



Australian Government

Department of Health and Ageing

Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 080/2007**

**Limited and controlled release of wheat genetically
modified for drought tolerance**

Applicant: Victorian Department of Primary Industries

June 2008

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Executive Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of up to 50 lines of wheat modified for drought tolerance into the environment in respect of application DIR 080/2007 from the Victorian Department of Primary Industries (DPI Victoria).

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Acting Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public¹.

The application

DPI Victoria applied for a licence for dealings involving the intentional release of up to 50 lines of genetically modified (GM) wheat on a limited scale and under controlled conditions. The wheat lines have been genetically modified to enhance drought tolerance. The trial is authorised to take place at two sites in the local government areas of Horsham and Mildura, Victoria, on a maximum total area of 0.4 hectares² per year between 2008 and 2010.

The GM wheat lines contain one of fifteen different introduced genes derived from the plants thale cress and maize, a moss and a yeast. The introduced genes encode proteins that are intended to enable normal plant growth with reduced amounts of water (drought tolerance) either by regulating gene expression or modulating biochemical pathways in the wheat plants.

The GM wheat lines also contain a herbicide tolerance gene and an antibiotic resistance gene that were used as markers to select for successful genetic modifications during initial research and development work in the laboratory. The applicant does not intend to apply the herbicide to which the GM plants are tolerant during the trial and the antibiotic resistance gene will not be expressed in the plants.

The purpose of the trial is to conduct proof of concept research, including continuing assessment of some lines that were initially authorised for release under DIR 071/2006. The agronomic performance, including yield, of the GM wheat lines will be evaluated under rain-fed, drought prone conditions. Seed and tissue samples would be collected and retained for analysis and possible future trials of lines that may be selected for further development, subject to further approval(s). The GM wheat will not be used for human food or animal feed.

¹ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au>>), and in the Regulator's Risk Analysis Framework (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

² As a result of a request from the applicant, the total maximum size of the proposed trial was increased from 0.225 hectares per year to 0.4 hectares per year. The applicant also proposed changes to harvest and post harvest licence conditions. The proposed changes were considered when finalising this RARMP and no new risks to people or the environment were identified.

DPI Victoria proposed a number of controls to restrict the dissemination or persistence of the GM wheat lines and the introduced genetic materials in the environment that have been considered during the evaluation of the application.

Confidential Commercial Information

Some details, including the names, classes and specific functions of the introduced genes, the names and origins of the promoters (regulatory sequences), and data from previous international field releases of other plants expressing the same genes, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment takes into account information in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge, advice received from a wide range of experts, agencies and authorities consulted on the RARMP, submissions from the public and from the applicant.

A **hazard** identification process was used to determine potential pathways that might lead to harm to people or the environment as a result of gene technology.

Seven events were considered whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the seven events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM wheat lines into the environment are considered to be **negligible**. Hence, the Acting Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the seven events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, a range of measures have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the proposed release to the size, locations and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

The licence conditions require DPI Victoria to **limit** the release to a total area of 0.4 hectares per year at two sites between July 2008 and March 2010. The **control** measures to restrict the spread and persistence of the GMOs include preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with OGTR transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed³.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 50 GM wheat lines on a maximum total area of 0.4 hectares per season over two growing seasons in the Victorian local government areas of Horsham and Mildura poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the proposed release to the size, locations and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

³ The licence for DIR 080/2007 is available on the OGTR website
<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>> via the link to DIR 080/2007

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Abbreviations

the Act	<i>Gene Technology Act 2000</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
<i>bar</i>	gene encoding Phosphinothricin Acetyltransferase
<i>bla</i>	gene encoding β -lactamase
BLAST	Basic Local Alignment Search Tool
CCI	Confidential Commercial Information as declared under section 185 of the <i>Gene Technology Act 2000</i>
CaMV	Cauliflower mosaic virus
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
DPI Victoria	Victorian Department of Primary Industries
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
km	kilometre
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OGTR	Office of the Gene Technology Regulator
PAT	Phosphinothricin Acetyltransferase
qPCR	Quantitative Polymerase Chain Reaction
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the (Acting) Regulator	the (Acting) Gene Technology Regulator
RNA	Ribonucleic Acid
TGA	Therapeutic Goods Administration
US FDA	United States Food and Drug Administration
USDA APHIS	United States Department of Agriculture Animal and Plant Health Inspection Service
WUE	Water Use Efficiency

Technical Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence (DIR 080/2007) to the Victorian Department of Primary Industries (DPI Victoria) for dealings involving the intentional release of genetically modified (GM) wheat lines into the Australian environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Acting Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public⁴.

The application

DPI Victoria has applied for a licence for dealings involving the intentional release of up to 50 lines⁵ of wheat (*Triticum aestivum* L.) that have been genetically modified to enhance drought tolerance on a limited scale and under controlled conditions. The trial is authorised to take place at two sites in the local government areas of Horsham and Mildura, Victoria, on a maximum total area of 0.4 hectares⁶ per year over two growing seasons between July 2008 and March 2010.

The GM wheat lines were produced by transforming plants of the bread wheat cultivar Bobwhite 26, which is not grown commercially in Australia. Each line contains one of 15 different genes derived from the plants thale cress (*Arabidopsis thaliana*) and maize (*Zea mays*), a moss (*Physcomitrella patens*) and a yeast (*Saccharomyces cerevisiae*). The introduced genes encode proteins that are intended to improve drought tolerance by regulating gene expression or modulating biochemical and signal transduction pathways in the wheat plants. The identity and specific function of these genes are subject to a commercial confidential information declaration (see below).

The GM wheat lines also contain the herbicide tolerance gene, *bar*, which was used as a marker to select for modified plants in the laboratory. The *bar* gene encodes the phosphinothricin acetyltransferase (PAT) enzyme, which provides tolerance to herbicides with glufosinate ammonium as the active ingredient. The applicant does not intend to apply glufosinate ammonium during the field trial.

⁴ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

⁵ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

⁶ As a result of a request from the applicant, the total maximum size of the proposed trial was increased from 0.225 hectares per year to 0.4 hectares per year. The applicant also proposed changes to harvest and post harvest licence conditions. The proposed changes were considered when finalising this RARMP and no new risks to people or the environment were identified.

Additionally, the GM wheat lines contain the β -lactamase (*bla*) gene from *Escherichia coli*, which confers ampicillin resistance and was used to select for bacteria containing the desired genes in the laboratory. The *bla* gene is not expressed in the GM wheat lines as it is linked to a bacterial promoter that does not function in plants.

The purpose of the trial is to conduct proof of concept research, including continuing assessment of some lines that were initially authorised for release under DIR 071/2006. The agronomic performance, including yield, of the GM wheat lines will be evaluated under rain-fed, drought prone conditions. Seed and tissue samples would be collected and retained for analysis and possible future trials of lines that may be selected for further development, subject to further approval(s). The GM wheat will not be used for human food or animal feed.

DPI Victoria proposed a number of controls to restrict the dissemination or persistence of the GM wheat lines and their genetic material into the environment. These controls have been considered during the evaluation of the application.

Confidential Commercial Information

Some details, including the names, classes and specific functions of the introduced genes, the names and origins of the promoters (regulatory sequences), and data from previous international field releases of other plants expressing the same genes, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment considered information contained in the application, relevant previous approvals, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received from consultation with a wide range of prescribed experts, agencies and authorities on the application (summarised in Appendix B of the RARMP). No new risks to people or the environment were identified from the advice received on the consultation RARMP. However, feedback on the consideration of previously raised issues enabled their clarification in the final RARMP.

Advice received from the public on the consultation RARMP (four submissions) and how it was considered, is summarised in Appendix C.

A reference document, *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)*, was produced to inform the risk assessment process for licence applications involving GM wheat plants. The document is available from the OGTR or from the website <http://www.ogtr.gov.au>.

The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

Seven events were considered whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

The characterisation of the seven events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principle reasons for this include:

- limits on the size, locations and duration of the release proposed by DPI Victoria;
- suitability of controls proposed by DPI Victoria to restrict the dissemination or persistence of the GM wheat plants and their genetic material;
- limited ability and opportunity for the GM wheat lines to transfer the introduced genes to commercial wheat crops or other sexually related species;
- limited capacity of the GM wheat lines to spread and persist in the areas proposed for release;
- none of the GM plant materials or products will be used in human food or animal feed;
- widespread presence of the same or similar proteins encoded by, and end products produced as a result of the activity of, the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM wheat 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 environment⁷.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the seven events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, a range of measures have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the proposed release to the size, locations and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

Licence conditions to manage this limited and controlled release

The Acting Regulator has imposed a number of licence conditions including requirements to:

- conduct the release on a total area of up to 0.4 hectares per year at two sites in the local government areas of Horsham and Mildura, Victoria, between July 2008 and March 2010

⁷ As none of the proposed dealings were considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the *Gene Technology Act 2000* mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator allowed 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

- establish a 10 m monitoring zone around each trial site that is free of any related species and is maintained in a manner that does not attract or harbour rodents
- maintain an isolation zone of at least 200 m around each trial site free of any sexually compatible species
- enclose each trial site with a 1.2 m high fence with lockable gates
- conduct rodent baiting and/or trapping in and around each trial site
- locate the trial sites at least 50 m away from natural waterways
- harvest the GM wheat plant material by hand, or by machine, and separately from other crops
- not permit any materials from the release to be used in human food or animal feed
- destroy all plant materials not required for further analysis
- following harvest, clean the sites, monitoring zones and equipment used on the sites
- after harvest, apply measures to promote germination of any wheat seeds that may be present in the soil
- monitor the site for at least 24 months and destroy any wheat plants that may grow until no volunteers are detected for a continuous 6 month period.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs; Policy on transport and supply of GMOs*). Licence conditions based on these guidelines and policies have also been proposed to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standard Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)⁸.

FSANZ is responsible for human food safety assessment, including GM food. As the trial involves proof of concept research, the applicant does not intend any material from the GM wheat lines proposed for release to be used in human food. Accordingly, the applicant has not applied to FSANZ to evaluate any of the GM wheat lines. FSANZ approval would need to be obtained before they could be used in human food in Australia.

Although the GM wheat lines have been modified to be tolerant to glufosinate ammonium, the applicant does not intend to apply this herbicide during the trial and therefore no approval is required from APVMA.

⁸ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for a large scale or commercial release of any of these GM wheat lines that may be selected for further development, or to justify a reduction in containment conditions. This would include:

- characterisation of the introduced genetic material in the plants, including copy number and genotypic stability
- additional data on the potential toxicity of plant materials from the GM wheat lines
- additional data on the allergenicity of proteins encoded by the introduced genes
- data on the dispersal of viable wheat seeds by Australian birds
- data on the level of long distance gene flow under Australian conditions
- characteristics indicative of weediness including measurement of altered reproductive capacity; tolerance to drought and other environmental stresses; and disease susceptibility

Suitability of the applicant

The Regulator determined, at the commencement of the assessment process for this application, that DPI Victoria is suitable to hold a DIR licence under the requirements of section 58 of the Act. The Acting Regulator is satisfied that DPI Victoria remains suitable as no relevant convictions have been recorded, no licences or permits have been cancelled or suspended under OGTR legislation relating to the health and safety of people or the environment, and the organisation has confirmed its ability to comply with the licence conditions.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 50 GM wheat lines on a maximum total area of 0.4 hectares per season over two growing seasons in the Victorian local government areas of Horsham and Mildura poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the proposed release to the size, locations and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people or the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation⁹, details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

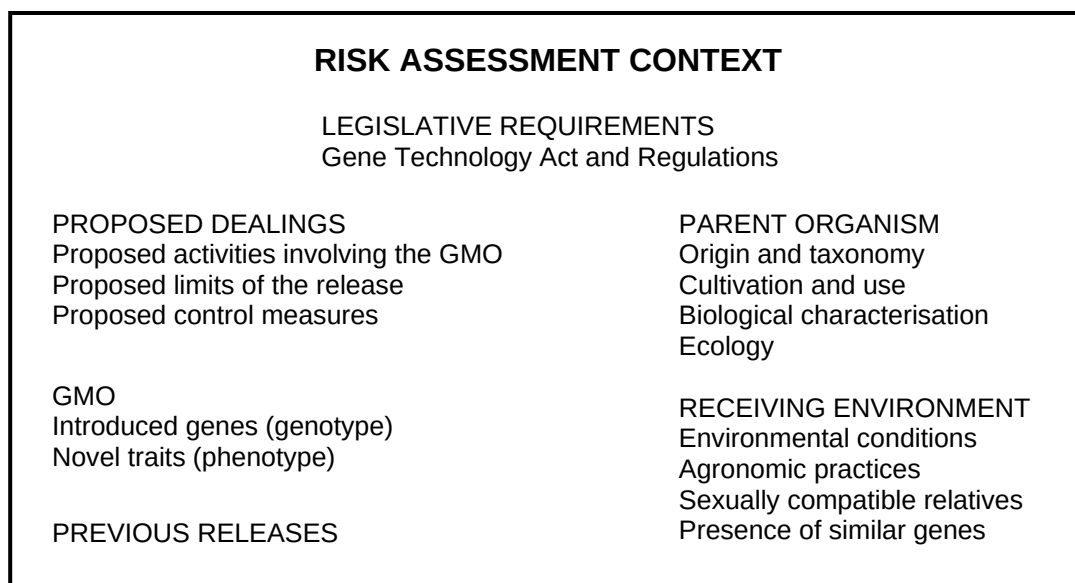


Figure 1. Components of the context considered during the preparation of the risk assessment

2. For this application, establishing the risk assessment context includes consideration of:
- the proposed dealings (Section 3.1)
 - the limits proposed by the applicant (Section 3.2)
 - the controls proposed by the applicant (Section 3.3)
 - characteristics of the parent organism (Section 4)
 - the nature and effect of the genetic modification (Section 5)
 - the environmental conditions in the locations where the release would occur (Sections 6.1 and 6.2)
 - relevant agricultural practices (Section 6.3)
 - the presence of related plants in the environment (Section 6.4)
 - the presence of the introduced or similar genes in the environment (Section 6.5)

⁹ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail on the Office of the Gene Technology Regulator (OGTR) website at <<http://www.ogtr.gov.au>> and in the *Risk Analysis Framework* (OGTR 2007) <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>

- any previous releases of these or other GMOs relevant to this application (Section 7).

