fire, plant competition and/or frost.

The GM wheat has been modified to achieve salt tolerance through having increased levels of proline as a result of overexpression of the *oat* gene. The GM wheat lines grown hydroponically in the glasshouse under salt-stress conditions (150 mM NaCl) show a two-fold increase in tiller number, seed number and seed weight relative to non-GM wheat. Under these salt-stress conditions growth of non-GM wheat is severely impaired.

Elevated proline levels are also thought to confer tolerance to some other environmental stresses, including frost and moisture stress. The GM wheat might therefore have some advantage over non-GM wheat in response to frost or drought as well as saline environments. However, the GM wheat plants would still be limited by water availability and the range of other environmental factors which normally limit the persistence of wheat plants in Australia.

The parental cultivars of the GM wheat lines do not have any significant seed dormancy. While the genetic modifications may provide the GM wheat with an advantage in some environmental conditions relative to non-GM wheat, they are unlikely to increase other characteristics normally associated with intrinsic weediness.

The applicant has not observed any unintended or secondary effects in the GM wheat lines grown under glasshouse conditions and reports that the growth characteristics of the GM wheat lines are similar to those of conventional wheat. However, under non-saline growth conditions the GM wheat plants are slightly smaller than non-GM plants. Therefore it is possible that there is a metabolic cost incurred through the overproduction of proline which may impact on the overall fitness of the GM wheat.

The *cah* gene introduced to the GM wheat lines confers tolerance to the herbicidal compound cyanamide. This tolerance was used to select transformed plants in the laboratory. Cyanamide is not registered for use as a herbicide in Australia and it would not be used during this release. Therefore the *cah* gene will not confer any advantage on the GM wheat plants.

The risk of the GM wheats establishing as problematic weeds in the release area is considered very low. The Regulator has imposed containment measures to minimise the spread and persistence of these GM wheats in the environment (refer to key licence conditions below).

### **Transfer of introduced genes to other organisms**

Wheat is predominantly self-pollinating with rates of out-crossing to other cultivated wheat plants of less than 5% between adjacent rows. Wheat pollen is relatively heavy compared to grass pollen and does not remain viable for long periods (under field conditions, up to 30 minutes) and its dispersal is via wind, rather than by insects. Because wheat is primarily self-pollinating and pollen movement is mediated by wind, an isolation zone is a more suitable measure than a pollen trap to limit pollen escape from the release site.

Wheat can cross-pollinate with a number of species within the genus *Triticum* and related genera such as *Aegilops*, *Elytrigia*, *Hordeum* and *Secale*. Out-crossing to these species will not occur as these plants will not be present near the release site, except for those deliberately planted as part of the trial.

Non-GM bread wheat will also be planted as part of the trial. While outcrossing from the GM wheat plants to these plants is possible, these plants and resultant seed would be treated in the same manner as the GM wheat. Licence conditions have been imposed to minimise the risk of transfer of the introduced genes to plants outside the release site including the use of an isolation zone (refer to key licence conditions below).

The risk of transfer of the introduced genes to naturalised wheat is negligible due to geographic isolation. The likelihood of transfer of the introduced genes to other organisms is negligible because of genetic incompatibility. Even if such transfer occurred, it would be unlikely to pose any risk to human health and safety and the environment.

### **THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)**

As part of the evaluation process for this licence application, a risk management plan has been developed (refer to Conclusion of the Risk Assessment, above). The applicant proposed a number of containment measures to minimise the spread and persistence of the GMOs and the introduced genes in the environment during the trial. The Regulator considered these proposals in selecting licence conditions that have been imposed to implement the risk management measures that will minimise the potential exposure of humans and other organisms and limit the likelihood of spread and persistence of the GMOs or the introduced genetic materials in the environment. 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 wheat seed being used as stockfeed;

- limit the scale and duration of the release;
- limit exposure to humans and other animals;
- destroy all GM materials not required for any possible future trials or research;
- securely transport and store the GMOs; and
- report adverse effects to the Regulator.

### **Weediness**

Licence conditions have been imposed which require the applicant to:

- limit the scale and duration of the release;
- locate the release site at least 50 m from natural waterways;
- contain the GM wheats with a 1.8 m fence to exclude rabbits and large animals;
- take measures to minimise rodent numbers, including mowing the 10 m monitoring zone around the trial site;
- use of bird proof netting to prevent birds entering site to minimise seed dispersal;
- securely transport and store the GM wheat material and seeds;
- clean the release site after harvest and equipment used at the site; and
- monitor the release site and the 10 m monitoring zone after harvest and destroy volunteers for at least 24 months.

### **Transfer of introduced genes to other organisms**

Licence conditions have been imposed which require the applicant to:

- limit the scale and duration of the release;
- surround the GM wheat lines with a 500 m isolation zone in which no other wheat or sexually compatible plants are planted;
- monitor the release site and the 10 m monitoring zone after harvest and destroy volunteers for at least 24 months; and
- clean the release site after harvest and equipment used at the site.

### **General conditions**

Any 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 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 imposed licence conditions are provided in Appendix 5.

### **Identification of issues to be addressed for future releases**

The limited and controlled release is a small scale, single-site ‘proof of concept’ trial over one growing season, from April 2005 – January 2006, to test the efficacy of proline overexpression as an osmoprotectant in saline field conditions and to compare the GM wheats’ field performance with conventional wheat. Hence, no research conditions have been imposed in the licence. However, the following information would be required from future applications, particularly to assess requests for larger scale releases of these GM wheat lines:

- the level of expression of the introduced genes and encoded OAT and CAH proteins, and the plant tissues (including pollen) and developmental stages in which they are being expressed;
- the level of free proline and metabolites present in various tissues at different developmental stages of the GM wheat plants under Australian conditions;
- genetic segregation and molecular characterisation of the introduced genes;
- the potential toxicity and allergenicity of the GM wheat, particularly the introduced OAT and CAH proteins;
- the magnitude of the tolerance of the GM wheat to salt and other abiotic stresses;
- agronomic characteristics of the GM wheats relating to fitness and potential weediness;
- the occurrence of gene flow from GM wheat to non-GM wheat under Australian field conditions; and
- any unintended or secondary effects resulting from the genetic modification.

It should be noted that provision of the above data during the release is not required to ensure the management of risks to human health and safety and the environment from this release. The risk management measures summarised in Chapter 2, Table 3 and given effect by the imposed licence conditions, will achieve this purpose

### **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 risk assessment and risk management plan document for this application, which can be obtained from the website of the Office of the Gene

Technology Regulator ([www.ogtr.gov.au](http://www.ogtr.gov.au)), or by calling 1800 181 030 (please quote application number DIR 053/2004).