



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 071/2006**

**Limited and controlled release of GM
drought tolerant wheat**

Applicant: Victorian Department of Primary Industries

June 2007

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the intentional release of genetically modified (GM) drought tolerant wheat lines into the environment, in respect of application DIR 071/2006 from the Victorian Department of Primary Industries (DPI Victoria).

The DIR 071/2006 licence permits the release of up to 30 GM wheat lines on a limited scale and under controlled conditions.

The *Gene Technology Act 2000* (the Act), the *Gene Technology Regulations 2001* (the Regulations) and corresponding State and Territory law govern the process undertaken by the Regulator before a decision is made on whether or not to issue a licence. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with the *Risk Analysis Framework* and in consultation with a wide range of experts, agencies and authorities, and the public.

More information on the comprehensive assessment undertaken for licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030) or at <http://www.ogtr.gov.au/>.

The application

DPI Victoria applied for a licence to release up to 30 lines of wheat (*Triticum aestivum* L.) that have been genetically modified to enhance drought tolerance into the environment under limited and 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 0.315 hectares¹ over one growing season (May 2007 – March 2008).

The GM wheat lines contain one of six different introduced 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 enable normal plant growth with reduced amounts of water (drought tolerance) either by regulating gene expression or modulating biochemical and signal transduction pathways in the wheat plants.

The GM wheat lines also contain a herbicide tolerance gene (*bar*, conferring tolerance to herbicides with glufosinate ammonium as the active ingredient) and an antibiotic resistance gene (*bla*, conferring resistance to ampicillin) 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 glufosinate ammonium during the trial and the *bla* gene does not function in plants.

The purpose of the trial is to conduct early stage ('proof of concept') research to evaluate the agronomic performance, including yield, of the GM wheat lines under rain-fed, drought prone conditions. Seed will be collected and retained for analysis and possible future trials of lines

¹ In its initial application, DPI-Victoria proposed individual sites of 0.15 ha in the local government area of Horsham and 0.075 ha in the local government area of Mildura. The applicant subsequently clarified the experimental design and as a consequence the area of the proposed site at Horsham has increased slightly to 0.24 ha.

that may be selected for further development (subject to additional approvals). No material from the release will be used in human food or animal feed.

DPI Victoria proposed a number of measures to limit the spread and persistence of the GM wheat lines and the introduced genetic materials that were considered during the evaluation of the application.

In accordance with the provisions of section 185 of the Act, DPI Victoria sought and received approval for some details of the application, including the names and classes 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, to be declared Confidential Commercial Information (CCI). The CCI was made available to the various prescribed experts and agencies that are consulted on the preparation of all RARMPs for DIR applications.

Risk assessment

The hazard identification process considered 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.

A hazard (source of potential harm) may be an event, substance or organism. A risk is identified when a hazard is considered to have some chance of causing harm. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

Fifteen events were identified and assessed whereby the release of the GM wheat lines might give rise to harm to people or the environment.

These 15 events 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. In addition, consideration was given to the opportunity for gene flow to other organisms, and its effects if this occurred.

All events were characterised in relation to both the magnitude and probability of harm in the context of the controls proposed by the applicant to limit the spread and persistence of the GMOs in both time and space. This detailed consideration concluded that none of the 15 events gave rise to an identified risk that required further assessment. The principal reasons comprise:

- the scale of the trial is limited in both area and duration
- containment, monitoring and disposal measures proposed by the applicant to limit the spread and persistence of the GM wheat plants
- 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 the introduced genes in the environment and lack of known toxicity or allergenicity from these proteins
- limited capacity of the GM wheat lines to spread and persist outside the release sites
- limited ability and opportunity for the GM wheat lines to transfer the introduced genes to commercial wheat crops or other sexually related species.

Therefore, any risk of harm to the health and safety of people, or the environment, from the limited and controlled release of the GM wheat lines into the environment is considered to be **negligible**.

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 15 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. However, conditions have been imposed on the licence to restrict the release to the size, duration and locations requested by the applicant, as these were an important part of establishing the context for assessing the risks.