Section 2 The legislative requirements

3. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom she must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions on licence applications. In addition, the Gene Technology Regulations 2001 (the Regulations) outline matters the Regulator must consider when preparing a RARMP.

4. In accordance with section 50A of the Act, the Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits have been proposed on the size, locations and duration of the release and controls have been proposed by the applicant to restrict the dissemination or persistence of the GMO and its genetic material in the environment. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application is considered to be a limited and controlled release and the Regulator has prepared a RARMP for this application.

5. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public. The advice from the prescribed experts, agencies and authorities and where it was taken into account is summarised in Appendix B. Four submissions were received from members of the public, and their consideration is summarised in Appendix C.

6. As a result of a request from the applicant, the total maximum size of the proposed trial was increased from 0.225 hectares per year to 0.4 hectares per year. The applicant also proposed changes to harvest and post harvest licence conditions. The proposed changes were considered when finalising this RARMP and no new risks to people or the environment were identified.

7. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a ‘significant risk’ to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d).

Section 3 The proposed dealings

8. The Victorian Department of Primary Industries (DPI Victoria) proposes to release up to 50 wheat lines¹⁰ that have been genetically modified (GM) to enhance drought tolerance into the environment under limited and controlled conditions.

3.1 The proposed activities

9. The applicant has stated that the purpose of the trial is to conduct proof of concept research to evaluate the agronomic performance, including yield, of the GM wheat lines under

¹⁰ The term ‘line’ is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

rain-fed, drought prone conditions. Some seed and tissue samples would be collected and retained for analysis and possible future trials of lines that may be selected for further development, subject to additional approvals. The GM wheat will not be used for human food or animal feed.

3.2 The proposed limits of the dealings (size, locations and duration)

10. The release is proposed to take place at sites within two Victorian Government Agricultural Research Stations in Victoria, in the shires of Horsham and Mildura, giving a total maximum area of 0.4 hectares per season, over two seasons between May 2008 and March 2010.

3.3 Proposed controls to restrict the dissemination or persistence of the GMOs and their genetic material in the environment

11. Only trained and authorised staff will be permitted access to the proposed locations.

12. The applicant has proposed a number of controls to restrict the dissemination or persistence of the GM wheat lines and their genetic material in the environment including:

- establish a 10 m monitoring zone free of plants around each release site
- maintain an isolation zone of at least 490 m (not including the 10 m monitoring zone) around each trial site free of any sexually compatible species
- enclose each trial site with a 1.2 m high fence with lockable gates
- conduct rodent baiting and/or trapping in and around each trial site
- locate the trial sites at least 50 m away from natural waterways
- harvest the GM wheat plant material by hand, or by machine, and separately from other crops
- not permit any materials from the release to be used in human food or animal feed
- destroy all plant materials not required for further analysis
- following harvest, clean the sites, monitoring zones and equipment used on the sites
- after harvest, apply measures to promote germination of any wheat seeds that may be present in the soil
- monitor the site for at least 24 months and destroy any wheat plants that may grow until no volunteers are detected for a continuous 6 month period.

13. These controls, and the limits outlined in Chapter 1, Section 3.2, have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.1.1.

Section 4 The parent organism

14. The parent organism is bread wheat (*Triticum aestivum* L.) which is exotic to Australia and is grown as an agricultural crop in most states of Australia. Bread wheat has been grown in Australia for over 200 years and is a significant food crop. Further detailed information about the parent organism is contained in a reference document, *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)*, which was produced in order to inform the risk assessment process for licence applications involving GM wheat plants. This document is available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

15. The GM wheat lines in the proposed release were derived from the wheat cultivar Bobwhite 26. The Bobwhite cultivar is not favoured as a commercial bread wheat as it is

considered to be of lower quality than most commercial cultivars (Bhalla et al. 2006), but is commonly used in genetic modification work because it is relatively easy to transform and has previously been used in conventional (non-GM) wheat breeding programs. Seed was obtained from seed sources provided by the wheat breeding program at the International Maize and Wheat Improvement Centre (CIMMYT).

Section 5 The GMOs, nature and effect of the genetic modification

5.1 Introduction to the GMOs

16. Some details of the application, including the names, classes and specific functions of the introduced genes, the names and origins of the promoters, and data from previous international field releases of other plants expressing the same genes, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was considered during the preparation of the RARMP and was made available to the prescribed expert groups and authorities that were consulted on this application.

17. Up to 50 GM wheat lines are proposed for release, with each line containing a single gene for drought tolerance derived from the plants *Arabidopsis thaliana* (thale cress) and *Zea mays* (maize), the moss *Physcomitrella patens* and the yeast *Saccharomyces cerevisiae*.

18. The GM wheat lines also contain the herbicide tolerance selectable marker gene, *bar*, and the antibiotic resistance selectable marker gene, *bla*. The *bar* gene, which was isolated from *Streptomyces hygroscopicus*, encodes the phosphinothricin acetyltransferase (PAT) enzyme and confers tolerance to herbicides with glufosinate ammonium as the active ingredient. The *bla* gene, encoding β -lactamase, was originally derived from the common gut bacterium *Escherichia coli* and confers ampicillin resistance. This bacterial marker gene is not expressed in the GM wheat lines as it is linked to a bacterial promoter that does not function in plants. The *bar* and *bla* genes were used during the initial selection of transformed plants and bacteria, respectively, in the laboratory. The applicant does not intend to apply glufosinate ammonium during the trial.

19. Short regulatory sequences (promoters and transcription termination sequences) that control expression of the introduced genes are also present in the GM wheat lines. These are derived from plants including *Z. mays*, the plant virus Cauliflower mosaic virus (CaMV), and the bacteria *Agrobacterium tumefaciens* and *E. coli*. Although CaMV and *A. tumefaciens* are plant pathogens, and *E. coli* is an opportunistic human pathogen, the regulatory sequences comprise only a small part of their total respective genomes, and are not capable of causing disease.

5.2 The introduced genes and their encoded proteins

5.2.1 Genes expected to enhance response to drought stress, and their encoded proteins

20. The GM wheat lines each contain one of fifteen different introduced genes that are subject to a CCI declaration. The genes were derived from the plants *Arabidopsis thaliana* (thale cress) and *Zea mays* (maize), the moss *Physcomitrella patens* and the yeast *Saccharomyces cerevisiae* (Table 1). The introduced genes encode proteins that are intended to enhance drought tolerance by regulating gene expression or modulating biochemical and signal transduction pathways in the wheat plants. The applicant states that the introduced genes have demonstrated the capacity to produce a water efficient phenotype in *A. thaliana*, *B. napus* and/or *Z. mays*.

Table 1. Genes used to genetically modify wheat

Genetic Element	Gene	Source organism	Function in the source organism
Gene 1	CCI	<i>Z. mays</i>	CCI
Gene 2	CCI	<i>A. thaliana</i>	CCI
Gene 3	CCI	<i>S. cerevisiae</i>	CCI
Gene 4	CCI	<i>P. patens</i>	CCI
Gene 5	CCI	<i>P. patens</i>	CCI
Gene 6	CCI	<i>S. cerevisiae</i>	CCI
Gene 7	CCI	<i>P. patens</i>	CCI
Gene 8	CCI	<i>P. patens</i>	CCI
Gene 9	CCI	<i>P. patens</i>	CCI
Gene 10	CCI	<i>P. patens</i>	CCI
Gene 11	CCI	<i>P. patens</i>	CCI
Gene 12	CCI	<i>P. patens</i>	CCI
Gene 13	CCI	<i>A. thaliana</i>	CCI
Gene 14	CCI	<i>P. patens</i>	CCI
Gene 15	CCI	<i>A. thaliana</i>	CCI
selectable marker	bar	<i>S. hygrosopicus</i>	Herbicide tolerance
selectable marker	bla	<i>E. coli</i>	Antibiotic resistance

21. Up to 50 GM wheat lines are proposed for release, with each line containing a single gene for enhanced drought tolerance. For six of the genes there are two constructs, one driven by a stress inducible promoter and one by a constitutive promoter, the remaining nine genes have only one construct each, driven by the constitutive promoter, giving a total of 21 constructs (for details see Table 2) which were used to produce the GM wheat lines proposed for release. Twelve of these were also assessed in detail in the RARMP for DIR 071/2006.

Table 2. Gene constructs used to generate the GM wheat lines proposed for release

Construct Number	Promoter	Gene Reference	Gene name	Terminator	Selectable marker cassette
1	constitutive	Gene 1	CCI	35st	Ubi::bar_nos
2	constitutive	Gene 2	CCI	35st	Ubi::bar_nos
3	constitutive	Gene 3	CCI	35st	Ubi::bar_nos
4	constitutive	Gene 4	CCI	35st	Ubi::bar_nos
5	constitutive	Gene 5	CCI	35st	Ubi::bar_nos
6	constitutive	Gene 6	CCI	35st	Ubi::bar_nos
7	inducible	Gene 1	CCI	35st	Ubi::bar_nos
8	inducible	Gene 2	CCI	35st	Ubi::bar_nos
9	inducible	Gene 3	CCI	35st	Ubi::bar_nos
10	inducible	Gene 4	CCI	35st	Ubi::bar_nos
11	inducible	Gene 5	CCI	35st	Ubi::bar_nos
12	inducible	Gene 6	CCI	35st	Ubi::bar_nos
13	constitutive	Gene 7	CCI	35st	Ubi::bar_nos
14	constitutive	Gene 8	CCI	35st	Ubi::bar_nos
15	constitutive	Gene 9	CCI	35st	Ubi::bar_nos
16	constitutive	Gene 10	CCI	35st	Ubi::bar_nos
17	constitutive	Gene 11	CCI	35st	Ubi::bar_nos
18	constitutive	Gene 12	CCI	35st	Ubi::bar_nos
19	constitutive	Gene 13	CCI	35st	Ubi::bar_nos
20	constitutive	Gene 14	CCI	35st	Ubi::bar_nos
21	constitutive	Gene 15	CCI	35st	Ubi::bar_nos

5.2.2 Toxicity/allergenicity of the introduced genes for drought tolerance

22. Homologues of all of the encoded proteins occur naturally in a range of organisms, including plants widely consumed by people and animals (see discussion in Section 6.5 of this

chapter). On this basis, people and other organisms have a long history of exposure to the proteins encoded by the introduced genes for drought tolerance.

23. No toxicity/allergenicity tests have been performed on any of the purified encoded proteins as the proposed trial is still at proof of concept stage. Such tests would have to be conducted if approval was sought for the GMOs to be considered for human consumption in Australia (Chapter 1, Section 7.1.2).

24. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The predicted amino acid sequences of the proteins encoded by each of the introduced genes for drought tolerance were compared to a database of known allergens. The results of this analysis did not indicate that any of the encoded proteins shared any significant sequence homology with any known allergens (information supplied by applicant).

25. A comprehensive search of the scientific literature also yielded no information to suggest that any of the encoded proteins are toxic or allergenic to people, or toxic to other organisms.

5.2.3 The herbicide tolerance marker gene (*bar*) and the encoded protein

26. All GM wheat lines proposed for release also contain the *bar* herbicide tolerance marker gene. The *bar* gene was isolated from *S. hygrosopicus*, a common saprophytic, soil-borne microorganism (Thompson et al. 1987). The *bar* gene encodes the PAT protein, which confers tolerance to glufosinate ammonium, the active component in a number of herbicides.

27. Glufosinate ammonium is widely used as a broad-spectrum herbicide and is registered for use in many countries. However, in Australia it is not a widely used herbicide and it is not registered for use on wheat. It is also not used as the main method to control wheat in other crops. Herbicides containing glufosinate ammonium as the active constituent are currently registered in Australia by the Australian Pesticides and Veterinary Medicines Authority (APVMA) as Basta® for horticultural use and non-agricultural use, Finale® for home garden and non-agricultural use, and Liberty® for use on GM InVigor® hybrid canola and GM Liberty Link® Cotton varieties.

28. Other regulatory agencies, both in Australia and in other countries, have previously assessed the *bar* gene, or related *pat* gene encoding the same PAT enzyme, as safe for use in human food. In addition, a number of GM crops, including food crops, containing the *bar* gene have been approved for commercial release both in Australia (DIR 021/2002, DIR 062/2005) and overseas. No adverse effects on humans, animals or the environment have been reported from any releases.

29. For more detailed information on the *bar* gene and the encoded protein refer to the RARMP prepared for DIR 062/2005 (Liberty Link® Cotton) available at <http://www.ogtr.gov.au>.

5.2.4 Toxicity/allergenicity of PAT

30. PAT proteins are widespread in the environment, through the presence of naturally occurring bacteria as well as in other GM crops approved for commercial release. The PAT protein expressed in the GM wheat plants proposed for release is the same as that present in commercially approved InVigor® hybrid canola (DIR 021/2002) and Liberty Link® Cotton (DIR 062/2005). Extensive toxicity studies using the purified form of the PAT protein have been conducted and have shown that the PAT protein is not likely to be toxic or allergenic to

humans. Detailed descriptions of the results of these studies are available in the RARMPs for DIR 021/2002 and DIR 062/2005.

31. Food Standards Australia New Zealand (FSANZ) has approved the use of food derived from other GM plants containing either the *bar* or *pat* gene, including GM cotton, corn, canola and soybean, concluding that the PAT protein is not toxic (ANZFA 2001a; 2001b; 2001c; and 2002; FSANZ 2003; 2004a; 2004b; 2005a; and 2005b). The studies submitted in support of the food uses for this protein indicate that it has none of the properties associated with protein toxins or allergens.

5.2.5 The antibiotic resistance marker gene (*bla*) and the encoded protein

32. The GM wheat lines also contain the β -lactamase (*bla*, also known as *amp*) antibiotic resistance marker gene. The *bla* gene is derived from *E. coli* (Spanu et al. 2002) and encodes the β -lactamase enzyme, which confers ampicillin resistance.

33. The β -lactamase enzyme is widespread in the environment and in food. Naturally occurring ampicillin-resistant microorganisms have been found in mammalian digestive systems (Spanu et al. 2002). The *bla* gene was originally isolated from antibiotic resistant strains of *E. coli* found in hospital patients.

34. The *bla* gene in the GM wheat lines is under the control of its own bacterial promoter and terminator from *E. coli* and therefore is not expressed in the GM wheat plants. The gene was used in the laboratory prior to the production of the GM wheat lines.

35. A number of GM food crops containing the *bla* gene have been approved for limited and controlled release both in Australia (DIRs 019/2002, 026/2002, 028/2002, 051/2004, 052/2004, 070/2006 and 071/2006) and overseas. No adverse effects on humans, animals or the environment have been reported from these releases.

5.2.6 The effects associated with the introduced genes

Drought and other abiotic stress tolerances

36. Drought stress is an abiotic stress; a nonliving factor that causes harmful effects to plants. Other types of abiotic stresses include salinity, temperature and nutrient deficiency or toxicity. Plants respond to different abiotic stresses often through an interconnecting series of signalling and transcription controls that ultimately aim to increase the plant's ability to tolerate the initial stress through different response mechanisms that include a range of biochemical and physiological processes. Cell signalling molecules can be activated by a number of stimuli and regulatory genes can be induced by more than one type of abiotic stress (eg drought, cold and salinity; Seki et al. 2002). Plant molecular responses to drought stress have been discussed in detail in the RARMP for DIR 071/2006 <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir071-2006>>.

37. Evidence of cross tolerance to different abiotic stresses has led researchers to conclude that the signalling pathways for abiotic stress tolerance are not strictly isolated (Yamaguchi-Shinozaki & Shinozaki 2006). This is supported by the finding that transcript levels of some genes are altered by several different abiotic stressors, with some transcript levels altered by three different abiotic stressors (drought, cold and salinity) (Mantri et al. 2007). Greater cross tolerance to drought and high saline soils has been reported than to cold and high saline soils (Seki et al. 2002).

38. The enhancement of tolerance in plants to other abiotic stresses as a result of increased tolerance to one abiotic stress has also been discussed in detail in the RARMP for DIR 071/2007 (Chapter 2, Event 7). It was also noted in this event that a gene expressed in a different species may not produce the same resultant phenotype as in the parent species.

5.3 The regulatory sequences

5.3.1 Regulatory sequences for expression of the genes for drought tolerance

39. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Two promoters have been used to control expression of the introduced genes for drought tolerance, a stress inducible promoter and a constitutive promoter. The details of these promoters have been declared CCI.

40. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA termination region for the introduced genes in the GM wheat lines is derived from the plant virus Cauliflower mosaic virus (CaMV) 35*st* terminator. Although CaMV is a plant pathogen, the regulatory sequence comprises only a small part of its total genome, and has not been reported to cause any adverse effects.

5.3.2 Regulatory sequences for the expression of the *bar* gene

41. Expression of the *bar* gene in the GM wheat lines is controlled by the promoter and first intron of the *Ubi1* ubiquitin gene from *Zea mays* (Christensen et al. 1992). The *Ubi1* promoter is considered a constitutive promoter, which means that genes that are linked to this promoter are generally expressed at relatively high levels throughout the growing season and in most tissues of the plant. However, *Ubi1* controlled gene expression is generally highest in young active wheat tissues and in pollen grains (Rooke et al. 2000).

42. The termination and polyadenylation signals that are also responsible for controlling *bar* gene expression are derived from the nopaline synthase (*nos*) gene of *Agrobacterium tumefaciens* (Bevan 1984). Although *A. tumefaciens* is a plant pathogen, the regulatory sequence comprises only a small part of its total genome, and has not been reported to cause any adverse effects.

5.4 Method of genetic modification

43. The drought tolerant GM wheat lines were each generated by biolistic transformation of wheat embryos (Pellegrineschi et al. 2002). This involved coating very small gold particles with the transformation vector containing the introduced genes. The particles were then ‘shot’ into zygotic embryos from *T. aestivum* cultivar Bobwhite 26. Transgenic plant tissues were recovered by survival on tissue media containing the selective agent phosphinothricin. Biolistic transformation has been widely used in Australia and overseas for introducing new genes into plants and is not known to cause any adverse effects on human health and safety or the environment.

44. All of the GM wheat lines were generated from independent transformation events, and therefore the introduced genes are expected to be located at different sites in the wheat genome for each line.

5.5 Characterisation of the GMOs

5.5.1 Stability and molecular characterisation

45. All transformation vectors used to produce the GM wheat lines have been fully sequenced. The exact location of the inserted genes within the bread wheat genome has not been determined. The inserted genes have been inherited as dominant Mendelian traits over at least four generations of plants grown in the glasshouse.

46. T₁ and T₂ generations of the GM wheat lines were obtained from primary transformants (T₀) by self-pollination and the genetic modification was characterised for the presence and expression of the introduced genes by Southern hybridisation analysis and by quantitative polymerase chain reaction (qPCR) at the T₁ generation.

47. Insertion copy number, determined by Southern hybridisation analysis, using probes of either the introduced gene for drought tolerance or the *bar* gene, has been performed on a subset of the events. Copy numbers in all of the GM wheat lines are still being determined. Analysis to date indicates a range of between one and seven copy numbers. Of these, the events are predominantly single locus. The GM wheat lines that have been selected are preferentially single locus homozygous for the introduced gene, although lines with a range of gene copy number are included.

48. Relative expression levels of the introduced genes were estimated for GM T₁ wheat plants using qPCR based on the comparative CT method (Livak & Schmittgen 2001). Expression of the introduced gene was normalised to the level of ubiquitin and estimated relative to either the lowest expressing GM line or to control samples. GM wheat lines showing a range of expression levels have been selected for the proposed trial.

5.5.2 Characterisation of the phenotype of the GMOs

49. One of the aims of the proposed trial is to compare agronomic performance of GM wheat lines and the non-GM parent under field conditions. The intended effect is improved drought tolerance in the field without unacceptable impacts on agronomic characteristics. Differences in growth characteristics were observed in the glasshouse within and between GM wheat lines for all of the introduced genes. The phenotypes predominantly observed include the development of more vegetative tillers and increased biomass under non-stressed conditions, and delayed flowering time.

50. All of the fifteen introduced genes for drought tolerance have been shown in *A. thaliana*, canola and/or maize to confer increased water use efficiency (WUE) or tolerance to water stress. In addition, the applicant states that the GM wheat lines selected showed drought tolerance characteristics under glasshouse conditions. Data on the tolerances of the GM wheat lines to drought stress and other abiotic stresses would be required for possible future applications involving large scale or commercial releases of these GM wheat lines. However, this information is not required for assessing the risks of this proposed release because of the containment measures proposed by the applicant to restrict the spread and persistence of the GM wheat lines, and the trial is limited in size, locations and duration.

Section 6 The receiving environment

51. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the geographic regions where the release would occur and any relevant biotic/abiotic properties of these locations; the intended agronomic practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2007).

6.1 Relevant abiotic factors

52. Wheat is grown across a wide range of environments around the world with the broadest adaptation of all the cereal crops species. The abiotic factors relevant to the growth and distribution of commercial wheat in Australia are discussed in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008).

53. The release is proposed to take place at two sites, both of which are typical of rain-fed, drought prone wheat production environments in Australia. The two sites are sufficiently distant from one another in Victoria (240 km) that if one site received considerably above average rainfall, the other site would have a large chance of still experiencing a more

representative level of drought. Average long-term rainfall in these locations varies from 250–350 mm. Selected climatic data for both proposed sites are given in Table 3.

Table 3. Climatic data for proposed drought tolerant GM wheat trial sites

	Horsham	Mildura
Average daily max/min temperature (Summer*)	29°C/13°C	31°C/15°C
Average daily max/min temperature (Winter*)	14°C/4°C	15°C/5°C
Average monthly rainfall (Summer*)	25.1 mm	22.6 mm
Average monthly rainfall (Winter*)	48.8 mm	33.3 mm

Source: <<http://www.bom.gov.au>>

* Summer averages were based on December to February and winter averages were based on June to August.

6.2 Relevant biotic factors

54. The biotic factors pertaining to the growth and distribution of commercial wheat in Australia are discussed in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008). Of relevance to this proposed release are the following points:

- wheat is grown both commercially and for research purposes in the regions surrounding both trial sites
- the applicant proposes that no sexually compatible species will be grown within 500 m of the trial sites
- invertebrates, vertebrates and microorganisms would all be exposed to the introduced genes, their encoded proteins and end products. In particular:
 - native and feral vertebrates including grain feeding birds, rodents and marsupials may visit the field location.
 - Although the control measures proposed by the applicant would minimise entry of vertebrates to the trial site, some unintended consumption of GM material may occur.

6.3 Relevant agricultural practices

55. The locations of the proposed limited and controlled release of the GM wheat lines are outlined in Section 3.2 of this chapter.

56. It is not anticipated that the agronomic practices for the cultivation of the GM wheat by the applicant will be significantly different from conventional practices for wheat growing. Conventional cultivation practices for wheat are outlined below and discussed in more detail in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008).

57. There are a number of pests and diseases of wheat (see OGTR 2008 for further details), which may require management (eg application of herbicide or pesticide) during the growing season. Weed control using specific classes of herbicides may involve a pre- or post-emergence application. However the applicant does not intend to apply glufosinate ammonium during the trial.

58. In Australia, spring wheat varieties are commonly grown as a winter crop and are usually planted May and June. Harvest of the mature wheat generally occurs from mid-November to late December. The applicant has indicated that all GM and non-GM plant materials from the field trial not required for further research will be destroyed by incorporation into the soil before the end of the first May following harvest to promote decomposition. The sites will also undergo three irrigations post harvest to promote seed bank depletion.

59. The applicant intends to trial the GM wheat alongside the non-GM parent and other varieties from major wheat growing regions in Australia. The additional varieties would include Wyalkatchem, an elite variety from WA, and Yitpi and Correll, elite varieties from SA. The applicant plans to irrigate part of the trial at the Horsham site, and the rest of the trials would be grown under rain-fed conditions.

6.4 Presence of related plants in the receiving environment

60. Bread wheat (*T. aestivum*) can cross pollinate with other *Triticum* species. Both bread wheat and, on a smaller commercial scale, durum wheat (*T. turgidum* ssp. *durum*), are grown in Australia. The applicant has indicated that there will be at least 500 m between the GM wheat and any planting of other wheat plants. Other *Triticum* species are not known to be present in Australia.

61. Intergeneric hybrids involving wheat and *Aegilops* spp., *Hordeum* spp., *Elytrigia* spp. *Secale* spp. and *Leymus* spp. are possible, but most hybrids between bread wheat and other genera were grown in embryo culture and were self sterile. Detailed discussion can be found the RARMP for DIR 071/2006 and *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008).

62. The applicant has indicated that no *Aegilops* species are present at either trial site, nor are there any naturalised *Aegilops* species recorded in Victoria. Additionally, if planted, rye (*Secale cereale*) would be at least 500 m (as indicated by the applicant) from the GM wheat. Barley is not known to hybridise with wheat under natural conditions (see OGTR 2008).

6.5 Presence of the introduced genes or similar genes and encoded proteins in the environment

63. All of the introduced genes are isolated from naturally occurring organisms that are already widespread and prevalent in the environment.

64. Four of the introduced genes for drought tolerance are derived from the plants *Z. mays*, a common crop plant, and *A. thaliana*, which is widely used in experimental studies. Two of the introduced genes for drought tolerance are derived from the yeast *S. cerevisiae*, which has been used since ancient times in baking and brewing. It is widespread in the environment and is one of the most intensively studied eukaryotic model organisms. The final nine genes for drought tolerance are derived from a moss, *P. patens*, which is widespread in the northern hemisphere (Tan 1978), has been isolated in NSW and VIC (AVH 2008) and is another common experimental organism.

65. The introduced genes for drought tolerance occur naturally and are all members of families that are common in eukaryotic organisms including plants and humans. Therefore, it is expected humans routinely encounter the introduced genes (or homologs) and their products.

66. The PAT protein is widespread in the environment, through the presence of the bacteria from which it is derived. PAT proteins are produced naturally by the common soil bacteria *Streptomyces viridochromogenes* and *S. hygrosopicus*, encoded by the *pat* and *bar* genes, respectively (Wohlleben et al. 1988; Strauch et al. 1988). These species of *Streptomyces* are saprophytic, soil-borne bacteria and are not considered pathogens of plants, humans or other animals (OECD 1999a). A search of the GenBank database reveals that other genes encoding PAT or similar enzymes are present in a wide variety of bacteria. Acetyltransferases, the class of enzymes to which PAT belongs, are common enzymes in all microorganisms, plants and animals. Different versions of PAT protein have also been expressed in other GM crop plants

trialled (DIRs 010/2001, 015/2002, 016/2002, 036/2003, 038/2003, 040/2003 and 044/2003) or commercially approved (canola DIR 021/2003 and cotton DIR 062/2005) in Australia.

67. The β -lactamase enzyme is also widespread in the environment and in food. The *bla* gene was originally isolated from antibiotic resistant strains of *E. coli* found in hospital patients. *E. coli* is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997).

Section 7 Australian and international approvals

7.1 Australian approvals of GM wheat lines

7.1.1 Previous releases approved by Genetic Manipulation Advisory Committee or the Regulator

68. The Regulator has previously issued a licence (DIR 071/2006) to DPI Victoria for the conduct of field trials (on 0.315 ha in Victoria) of GM drought tolerant wheat involving twelve of the GMOs in this current application.

69. Additionally, the Regulator has issued licences for the conduct of two field trials involving unrelated GM wheat lines under limited and controlled conditions: DIR 053/2004 was issued to Grain Biotech for GM salt tolerant wheat on an area of 0.45 ha in Western Australia and DIR 054/2004 was issued to CSIRO for GM wheat with altered starch content on 0.25 ha in the Australian Capital Territory.

70. Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been five field trials of different GM wheat ranging in size from 325–1500 plants: PR65 (1996) and PR107 (1999) were modified for herbicide tolerance, PR102 (1998) and PR102X (2000) were also herbicide tolerant, but with modified grain qualities, and PR66 (1996) had altered starch levels in the grain.

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

7.1.2 Approvals by other Australian government agencies

72. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the Australian Quarantine and Inspection Service (AQIS), APVMA and FSANZ. This is discussed further in Chapter 3.

73. FSANZ is responsible for human food safety assessment and food labelling, including GM food. The applicant does not intend to use materials from the GM wheat lines in human food, accordingly an application to FSANZ has not been submitted. FSANZ approval would need to be obtained before materials from these GM wheat lines could be used in food.

74. The APVMA, which has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia, has registered herbicides containing glufosinate ammonium as the active constituent. Although the GM wheat lines have been modified to be tolerant to glufosinate ammonium, the applicant does not intend to apply this herbicide during the trial. Therefore, no approval is required from APVMA.

7.2 International approvals

75. There have been no approvals for release of these GM wheat lines overseas. However, all of the genes for drought tolerance contained in the GM wheat lines proposed for release

have been authorised for field release in the U.S. or Canada in other crops (*Zea mays* or *B. napus*). Details of these trials have not been published.

76. No GM wheat lines have been approved for commercial release in any country. However, small scale field trials of different GM wheats have been approved in countries such as Canada, Egypt, Mexico, Morocco, South Africa, and the U.S. (USDA-APHIS 1994; Pellegrineschi et al. 2004; AGBIOS 2005; Canadian Food Inspection Agency 2006; Moola & Munnik 2007) and also in some member countries of the EU (Directorate General for the Environment & European Commission 2004a; 2004b; 2004c; 2004d; and 2006).

77. An application for non-regulated status for glyphosate tolerant GM wheat was received by the US regulatory agencies in 2002. The application to the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) was withdrawn by the applicant prior to a decision being made. However, the United States Food and Drug Administration (US FDA) approved the use of the GM wheat for food and feed (US FDA 2004).

Chapter 2 Risk assessment

Section 1 Introduction

78. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 2) considers risks from the proposed dealings with the GMOs that could result in harm to the health and safety of people or the environment posed by, or as a result of, gene technology. It takes into account information in the application, relevant previous approvals and current scientific knowledge.

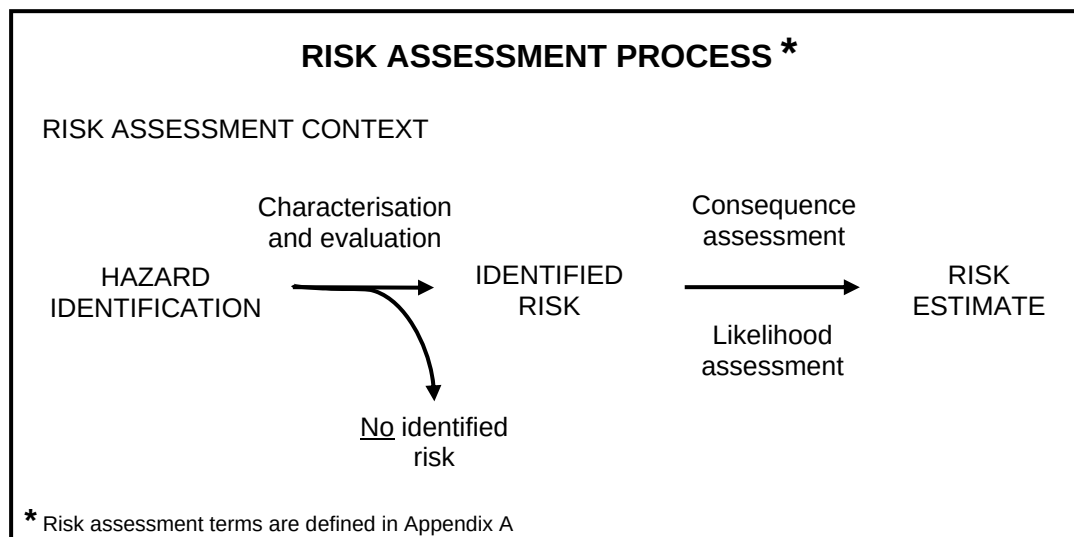


Figure 2. The risk assessment process.

79. Once the risk assessment context has been established (see Chapter 1) the next step is hazard identification to examine what harm could arise and how it could happen during a release of these GMOs into the environment.

80. It is important to note that the word 'hazard' is used in a technical rather than a colloquial sense in this document. The hazard is a source of *potential* harm. There is no implication that the hazard will *necessarily* lead to harm. A hazard can be an event, a substance or an organism (OGTR 2007).

81. Hazard identification involves consideration of events (including causal pathways) that may lead to harm. These events are particular sets of circumstances that might occur through interactions between the GMOs and the receiving environment as a result of the proposed dealings. They include the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

82. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2007). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

83. The hazard identification process results in the compilation of a list of events. Some of these events lead to more than one adverse outcome and each adverse outcome can result from more than one event.

Section 2 Hazard characterisation and the identification of risk

84. Each event compiled during hazard identification is characterised to determine which events represent a risk to the health and safety of people or the environment posed by, or as a result of, gene technology.

85. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2007). Harm is assessed in comparison to the parent organism and in the context of the proposed dealings and the receiving environment. Wherever possible, the risk assessment focuses on measurable criteria for determining harm.

86. The following factors are taken into account during the analysis of events that may give rise to harm:

- the proposed dealings, which may be for the purpose of experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- the proposed limits
- the proposed controls
- characteristics of the non-GM parent
- 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 biotic and abiotic environment at the site(s) of release
- agronomic management practices for the GMOs.

87. Twelve of the GMOs in this application have previously been approved for trial under limited and controlled conditions by licence DIR 071/2006.

88. The seven events that were characterised are discussed in detail later in this Section. They are summarised in Table 4 where events that share a number of common features are grouped together in broader hazard categories. None were considered to lead to an identified risk that required further assessment.

89. As discussed in Chapter 1, Sections 5.2.3 to 5.2.5, the GM wheat lines contain the herbicide tolerance selectable marker gene, *bar*, and the antibiotic resistance selectable marker gene, *bla*. The *bla* gene, encoding β -lactamase, is not expressed in the GM wheat lines as it is linked to a bacterial promoter that does not function in plants, and therefore it will not be assessed further.

90. The *bar* gene, and its product, PAT, has already been considered in detail in the RARMP prepared for DIR 062/2005 (Liberty Link[®] Cotton) and by other regulators and was found to pose no risks to either people or the environment. Therefore, it will only be assessed in brief.

Table 4. Summary of events that may give rise to an adverse outcome through the expression of the introduced genes for drought tolerance.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Ingestion of, contact with, or inhalation of GM plant material containing proteins encoded by the introduced genes or their end products.	Allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The encoded proteins are widespread in the environment and are unlikely to be toxic/allergenic to people or toxic to other organisms. The limited scale, short duration and other proposed limits and controls, further reduce exposure of people and other organisms to products of the introduced genes.
Section 2.2 Spread and persistence of the GM wheat lines in the environment	2. Expression of the introduced genes improving the survival of GM wheat plants.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Cultivated wheat is not considered to be weedy and the genetic modifications are not expected to change the weediness characteristic of the GMOs. Many factors other than water availability limit the spread and persistence of wheat in the areas proposed for release. Glufosinate ammonium is not used as the main method to control wheat in other crops. The limits and controls proposed for the release would minimise persistence.
	3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> As discussed in Event 1 the encoded proteins and their end products are already widespread in the environment. Wheat lacks seed dispersal characteristics, which are not expected to be changed in the GMOs. The GM wheat lines proposed for release are in white wheat parental backgrounds, which have a thin seed coat and are readily digested by birds and mammals. The proposed limits and controls would minimize dispersal.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced genes or regulatory sequences in other wheat plants or in other sexually compatible plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Wheat is predominately self-pollinating and outcrossing is limited. The applicant proposes an isolation zone around the release site in which no other <i>Triticaceae</i> plants are grown which would limit the potential for vertical gene flow.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The introduced genes or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms. Events 1–4 did not constitute identified risks for people or the environment associated with expression of the introduced genes.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.5 Unintended changes in biochemistry, physiology or ecology	6. Changes to biochemistry, physiology or ecology of the GM wheat lines resulting from expression, or random insertion, of the introduced genes.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Unintended, adverse effects, if any, would be minimised by the proposed limits and controls. Unexpected alterations are likely to be detected and eliminated during the selection process
Section 2.6 Unauthorised activities	7. Use of the GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	<ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

2.1 Production of a substance toxic/allergenic to people or toxic to other organisms

91. 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).

92. Allergenicity is the potential of a protein 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).

93. A range of organisms may be exposed directly or indirectly to the proteins encoded by the introduced genes for drought tolerance. Workers cultivating the wheat would be exposed to all plant parts. Organisms may be exposed directly to the proteins through biotic interactions with GM wheat plants (vertebrates, insects, symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include organisms that feed on organisms that feed on GM wheat plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

Event 1. Ingestion of, contact with, or inhalation of GM plant materials containing proteins encoded by the introduced genes, or their end products occurring as a result of the genetic modification.

94. Expression of the introduced genes for drought tolerance could potentially result in the production of novel toxic or allergenic compounds in the GM wheat lines, or alter the expression of endogenous wheat proteins. 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 humans or other organisms.

95. Non-GM wheat is not known to be toxic to humans or other organisms, although non-GM wheat flour can produce allergic responses in susceptible individuals on inhalation or ingestion. Ingestion of wheat flour by coeliac disease sufferers will trigger a sensitivity response. This response is caused by the prolamin fraction of the storage protein complex, gluten (reviewed in OGTR 2008). It is not known if any of the introduced genes for drought tolerance are involved in the expression of proteins in the gluten production pathway. However, expression of gluten protein genes is altered in response to various abiotic stresses including drought stress (Altenbach et al. 2002; Dupont & Altenbach 2003).

96. Although no toxicity studies on the GM wheat plant material or encoded proteins have been performed, all of the introduced genes are isolated from naturally occurring organisms that are already widespread and prevalent in the environment. With the exception of one of the genes from *S. cerevisiae*, all of the introduced genes are likely to be homologous to genes in all plants; including wheat (see Chapter 1, Section 6).

97. No information was found to suggest that the proteins encoded by the introduced genes are toxic or allergenic to people or to other organisms (Chapter 1, Sections 5.1 and 5.2.4) or could affect the production of endogenous wheat allergens and therefore exposure to the GM plant materials is not expected to adversely affect the health of humans or other organisms.

98. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. Public exposure to GM plant materials via ingestion, skin contact or inhalation would be minimal as no GM plant material will be used as human food or animal feed and public access to the trial site is restricted. Although preliminary glasshouse studies suggest that some plants may show a variation in flowering time compared to the non-GM parent plants, each GM wheat plant is expected to be in flower for the same length of time as the non-GM parent, and produce the same amount of pollen. Therefore the total potential exposure of workers to pollen would be the same as for non-GM wheat. Human exposure to the GM plant materials would be limited to trained and authorised staff associated with the field trial.

99. The proposed trial sites will be surrounded by 1.2 m high fences with access to the trial sites being via locked gates, which limits exposure of the public and larger animals to the GM plant material. Livestock would not be intentionally exposed as the GM plant material will not be used as feed. Exposure of rodents would be limited by the use of a 10 m monitoring zone of reduced plant cover around the trials and other rodent control measures.

100. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms as a result of consumption of, contact with, or inhalation of, GM plant materials containing proteins encoded by the introduced genes as a result of the genetic modification is **not an identified risk** and will not be assessed further.

2.2 Spread and persistence of the GM wheat lines in the environment

101. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM wheat plants in particular, is given in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008). In summary, wheat shares some characteristics with known weeds, such as wind-pollination (although it is predominantly self-pollinating) and the ability to germinate or to produce some seed in a range of environmental conditions. However, wheat lacks most characteristics that are common to many weeds, such as the ability to produce a persisting seed bank, rapid growth to flowering, continuous seed production as long as growing conditions permit, high seed output, high volume seed dispersal and long-distance seed dispersal (Keeler 1989). In addition, wheat has been bred to avoid seed shattering and white wheats are prone to pre-harvest sprouting and therefore have little seed dormancy (OGTR 2008).

102. Scenarios that could lead to increased spread and persistence of the GM wheat lines include expression of the introduced genes conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These events could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins.

Event 2. Expression of the introduced genes improving the survival of the GM wheat plants

103. If the GM wheat lines were to establish or persist in the environment they could increase the exposure of humans and other organisms to the GM plant material. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials, the encoded proteins or end products has been considered in Event 1 and was not considered an identified risk.

104. If the expression of the introduced genes for drought tolerance were to provide the GM wheat plants with a significant selective advantage over non-GM wheat plants and they were able to establish and persist in favourable non-agricultural environments, this may give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. Similarly, the GM wheat plants could adversely affect agricultural environments if they exhibited a greater ability to establish and persist than non-GM wheat.

105. The impact of the genetic modifications on survival of the GM wheat lines is uncharacterised under field conditions. However, the applicant states the introduced genes have demonstrated the capacity to produce a drought tolerant phenotype in the GM wheat lines grown in glasshouse experiments and in other plants. In an environment in which water availability was the main factor limiting the spread and persistence of wheat, expression of the genes for drought tolerance could result in increased weediness of the GM wheat lines relative to non-GM wheat.

106. Plants often respond to different stresses through an interconnecting series of signalling and transcription controls. Therefore, the regulatory nature of some of the introduced genes for drought tolerance may mean that the encoded proteins could also confer tolerances to other environmental stresses, such as extremes of temperature or soil salinity. Furthermore, the GM wheat lines may possess other characteristics under stress such as increased seed dormancy, viability, or improved seedling germination rates which may impact on their weediness potential. Expression of the introduced genes for drought tolerance could also impact on biotic stress tolerances and the way the GM wheat lines interact with plant pests and pathogens.

107. However, modern wheat cultivars are not recognised as a significant weed risk in Australia, and there have been no reports of bread wheat becoming an invasive pest in Australia or overseas. Additionally, the survival of the GM wheat plants would still be limited by lack of seed shattering, temperature, low intrinsic competitive ability, nutrient availability, pests and diseases and other domestication and environmental factors that normally limit the spread and persistence of wheat plants in Australia (Slee 2003; Condon 2004). Furthermore, in the unlikely instance that cross tolerance is conferred, the GM plants will most likely be less fit as compared to other commercially available wheat varieties because of the potential metabolic/physiological burdens (eg as discussed in Pretty 2001). For example, the wheat may have stunted growth, produce less seeds, and have a decreased ability to tolerate competition from other plants. Therefore, the expression of the introduced genes for drought tolerance is not expected to provide the GM wheat plants with a significant selective advantage over non-GM wheat plants.

108. The GM wheat lines also contain the introduced *bar* gene. The PAT protein, which is encoded by the *bar* gene, confers tolerance to the herbicide glufosinate ammonium. Expression of the *bar* gene could confer a selective advantage in areas where glufosinate ammonium is used to control weeds. Glufosinate ammonium is widely used internationally as a broad-spectrum herbicide and is registered for use in many countries. However, in Australia it is not as widely used as some other commonly used herbicides. Therefore, the genetic

modification is not expected to confer a selective advantage to the GM wheat lines. However, it should be noted that the growing of commercial InVigor[®] canola and Liberty Link[®] cotton may lead to an increase in the use of this herbicide in the future.

109. Altered flowering time and increased tillering has been seen in several of the GM lines proposed for release. As wheat is primarily self-pollinating, a delay in flowering time is unlikely to affect the fertilisation of the GMOs. However, increased tillering during early development could lead to an increase in the number of seed heads and seed yield per plant.

110. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM wheat lines proposed for release. The release would be of limited size and short duration and the applicant proposes a number of control measures, including destruction of all plant materials not required for further analysis, post harvest irrigation of the site and post harvest monitoring of the proposed site (and destroying any germinated seed) for at least two years and until no wheat plants have been found on the site for at least the last six months of the monitoring period.

111. Wheat stubble and other GM plant material remaining on the site after harvest is expected to be destroyed by microbial degradation after the site is cultivated, and any tiller regrowth that may occur will be killed with an appropriate herbicide.

112. The purpose of the proposed release is to conduct proof of concept experiments with the GM wheat lines to assess growth and yield characteristics. Thus, any characteristics that may impact on the survivability of the GM plants including tolerance to other abiotic stresses will be closely monitored during the proposed trial.

113. **Conclusion:** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced genes for increased drought tolerance improving the survival of the GM wheat lines is **not an identified risk** and will not be assessed further.

Event 3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions

114. If the GM wheat lines were to be dispersed from the release site they could increase the exposure of humans and other organisms to the GM plant material and/or establish and persist in the environment. The effects of contact, inhalation or ingestion of the GM wheat lines have been assessed in Event 1 and were not an identified risk. The introduced genes improving survival of the GM wheat lines in the environment was assessed in Event 2 and was also found not to be an identified risk. Therefore the dispersal of reproductive GM plant material is not expected to adversely affect the health of humans or other animals; or to increase the survival of the GM wheat lines compared to non-GM wheat.

115. Wheat lacks seed dispersal characteristics such as stickiness, burrs, and hooks, which contribute to seed dispersal via animal fur (Howe & Smallwood 1982), or the possession of small dormant seeds which is vital for seeds to survive chewing and digestion (Malo & Suárez 1995). The GM wheat lines proposed for release are in a white wheat parental background, which have large seeds with low dormancy and a thin seed coat (Hansen 1994), and are therefore likely to be easily broken down in the digestive system of mammals. Seed production, dispersal and digestibility characteristics are not expected to be altered in the GM wheat lines compared to non-GM parental wheat lines.

116. The proposed release sites will be surrounded by 1.2 m fences with access through a locked gate limiting the possibility of seed dispersal by any large animals or by unauthorised people. Dispersal by authorised people entering the proposed trial sites would be minimised

by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing.

117. Habitat modifications such as reduced plant cover have been reported to be a deterrent to the movement of mice (White et al. 1998; Central Science Laboratory 2001; AGRI-FACTS 2002; Brown et al. 2004) and therefore the 10 m monitoring zones of reduced plant cover around the trial sites are expected to discourage dispersal by mice. Rodent baits and/or traps will be placed at the sites, which will further limit seed dispersal by rodents.

118. The possibility of dispersal of GM plant materials by birds was considered in detail in the previous assessment for RARMP for DIR 071/2006 which is available from the OGTR or from the website and is discussed in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008). To briefly summarise, bird damage has been reported for wheat crops although birds appear to prefer softer plant parts, and are more likely to eat wheat or grain on site rather than carry it elsewhere for storage or consumption. However, there are no reports on the ability of birds such as cockatoos and galahs, which are known to consume loose seeds found on the soil surface following harvest, to disperse viable seeds. Reports of seed dispersal via birds are generally confined to fruit eating birds which consume succulent fruits and berries and then disseminate the undigested seeds at considerable distances (McAtee 1947; Barnea et al. 1991; VanDer Wall et al. 2005), rather than grain eating birds which intentionally consume seed grains including wheat and barley (Diaz 1990; Thompson et al. 1991). The thin seed coat of the wheat cultivar used in this trial will promote digestion of the seeds during passage through bird and animal intestines and therefore dispersal of viable GM wheat seed is likely to be low.

119. Extremes of weather may cause dispersal of plant parts. However, control measures have been proposed by the applicant to minimise dispersal. These include locating the proposed release site 50 m away from natural water ways in the event of flooding, and having an isolation zone in which there are no other wheat or related plants in the event of strong winds dispersing pollen or seeds.

120. All GM plant material will be transported in accordance with the OGTR transport guidelines which will minimise the opportunity to disperse the GM material.

121. Dispersal of any GM seed is not expected to result in the establishment of volunteer plants as they would need appropriate environmental conditions for germination, survival and persistence. The spread and persistence of the GM wheat lines outside of the trial site would be limited by multiple factors including temperature, low intrinsic competitive ability, nutrient availability, and pests and diseases (see Event 2).

122. **Conclusion:** The potential for allergenicity, toxicity or increased weediness due to the dispersal of reproductive (sexual or asexual) GM plant materials through various means including animals and extreme weather conditions is **not an identified risk** and will not be assessed further.

2.3 Vertical transfer of genes or genetic elements to sexually compatible plants

123. Vertical gene flow is the transfer of genetic information 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 (Waines & Hedge 2003). For GM crops, vertical gene flow could therefore occur via successful crosspollination between the crop and neighbouring crops, related weeds or native plants (Glover 2002).

124. Baseline information on vertical gene transfer associated with non-GM wheat plants can be found in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008). In summary, wheat plants are primarily self-pollinating and while natural hybrids with other species can occur at low frequencies, they are usually sterile.

Event 4. Expression of the introduced genes and regulatory sequences in other wheat plants or in other sexually compatible plants

125. Transfer and expression of the introduced genes for drought tolerance in other wheat or sexually compatible plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

126. However, as discussed in Event 2, the survival of the GM wheat plants proposed for release would be limited by factors such as lack of seed shattering, temperature, low intrinsic competitive ability, nutrient availability, pests and diseases and other domestication and environmental factors that normally limit the spread and persistence of wheat plants in Australia. Therefore, similar to the GM wheat plants, expression of the introduced genes in other wheat plants would also result in plants limited by these factors. The expression of the introduced genes in other sexually compatible species is also unlikely to give these plants a significant selective advantage. The conditions that limit the spread and persistence of any hybrids between non-GM wheat and other sexually compatible plants would be expected to limit the spread and persistence of any hybrids between the GM wheat and other sexually compatible species.

127. If the introduced genes were transferred to wheat plants in a non-GM wheat breeding trial, the resulting seeds could be propagated as part of the non-GM trial and incorporated into one or more lines being developed for commercial release. However, as a range of specific traits are selected for in breeding trials (such as improved yield, disease resistance, drought tolerance, salt tolerance, grain and flour qualities), selection and propagation of any hybrids produced as a result of gene flow would only occur if the hybrid plants displayed the relevant trait(s) being developed. It should be noted that the GM wheat lines in the proposed release were derived from the wheat cultivar Bobwhite which is considered to be of lower quality than most commercial cultivars (Bhalla et al. 2006) and consequently any hybrids are likely to be of lower quality and less likely to be selected for further propagation.

128. Additionally, new cultivars being developed for commercial release are evaluated for agronomic performance under a range of environmental conditions. As discussed in Event 2, the GM plants (and potentially any resulting hybrids) may have a selective advantage under drought and other abiotic stresses. Conversely, the GM plants and hybrids could also be less fit as compared to other commercially available wheat varieties because of the potential metabolic/physiological burdens resulting from the expression of the introduced genes. Any hybrids produced as a result of gene flow would have to be agronomically superior to be selected for further development. Therefore, it is unlikely that, in the event of gene flow to breeding trials, that the resulting hybrid wheat plants would be further developed.

129. As discussed in Event 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM wheat plants by the introduced genes or regulatory sequences. This will be the same if the introduced genes are expressed in other wheat plants. Similarly, if the introduced genes are expressed in other sexually compatible species, allergenicity and toxicity are not expected to be altered.

130. All of the introduced regulatory sequences are expected to operate in the same manner as regulatory elements endogenous to the wheat and barley plants. While the transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects, the

impacts from the introduced regulatory elements are likely to be equivalent and no greater than the endogenous regulatory elements.

131. Wheat is predominantly self-pollinating (cultivars vary from 94-99.7%, Hucl 1996) and its pollen is not generally dispersed by insects, and therefore wind pollination plays an important role in any outcrossing. Laboratory experiments have shown that pollen can travel a distance of about 60 m at a height of 1 m (D'Souza 1970). Under field conditions, wheat pollen has a viable lifespan of less than 30 minutes (OECD 1999b) and a majority of studies suggest that more than 90% of wheat pollen falls within 3 m of the source (reviewed by Hedge & Waines 2004). Field conditions including temperature, relative humidity and wind intensity also have a great influence on pollen viability and pollen movement.

132. Gene flow rates in wheat have been studied at both the experimental and commercial scale (reviewed in OGTR 2008) with gene flow in experimental scale fields detected over much shorter distances than in commercial fields (Matus-Cadiz et al. 2004; 2007; Gaines et al. 2007). The majority of gene flow in an experimental scale field occurs within three to ten metres of the pollen source, although low levels of gene flow may be detected as far as 100 m away. There has also been a single recorded instance of gene flow occurring at 300 m (Matus-Cadiz et al. 2004). Although there has been a small scale study of pollen-mediated gene flow in wheat under Australian conditions (Gatford et al. 2006), gene flow was only monitored within 12 m of the source. Higher levels of gene flow, at longer distances, have been detected for larger scale and commercial wheat plantings, although gene flow levels are highly variable. Therefore, the introduced genes and regulatory sequences could be transferred to sexually compatible plants within ten meters of the trial site. However, the likelihood of gene transfer declines rapidly as the distance from the pollen source increases.

133. As discussed in Chapter 1 Section 6.2, the applicant has indicated the presence of commercial and research crops of wheat at or near the proposed release site. The applicant has also stated that while wheat breeding trials may take place on the research stations, there will be no breeding trials of wheat within 500 m of the trial site.

134. As identified in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008), there are few species outside the *Triticum* genus (for example, *Aegilops cylindrica*, *A. ovata*, *A. biuncialis*, *Hordeum marinum* and *Secale cereale*) that are sexually compatible with wheat and known to form hybrids under natural conditions although these hybrids are usually sterile.

135. The related species that the applicant has identified at or near the proposed release site are *S. cereale* (rye) and *Hordeum marinum* (sea barley). The potential for gene transfer to rye and sea barley was considered in detail in the previous assessment for the RARMP for DIR 071/2006 which found that while hybridisation could occur, introgression of the introduced genes for drought tolerance into rye or sea barley was unlikely due to the genomic differences between the species.

136. Although the altered flowering time observed in some lines expressing the drought tolerance genes could produce an advantageous alteration in flowering synchronicity with sexually compatible plants, the likelihood of vertical gene transfer occurring will be reduced by the close monitoring of the area surrounding the proposed release for related species during the flowering of the GM wheat lines.

137. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for pollen flow and gene transfer to sexually compatible plants. In particular, the applicant proposes to isolate the trial site from other wheat plants, sexually compatible species and breeding trials, and the majority of the pollen is expected to fall within

the trial site or the 10 m cleared area directly surrounding the trial site. The applicant also proposes to perform post harvest monitoring of the site for twenty four months and until the site has been clear of volunteers for at least the last six months of the monitoring period and destroy any volunteer plants found in either the site or the cleared zone. This would ensure any remaining GM wheat seeds, or plants that were potentially the product of gene flow, in these areas would be destroyed.

138. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genes and regulatory sequences in other wheat plants or other sexually compatible plant species as a result of gene transfer is **not an identified risk** and will not be assessed further.

2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms

139. Horizontal gene transfer is the movement of genetic information (DNA) between sexually unrelated organisms (Thomson 2001). In the context of genetic modification, the major concern has been whether DNA introduced into crops could transfer into bacteria in the soil or into the cells of organisms that may eat the crops. Horizontal gene transfer has been considered in previous RARMPs (including in detail in DIR 057/2004), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. These assessments have concluded that horizontal gene transfer from plants to sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. There are no more recent reviews that alter this conclusion.

Event 5. Presence of the introduced genes, or the introduced regulatory sequences, in unrelated organisms as a result of gene transfer

140. The probability of transferring introduced genes contained in the GM wheat plants is no greater than that of transferring any of the native genes. Non-GM wheat is expected to contain homologues of all of the introduced genes, with the exception of the marker genes, *bar* and *bla* and one of the introduced genes from *S. cerevisiae*. The introduced *bar* and *bla* and *S. cerevisiae* genes are found in common microorganisms and the *bar* gene can also be found in commercially approved GM cotton and canola plants (Liberty Link[®] cotton and InVigor[®] canola). In addition, homologues of most of these genes occur in all plant species and thus are widespread in the environment. Therefore these genes and regulatory sequences are already available for transfer via demonstrated natural mechanisms (Chapter 1, Section 6.5).

141. Reports of horizontal gene transfer from plants to bacteria occurring during laboratory experiments have relied not only on the use of highly similar sequences to allow homologous recombination to occur, but also on conditions designed to enhance the selective advantage of gene transfer events (Mercer et al. 1999; Gebhard & Smalla 1998; Nielsen et al. 2000; Nielsen 1998; De Vries et al. 2001). This suggests that the likelihood of natural transfer is remote.

142. A key consideration in the risk assessment process should be the safety of the protein product(s) resulting from the expression of the introduced gene(s) rather than horizontal gene transfer *per se* (Thomson 2001). If the protein products are not associated with any risk then even in the unlikely event of horizontal transfer occurring, it should not pose any risk to humans, animals or the environment. Conclusions reached for Events 1–4 associated with the expression of the introduced genes or end products did not represent an identified risk.

143. **Conclusion:** The potential for an adverse outcome as a result of horizontal gene transfer is **not an identified risk** and will not be assessed further.

2.5 Unintended changes in biochemistry, physiology or ecology

144. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy¹¹ (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such pleiotropic effects may include:

- altered expression of an unrelated gene at the site of insertion
- altered expression of an unrelated gene distant to the site of insertion, for example, due to the encoded protein of the introduced gene changing chromatin structure, affecting methylation patterns, or regulating signal transduction and transcription
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions of the protein encoded by the introduced gene product with endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in the biochemical pathway incorporating the protein encoded by the introduced gene.

145. Such unintended pleiotropic effects might result in adverse outcomes such as toxicity or allergenicity; weediness, altered pest or disease burden; or reduced nutritional value as compared to the parent organism. However, accumulated experience with genetic modification of plants indicates that, as for conventional (non-GM) breeding programs, the process has little potential for unexpected outcomes that are not detected and eliminated during the early stage of selecting plants with new properties (Bradford et al. 2005).

Event 6. Changes to biochemistry, physiology or ecology of the GM wheat lines resulting from expression or random insertion of the introduced genes

146. Some altered physiology has been observed in the GM wheat lines under glasshouse conditions. These are outlined in Chapter 1, Section 5.5.2 and discussed in Event 2. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced genes, have already been discussed in Events 1 to 3, and were not considered identified risks.

147. Various biochemical pathways of the GM wheat plants could be altered by the predicted higher regulatory functions of proteins encoded by some of the introduced genes, resulting in the production of novel or higher levels of endogenous toxins, allergens or anti-nutritional compounds. Non-GM wheat, particularly the green leaf material, can be toxic to animals if consumed in large quantities (due to nitrate poisoning), and wheat flour can be allergenic to people with gluten intolerance. For further discussion regarding the toxicity and allergenicity of non-GM wheat see *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008).

148. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered capacity to deal with environmental stress, production of novel substances, and changes to levels of endogenous substances. However, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

¹¹ Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

149. The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the scale and duration of the trial would limit the potential for adverse effects. The proposed trial sites will be surrounded by 1.2 m high fences with access to the trial sites being via locked gates, which limits exposure of the public and larger animals to the GM plant material. Livestock would not be intentionally exposed as the GM plant material will not be used as feed. Exposure of rodents would be limited by the use of a 10 m monitoring zone of reduced plant cover around the trials and other rodent control measures.

150. **Conclusion:** The potential for an adverse outcome as a result of altered biochemistry, physiology or ecology is **not an identified risk** and will not be assessed further.

2.6 Unauthorised activities

Event 7. Use of GMOs outside the proposed licence conditions (non-compliance)

151. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM wheat lines outside of the proposed release areas. The adverse outcomes that this event could cause are discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

152. **Conclusion:** The potential for an adverse outcome as a result of unauthorised activities is **not an identified risk** and will not be assessed further.

Section 3 Risk estimate process and assessment of significant risk

153. The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

154. Seven events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

155. A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

156. The characterisation of the seven events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principle reasons for this include:

- limits on the size, locations and duration of the release proposed by DPI Victoria;
- suitability of controls proposed by DPI Victoria to restrict the dissemination or persistence of the GM wheat plants and their genetic material;
- limited capacity of the GM wheat lines to spread and persist outside the areas proposed for release;

- limited ability and opportunity for the GM wheat lines to transfer the introduced genes to commercial wheat crops or other sexually related species;
- none of the GM plant materials or products will be used in human food or animal feed;
- widespread presence of the same or similar proteins encoded by, and end products produced as a result of the activity of, the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM wheat lines 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 environment¹².

Section 4 Uncertainty

157. 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 (i.e. consequence and likelihood) are always uncertain to some degree.

158. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹³. For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily treating an identified risk.

159. For DIR 080/2007 which involves proof of concept research, uncertainty exists in relation to the characterisation of:

- Event 1, regarding potential increases in allergenicity or toxicity through contact with plant material containing proteins encoded by the introduced genes;
- Event 2, associated with a potential for increased survival of the GMOs;
- Event 3, regarding potential for birds to disperse mature seed following harvest; and
- Event 4, regarding the level of long distance gene flow under Australian conditions.

160. Additional data, including information to address these uncertainties, would be required to assess possible future applications for a larger scale trial, reduced containment conditions, or the commercial release of any of these GM wheat lines that may be selected for further development.

161. Chapter 3, Section 5 discusses information that may be required for future releases.

¹² As none of the proposed dealings were considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the *Gene Technology Act 2000* mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator allowed 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

¹³ A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

Chapter 3 Risk management

162. Risk management includes evaluation of risks identified in Chapter 2 to determine whether or not specific treatments are required to mitigate harm to human health and safety, or the environment, that may arise from the proposed release. Other risk management considerations required under the Act are also addressed in this chapter. Together, these risk management measures are used to inform the decision-making process and determine licence conditions that may be imposed by the Regulator under the Act. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

163. 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. All licences are required to be subject to three conditions prescribed in the Act.

164. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. Other mandatory statutory conditions contemplate the Regulator maintaining oversight of licensed dealings. For example, section 64 requires the licence holder to provide access to premises to OGTR monitors, 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.

165. It is a further requirement that the licence be 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 and the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Responsibilities of other Australian regulators

166. Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Health and Medical Research Council (NHMRC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹⁴.

167. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. The *Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

¹⁴ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

168. FSANZ is responsible for human food safety assessment, including GM food. As the trial involves proof of concept research, the applicant does not intend any material from these GM wheat lines to be used in human food. Accordingly the applicant has not applied to FSANZ for evaluation of any of the GM wheat lines for use in human food. FSANZ approval would need to be obtained before they could be used in food.

169. The APVMA, which has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia, has registered herbicides containing glufosinate ammonium as the active constituent. Although the GM wheat lines have been modified to be tolerant to glufosinate ammonium, the applicant does not intend to apply this herbicide during the trial. Therefore, no approval is required from APVMA.

170. No other approvals are required.

Section 3 Risk treatment measures for identified risks

171. The risk assessment of events listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed trial of GM wheat. The *Risk Analysis Framework* (OGTR 2007), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

172. These events were considered in the context of the scale of the proposed release (a maximum total area of 0.4 hectares per season over two growing seasons (July 2008 – March 2010) on two sites in the local government areas of Horsham and Mildura, Victoria, the containment measures (Chapter 1, Section 3), and the receiving environment (Chapter 1, Section 6).

Section 4 General risk management

173. Licence conditions have been proposed to control the dissemination and persistence of the GMOs and their genetic material in the environment and limit the release to the size, location and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are summarised in Sections 4.1.2 and 4.1.3.

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by DPI Victoria

174. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls proposed by DPI Victoria in their application, and discussed in the events characterised for the release in Chapter 2. The appropriateness of these limits and controls are considered further below.

175. The proposed release would be confined to two sites, which occur within Victorian Government Agricultural Research Stations currently encompassing the trial sites for DIR 071/2006. These stations are staffed by personnel who receive appropriate training in practices relevant to the handling and disposal of GMOs. Additionally, the applicant does not intend to use any of the GM plant material as human food or animal feed. Furthermore, the duration of the proposed release will be limited to two growing seasons. These measures will limit the potential exposure of humans and vertebrates to the GMOs (Event 1) and the potential for the GM wheat lines to persist or to establish outside the proposed release site (Event 3).

176. The trial sites will be located more than 50 m from the nearest waterways which will minimise the chance of plant material being washed away from the sites (Event 3).

177. The applicant's proposal to limit gene flow from the GM wheat (Event 4) include surrounding the proposed release site with a 10 m monitoring zone cleared of vegetation and a 490 m isolation zone in which no wheat or related species are grown.

178. Differences in pollen flow have been observed between field and commercial trial size wheat releases. A number of variables, particularly pollen source size, climatic conditions and the difficulty of detecting rare events, could influence the accuracy and reproducibility of these measurements. For small scale trials, low rates of outcrossing occur up to 100 m with rare occurrences up to 300 m, while at the commercial scale gene flow was found up to 2720 m from the pollen source.

179. Field trial releases of GM wheat in Canada require a 30 m isolation distance between the GM plants and other wheat plants, while in the United States the isolation distance is reduced to 20 feet (approximately 6.1 m) (USDA-APHIS 1994; Canadian Food Inspection Agency 2006). In both Germany and Spain trials with GM wheat plants are required to be surrounded by a 5 m border of either non-GM wheat or a non related species, while separation distances vary from 10–50 m (Directorate General for the Environment & European Commission 2004a; 2004c; and 2006).

180. Both basic and certified wheat seed in Australia is separated from other cereals by at least a two metre strip or a physical barrier such as a fence to prevent any mixture of seed during harvest (Smith & Baxter 2002). The acceptable level of off-types or other cultivars of the same species are 0.1% for basic seed and 0.3% for certified seed. Basic seed allows no contamination from other cereal species while in certified seed other cereal seeds may be present at a level of one seed in every two thousand (Smith & Baxter 2002). The OECD rules relating to the production of basic and certified seed from self-pollinated cereal state the same requirements (OECD 2008). Similarly, the United States *Federal Seed Act Regulations* does not specify an isolation distance for wheat used for seed production. However, for hybrid seed production (where the phenotype may be variable and determination of contamination levels is difficult) a distance of 300 feet (approximately 100 m) is required for the US and 25–100 m for the OECD (Code of Federal Regulations 2006; OECD 2008).

181. On the basis of the scientific literature on gene flow, international containment measures for GM wheat trials, and the rules for producing basic and certified seed, a 200 m isolation zone clear of sexually compatible species is considered adequate to minimise gene flow from the GM wheat plants to other wheat plants or other sexually related species (Event 4) and is therefore proposed as a licence condition.

182. The applicant proposes to surround the trial with a 1.2 m high fence and conduct rodent baiting and trapping within the fenced area during the trial. However, since viable seed may remain on the soil surface after harvest, a licence condition has been imposed requiring rodent reduction measures to continue after harvest and until all remaining seeds have been incorporated into the soil through post harvest tillage. These measures will aid in excluding grazing livestock and in reducing the size of the rodent population which may have access to the GM wheat lines. Additionally the proposed 10 m monitoring zone cleared of vegetation will serve as a measure to control rodent damage/feeding at the proposed release site (Event 3). Whilst there are differing reports regarding the average territory size of mice, the use of reduced vegetation has been shown to help reduce rodent numbers in agricultural settings. This will limit the potential exposure of vertebrates to the GMOs (Event 1) and the potential dispersal of the GMOs (Event 3).

183. The applicant has proposed to harvest the GM wheat plants by hand or with a plot harvester. The use of a plot harvester may increase the amount of residual seed and intact seed heads remaining on site after harvest.

184. The applicant has proposed a number of conditions to minimise the persistence of any GM wheat plants and seeds in the seed bank at the proposed release site after harvest of the proposed trial (Event 2). These conditions include facilitating post harvest ripening of seed by retaining any seed remaining after harvest on the soil surface prior to irrigation (Anderson & Soper 2003), and two irrigations, the second combined with shallow cultivation, to promote germination of the majority of viable seed. The applicant has also proposed to monitor the proposed release site for 24 months after harvest and until the site has been clear of any volunteers for at least the last six months of the monitoring period. All volunteers will be destroyed by hand pulling or by herbicide application.

185. Viable wheat seeds have been detected in the soil over longer periods under dry conditions than under moist conditions and wheat seeds present as un-threshed ears have longer dormancy than that of loose seeds (Komatsuzaki & Endo 1996). The minimum level of moisture necessary for germination of wheat seeds is 35 to 45% of the kernel dry weight (OGTR 2008). In field studies of wheat, volunteer seedlings were still emerging 16 months after harvest and seedlings were observed 2 years after harvest (Anderson & Soper 2003; Harker et al. 2005). Shallow tillage after harvest, combined with irrigation, will germinate much of the small grain seed lying on the surface (Ogg & Parker 2000). However, deep cultivation in certain soil types can prevent emergence by encouraging prolonged dormancy in seeds as a result of low oxygen availability but can also reduce the viability of shed seeds (Pickett 1989; Ogg & Parker 2000). Exposure to periods of rain interspersed with dry conditions may encourage germination in grains on the soil surface.

186. It is considered that three irrigations, combined with an appropriate tillage regime, and monitoring for and destruction of volunteers for at least 24 months would effectively reduce survival and persistence of viable wheat seeds in the soil. The initial irrigation should take place within 60 days of harvest and will encourage surface seed to germinate. The second irrigation, at least 28 days later and before the end of the first May following harvest, will further assist volunteer seed germination. The third irrigation to occur 10 to 18 months after the proposed trial is harvested is designed to encourage germination of any remaining viable seeds. Tillage to the original sowing depth at the time of the second and third irrigations will further promote germination by ensuring any remaining seeds are exposed to sufficient moisture and placed at an appropriate depth. This will also encourage the microbial decomposition of any residual seed. These measures will minimise the persistence of the GMOs in the environment (Event 2).

187. As viable wheat seeds have been observed to persist in soil for periods greater than one growing season, post harvest monitoring of the proposed release site for at least 24 months after harvest with no volunteers observed in the most recent six months, needs to be completed before an application that inspection conditions no longer apply can be made to the Regulator. These measures will minimise the persistence of the GMOs in the environment (Event 2).

188. The applicant has stated that any plant material taken off-site for experimental analysis will be transported according to the OGTR *Guidelines for the transport of GMOs*, <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/transport-guide-1>> and will be destroyed by autoclaving immediately after analysis. These are standard protocols for the handling of GMOs to minimize exposure of the GMO to human and other organisms (Event 1), dispersal into the environment (Event 3), and gene flow/transfer (Events 4 and 5).

4.1.2 Summary of measures imposed by the Regulator to limit and control the proposed release

189. A number of licence conditions have been imposed by the Acting Regulator to limit and control the release, including requirements to:

- conduct the release on a total area of up to 0.4 hectares per year at two sites in the local government areas of Horsham and Mildura, Victoria, between July 2008 and March 2010
- establish a 10 m monitoring zone around each trial site that is free of any related species and is maintained in a manner that does not attract or harbour rodents
- maintain an isolation zone of at least 200 m around each trial site free of any sexually compatible species
- enclose each trial site with a 1.2 m high fence with lockable gates
- conduct rodent baiting and/or trapping in and around each trial site
- locate the trial sites at least 50 m away from natural waterways
- harvest the GM wheat plant material by hand, or by machine, and separately from other crops
- not permit any materials from the release to be used in human food or animal feed
- destroy all plant materials not required for further analysis
- following harvest, clean the sites, monitoring zones and equipment used on the sites
- after harvest, apply measures to promote germination of any wheat seeds that may be present in the soil
- monitor the site for at least 24 months and destroy any wheat plants that may grow until no volunteers are detected for a continuous 6 month period.

4.1.3 Measures to control other activities associated with the trial

190. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, Policy on transport and supply of GMOs*). Licence conditions based on these guidelines and policies have been proposed regarding transportation and storage, and to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

191. Conditions applying to the conduct of experimental analyses are also included in the licence conditions.

4.2 Other risk management considerations

192. All DIR licences issued by the Regulator contain a number of general conditions that relate to general risk management. These include, for example:

- applicant suitability
- contingency and compliance plans
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment
- a requirement that the applicant allows access to the trial sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

193. 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 applicant's history of compliance with previous approved dealings
- the capacity of the applicant to meet the conditions of the licence.

194. On the basis of information submitted by the applicant and records held by the OGTR, the Acting Regulator considers DPI Victoria suitable to hold a licence.

195. The licence conditions include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.

196. DPI Victoria must continue to have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Compliance and contingency plans

197. Prior to planting the GM wheat lines, DPI Victoria is required to submit a plan detailing how it intended to ensure compliance with the licence conditions and document that compliance. This plan would be required before the planting of the GM wheat lines could occur.

198. DPI Victoria is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan would detail measures to be undertaken in the event of any unintended presence of the GM wheat lines outside of the permitted areas.

199. DPI Victoria is also 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 detection method is required within 30 days of the issue date of the licence.

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

200. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence.

4.2.4 Reporting structures

201. 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 trial
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

202. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

203. A number of written notices are required under the licence that will assist the OGTR in designing and implementing a monitoring program for all licensed dealings. The notices include:

- expected and actual dates of planting
- expected and actual dates of commencement of flowering
- expected and actual dates of harvest and cleaning after harvest.

4.2.5 Monitoring for Compliance

204. 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. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

205. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

206. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. These include the provision 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 5 Issues to be addressed for future releases

207. Additional information has been identified that may be required to assess an application for a large scale or commercial release of any of these GM wheat lines that may be selected for further development, or to justify a reduction in containment conditions. This includes:

- characterisation of the introduced genetic material in the plants, including copy number and genotypic stability;
- additional data on the potential toxicity of plant materials from the GM wheat lines;
- additional data on the allergenicity of proteins encoded by the introduced genes;
- data on the dispersal of viable wheat seeds by Australian birds;
- data on the level of long distance gene flow under Australian conditions; and
- characteristics indicative of weediness including measurement of altered reproductive capacity; tolerance to drought and other environmental stresses; and disease susceptibility.

Section 6 Conclusions of the RARMP

208. The risk assessment concludes that this limited and controlled release of up to 50 GM wheat lines on a maximum total area of 0.4 hectares per season over two growing seasons in the Victorian local government areas of Horsham and Mildura poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

209. The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to

limit the proposed release to the size, locations and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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Appendix A Definitions of terms in the *Risk Analysis Framework* used by the Regulator

(* terms defined as in Australia New Zealand Risk Management Standard AS/NZS 4360:2004)

Consequence

outcome or impact of an adverse event

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

Event*

occurrence of a particular set of circumstances

Hazard*

source of potential harm

Hazard identification

the process of analysing hazards and the events that may give rise to harm

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

to check, audit, review and evaluate the progress of an activity, process or system on an ongoing basis to identify change from the performance level required or expected and opportunities for improvement

Risk

the chance of something happening that will have an undesired impact

Negligible: risk is insubstantial and there is no present need to invoke actions for mitigation

Low: risk is minimal but may invoke actions for mitigation beyond normal practices

Moderate: risk is of marked concern requiring mitigation actions demonstrated to be effective

High: risk is unacceptable unless actions for mitigation are highly feasible and effective

Risk analysis

the overall process of risk assessment, risk management and risk communication

Risk analysis framework

systematic application of legislation, policies, procedures and practices to analyse risks

Risk assessment

the overall process of hazard identification and risk estimation

Risk communication

the culture, processes and structures to communicate and consult with stakeholders about risks

Risk Context

parameters within which risk must be managed, including the scope and boundaries for the risk assessment and risk management process

Risk estimate

a measure of risk in terms of a combination of consequence and likelihood assessments

Risk evaluation

the process of determining risks that require treatment

Risk management

the overall process of risk evaluation, risk treatment and decision making to manage potential adverse impacts

Risk management plan

integrates risk evaluation and risk treatment with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

those people and organisations who may affect, be affected by, or perceive themselves to be affected by a decision, activity or risk

States

includes all State governments, the Australian Capital Territory and the Northern Territory governments

Uncertainty

imperfect ability to assign a character state to a thing or process; a form or source of doubt

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities¹⁵ on the consultation RARMP for DIR 080/2007

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

Summary or issues raised	Comment
Suggested that the isolation zone be increased from 200 m to 1,000 m due to concern over potential pollen flow to non-GM plants.	The scientific literature referenced in the submission was considered in the preparation of the RARMP (Events 4 & 5). The control measures imposed are considered to be adequate to restrict gene flow to non-GM plants in relation to this application.
Notes that if any future applications were made that involve produce being used in food or feed the OGTR would need to examine the potential for altered allergenicity or toxicity, or for altered metabolism of pesticides applied to the GM plants. If altered metabolism resulted in changes in the nature/quality of chemical residues a revised Acceptable Daily Intake may have to be established, and the definition of the herbicide residue in and on products may have to be reset, based on relevant toxicology and metabolism data.	Noted. Toxicity and allergenicity of the GMOs has been considered in Event 1 and in addition, has been identified as a future research requirement. APVMA is responsible for pesticide use and chemical residues.
Recommend changes in paragraphs 95 and 134 [of the expurgated RARMP] to reflect the lack of known plant homologues for one of the introduced genes for drought tolerance from <i>S. cerevisiae</i> .	Noted. The relevant paragraphs have been amended.
Declaring CCI may support public concerns that GM work is high risk, harmful to the people and the environment. Greater transparency of the review process could assist to reduce concerns. The commercial development of the GMOs is well advanced and one wonders whether there is a need to declare CCI.	The Regulator was satisfied that the information specified in the application for DIR 080/2007 qualified as confidential commercial information (CCI) under section 185 of the Act. The CCI declaration is limited to: <ul style="list-style-type: none"> the identity, classes and specific functions of the introduced genes; the names and origins of the promoters; and data from previous international field releases of other plants expressing the same genes. A comprehensive literature search found no previous disclosure of the information declared to be CCI in this application.

¹⁵ GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment, Heritage & the Arts and the Local council(s) where the release may occur.

Summary or issues raised	Comment
Notes that the GM wheat plants and their products will not be used for human food or animal feed. However, if DPI Victoria envisages a future application for permission for the use of food derived from these GM wheat lines, they should contact the relevant regulatory agency to discuss specific data requirements to support a safety assessment.	Noted. FSANZ is responsible for human food safety assessment, including GM food.
Consideration should be given to whether the use of a mechanical harvester is likely to lead to increased spread and persistence of the GMO compared with hand harvesting.	The risk of harm occurring as a result of increased spread and persistence of the GM wheat lines was discussed in Event 2, which was not considered to be an identified risk. The imposed post harvest monitoring conditions, and in particular seed germination measures, are considered to be adequate to restrict the spread and persistence of the GM wheat lines in the environment.
Consideration should be given to risks that might be posed by the dispersal of seed by wildlife or strong winds.	Dispersal of GM wheat has been considered in detail in previous RARMPs. A detailed consideration was undertaken for DIR 071/2006 (Event 8), and risks were considered negligible. As discussed in Event 2, the spread and persistence of the GMOs outside of the trial site would be limited by multiple factors including temperature, low intrinsic competitive ability, nutrient availability, and pests and diseases. The control measures imposed are considered to be adequate to restrict the dispersal of the GM wheat lines in the environment.
Consideration should be given to risks that might be posed by gene flow from the GMOs to non-GM wheat breeding material.	The risk of harm occurring as a result of gene flow from the GM wheat lines to other sexually compatible plants, including those in non-GM wheat breeding trials was discussed in Event 4, which was not considered to be an identified risk. The control measures imposed are considered to be adequate to restrict gene flow to non-GM plants in relation to this application.

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 080/2007

The Regulator received four submissions from the public on the consultation RARMP. These submissions, summarised in the table below, raised issues relating to human health and safety and the environment. These 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.

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

Issues raised: **A:** administration; **B:** benefits of gene technology; **C:** Containment; **CCI:** Commercial Confidential Information; **DR:** data requirements; **EA:** expert advice; **EN:** Environmental risks; **GT:** gene transfer; **H:** Human health and safety; **HGT:** Horizontal Gene Transfer; **HR:** herbicide resistance; **M:** Marketing; **OSA:** outside scope of the assessment; **RA:** Risk Assessment process; **Res:** further research; **RM:** risk management; **S:** Segregation; **UE:** Unintended effects

Other abbreviations: **the Act:** the *Gene Technology Act 2000*; **Ch:** Chapter; **FSANZ:** Food Standards Australia New Zealand; **GM:** Genetically Modified; **GMO:** Genetically Modified Organism; **GTR:** the Gene Technology Regulator; **OGTR:** Office of the Gene Technology Regulator; **RARMP:** Risk Assessment and Risk Management Plan

Type: **A:** Agricultural/industry organisation; **IG:** interest group; **I:** individual

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	x	none	Objects to the release of GM wheat into the Australian environment	Noted.
			EN	The risks posed by the release are unknown due to lack of experience with the GM wheat lines. Post release identification of risks may occur too late for recall.	Twelve of the GMOs in this application have previously been approved for trial under limited and controlled conditions by licence DIR 071/2006 and no adverse effects have been reported. Risks to human health and safety and to the environment were assessed as negligible. The Regulator has imposed a range of measures to restrict the dissemination and persistence of the GMOs and their genetic material in the environment and to limit the trial to the proposed size, locations and duration.
			H	GM wheat would be impossible to avoid for those not wishing to consume it. This would mean that there would be no control population of non consumers for epidemiological studies in the future.	It is a condition of the licence that plant material from the GM wheat not be used as human food or animal feed. FSANZ approval would need to be obtained before GM wheat could be consumed by humans.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
2	I	x	Administrative processes		
			A	Concerned about administrative processes in the OGTR and the public service in general.	Noted.
			CCI	Feels that secrecy about introduced genes stifles debate and "Commercial in Confidence" claims must be justified in areas of public debate.	The GTR considers each application for CCI in accordance with the Act. CCI in relation to this application was granted by the Regulator on 31 January 2008. A comprehensive literature search found no previous disclosure of the information declared to be CCI in this application.
			EA	Disclosure of interests of experts and advisors should be made public.	All committee members are subject to strict disclosure of interest provisions which are contained in the Gene Technology Regulations 2001. OGTR staff are subject to the same disclosure of interest provisions as all Australian public service employees.
				The GTR is being tested by applicants and assistance from the OGTR and other experts is inadequate. GTR must seek advice from unbiased, competent experts and ensure the OGTR is staffed by competent persons who keep up to date with the literature and submissions.	Members of GTTAC are experts that are appointed on the basis of their skills and experience in a range of subject areas relevant to gene technology. Scientific staff within the OGTR have postgraduate qualifications and research experience in a number of subject areas relevant to gene technology. The Office has ongoing programs for professional development including ensuring that staff have access to the latest scientific information in relevant areas of gene technology.
			Horizontal Gene Transfer (HGT)		
			HGT	Concerned about the conclusion of the risk assessment of HGT in the current DIR 077/2007 and 080/2007 RARMPs (and their reference to DIR 057/2004). Considers that the OGTR and its experts, advisors and committees have not dealt appropriately with HGT and the risk assessment is biased against HGT. Notes that a current literature review on HGT has not been prepared by either the OGTR or the applicant.	The risk harm occurring as a result of HGT from the GMOs to microorganisms in the soil has been considered in the context of this limited and controlled release (Event 5) A scientist on the OGTR staff has recently had a review of the risks that might arise as a result of HGT from GMOs accepted for publication in an international, peer reviewed journal. This paper is a comprehensive review of the current scientific, peer reviewed literature on HGT and cites over a hundred independent papers, including a number of papers published in 2008.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			GT H UE	<p>Reasons provided for concern about HGT include;</p> <p>Genes introduced by genetic modification are not as stable as natural genes in genomes and are therefore more likely to be transferred.</p> <p>A multitude of microenvironments would exist at the trial site including conditions that may be suitable for transformation.</p> <p>Sufficient sequence homology may exist for homologous recombination to occur between the DNA introduced into the GMOs and microorganisms and for the sequences to be expressed.</p> <p>Calculates that more than 10⁹ new drought tolerant species of microorganisms could be produced as a result of the proposed release for DIR 080/2007.</p> <p>Natural environmental conditions could provide selective pressure for microorganisms to retain abiotic stress tolerance genes as a result of HGT.</p> <p>HGT of introduced abiotic tolerance genes from GM plants to microorganisms could lead to microorganisms more tolerant to abiotic stresses and could result in new and tougher species of pathogens with greater virulence, the consequences of which may not be immediately obvious but could have a significant impact on human health and safety.</p>	<p>The risk arising from such transfer from GMOs to microorganisms was assessed using the available evidence and was considered to be negligible (Event 5). Reasons include:</p> <ul style="list-style-type: none"> • There is no evidence that the introduced genes are unstable or more likely to transfer to other organisms. However, further evidence would be required if a larger scale release application was made. • Transfer of plant DNA to microorganisms is extremely rare; although examples have been identified the genome sequencing of bacteria shows a lack of plant genes. • There are a large number of events that must successfully occur before an adverse outcome could arise as a result of HGT, should it occur. Many of these events were considered unlikely. • The introduced genes are from common plants and organisms. Therefore, these genes have been in the environment and available for transfer for a significant amount of time. <p>Even so microorganisms have preferentially evolved other mechanisms to deal with drought and other adverse conditions. For example fungi develop spores which allow them to survive dry conditions.</p> <p>The small size and short duration of the proposed trial greatly reduce the chance of any adverse effect occurring as a result of HGT, should it occur.</p>
			Dispersal of GM Plant Material		
			C	<p>Bird dispersal of GM seed was not considered in the RARMP. Use of bird scarers or nets should be considered.</p>	<p>Dispersal of GM plant material has been considered in detail in previous RARMPs and reference documents prepared by the OGTR. A detailed consideration was undertaken for DIR 071/2006 (Event 8), and risks were considered negligible.</p> <p>A recent literature search was unable to find reports of wheat or other grain seeds being dispersed by birds in Australia.</p> <p>Bird damage has been reported for wheat crops. However, birds appear to prefer softer plant parts and are more likely to eat the GM wheat or grain on site rather than carry it elsewhere for storage or consumption.</p> <p>Dispersal of any GM seed is not expected</p>

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
					to result in the establishment of volunteer plants as they would need appropriate environmental conditions for germination, survival and persistence. The spread and persistence of the GMOs outside of the trial site would be limited by multiple factors including temperature, low intrinsic competitive ability, nutrient availability, and pests and diseases (Event 2).
			Data Requirements		
			DR	A number of lines are proposed for release but exact information (including interpretable Southern and Northern blots) on gene copy number, insertion sites, stability; and about phenotypic features, which should have been identified in laboratory and greenhouse trials, are not provided for each of them.	Some information on gene copy number was provided as was information on relevant phenotypic features. This is a 'proof of concept' trial and issues to be addressed by the applicant for future releases have been identified in the RARMP, including further characterisation of the introduced genetic material in the plants.
				Suggested experimental tests that the OGTR should have required the applicant to undertake to test for HGT to soil microorganisms. Further data, such as transcription/expression in <i>Escherichia coli</i> (and possibly yeast) and colony lifts of microorganisms in soil cultured with the construct DNA, should be required by the OGTR.	Risks to the health and safety of people or the environment that might arise as a result of HGT from the GMOs to microorganisms in the soil has been considered in the context of this limited and controlled release (Event 5). Information suggested was not required for this release.
			Other concerns		
			B	Considers that greater corporate control, larger profits and royalties provide some financial benefit to others but no real benefit to the public or consumers. On the other hand there is a significant risk of establishing new and unpredictable pathogenic microorganisms.	Risks to the health and safety of people or the environment that might arise as a result of HGT from the GMOs to microorganisms in the soil has been considered in the context of this limited and controlled release (Event 5). The consideration of financial benefits of gene technology are outside the scope of issues the GTR must have regard to when deciding whether or not to issue a licence.
			HR	Notes that the construct contains a herbicide resistance gene. Any transfer of the drought tolerance genes to weeds will also carry a limitation on possible control of those weeds (herbicide resistance) even if not a commonly used herbicide at the present time.	Expression of the introduced herbicide tolerance gene is not expected to alter susceptibility to major biotic and abiotic factors that limit the spread and persistence of all wheat in Australia. Licence conditions have been imposed to minimise gene flow to related species.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
3	I	n	none	Commend the efforts taken by the applicant and the Gene Technology Regulator in investigating and minimising the hazards and subsequent risks that might accrue due to the planting of the drought-tolerant genetically modified wheat, and we would welcome their preparedness to incorporate our additional recommendation when undertaking the trials.	Noted
			HGT	Concerned about HGT of antibiotic genes to soil microorganisms. Recommends that the applicants carry out studies on soil samples taken from the fields to re-assure the community that these transformative events do not pose an undue (short and long term) risk.	The risk of harm occurring as a result of HGT from the GMOs to microorganisms in the soil has been considered in the context of this limited and controlled release (Event 5) and was considered negligible.
			C	Supports the Regulator's suggestions to erect a 1.2 m high fence, 10 m exclusion zone, 200 m isolation zone and rodents control measures. Concerned about birds, insects and water dispersing the GMOs. Suggests trial site should be located much more than 50 m from waterway or surrounded by channels to divert excess water. Any physical confinement strategies should not only be put in place, they should also be monitored frequently and evaluated with regards to their stability.	Dispersal of GM plant material has been considered in Event 3 of this RARMP and in detail in previous RARMPs (eg DIR 071/2006; Event 8) and reference documents prepared by the OGTR and risks were considered negligible. Neither of the trial sites have any history of flooding. Licence conditions have been imposed requiring regular inspection of all fences and rodent control measures.
			GT	Notes that <i>Triticum aestivum</i> L. em Thell has a low rate of out-crossing and that if out-crossing occurs, it is facilitated by wind dispersal, and in turn is dependent on prevailing weather patterns and conditions. Wind-borne cross fertilisation of wheat is higher in warm, dry weather, which has to be assumed, given the environmental conditions prevalent in the location the wheat is going to be trialled. Suggests the 10 m monitoring zone be monitored during the trial and the 200 m isolation zone be increased.	The scientific literature referenced in the submission was considered in the preparation of the RARMP (Events 4 & 5). The control measures imposed are considered to be adequate to restrict gene flow to non-GM plants in relation to this application.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			EN	Concerned about the persistence of GMO plant material (including pollen) in the environment after harvest.	The persistence of the GMOs in the environment was assessed in Event 2 and the risks were considered negligible. Plant material from the GM wheat lines are not expected to be any more toxic or allergenic than non-GM wheat. The Regulator has imposed a range of measures to restrict the persistence of the GMOs and their genetic material in the environment.
4	AG	x	M S	Concerned about future commercial release of GM wheat. States that the Australian animal industries have a potential market advantage over international competitors through the ability to avoid GM feed stocks. Feels that, while beyond the scope of the current RARMP, more research should be conducted into improving on-farm safety and tracking systems for segregation of GM and non-GM feed stocks.	Noted. Agricultural production, segregation and marketing concerns are outside the scope of assessments required by the act.