The licence conditions require the applicant to limit the size and duration of the release to a maximum total area of 0.315 hectares on two sites over one winter wheat growing season (2007/08) and prevent the use of the GMOs, or materials from the GMOs for any other purposes. Containment measures include maintaining physical isolation of the release sites; transport requirements; and the conduct of post-harvest monitoring to ensure GMOs are destroyed².

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 30 GM wheat lines modified to enhance drought tolerance poses **negligible** risks to the health and safety of people and 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 contain the release to the size, duration and locations requested by the applicant, as these were important parameters in establishing the context for assessing the risks.

² The licence for DIR 071/2006 is available on the OGTR website (<http://www.ogtr.gov.au/gmorec/ir.htm#table>), via the link to DIR 071/2006).

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Abbreviations

ABA	Abscisic Acid
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
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
FAO	Food and Agricultural Organization of the United Nations
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
kDa	kilodalton
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
PPT	Phosphinothricin
qPCR	Quantitative Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RARMP	Risk Assessment and Management Plan
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
TGA	Therapeutic Goods Administration
WHO	World Health Organisation
WUE	Water Use Efficiency

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Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has decided to issue a licence (DIR 071/2006) to the Victorian Department of Primary Industries (DPI Victoria) for dealings involving the intentional release of genetically modified (GM) drought tolerant wheat into the environment, on a limited scale and under controlled conditions.

The *Gene Technology Act 2000* (the Act), the *Gene Technology Regulations 2001* (the Regulations) 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 Regulator's *Risk Analysis Framework* explains the approach used to evaluate licence applications and to develop the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions³.

This RARMP for DIR 071/2006 has been finalised in accordance with the gene technology legislation. Matters raised in the consultation process regarding risks to the health and safety of people or the environment from the proposed dealings were taken into account by the Regulator in deciding to issue a licence and the licence conditions that have been imposed.

Application

Title:	Limited and controlled release of GM drought tolerant wheat*
Applicant:	Victorian Department of Primary Industries (DPI Victoria)
Common name of the parent organism:	Bread wheat
Scientific name of the parent organism:	<i>Triticum aestivum</i> L.
Modified traits:	Drought tolerance and herbicide tolerance
Identity of the genes responsible for the modified traits:	Each wheat line contains: <ul style="list-style-type: none"> One of six different genes for drought tolerance derived from the plants <i>Zea mays</i> and <i>Arabidopsis thaliana</i>, the moss <i>Physcomitrella patens</i>, and the yeast <i>Saccharomyces cerevisiae</i> <i>bar</i> gene (herbicide tolerance selectable marker)
Proposed locations:	Two sites in the local government areas of Horsham and Mildura, Victoria
Proposed release size:	Up to 0.315 [†] hectares
Proposed time of release:	May 2007 to March 2008

*The title of the licence application submitted by DPI Victoria is *Field assessment of candidate genes for drought tolerance in transformed wheat*.

[†] In its initial application, DPI-Victoria proposed individual sites of 0.15 ha in the local government area of Horsham and 0.075 ha in the local government area of Mildura. The applicant subsequently clarified their experimental design and as a consequence the area of the proposed site at Horsham has increased slightly to 0.24 ha.

DPI Victoria applied for a licence to release up to 30 lines of wheat that have been genetically modified to enhance drought tolerance into the environment under limited and controlled conditions. The trial is authorised to take place at two sites in the local government areas of

³ More information on the assessment of licence applications and copies of the *Risk Analysis Framework* are available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <http://www.ogtr.gov.au/ir/process.htm> and <http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>, respectively.

Horsham and Mildura, Victoria, on a maximum total area of 0.315 hectares over one growing season (May 2007 – March 2008).

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 six different genes derived from the plants *Arabidopsis thaliana* and *Zea mays*, the moss *Physcomitrella patens* and the 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 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.

Additionally, the GM wheat lines contain the beta-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 early stage ('proof of concept') research to evaluate the agronomic performance, including yield, of the GM wheat lines under rain-fed, drought prone conditions. Seed will also be collected and retained for analysis and possible future trials of lines that may be selected for further development (subject to additional approvals).

The applicant proposed measures to limit the spread and persistence of the GM wheat lines in the environment. These were taken into account in establishing the risk assessment context for the release, and their suitability for limiting the release to the size, duration and locations proposed by the applicant was considered as part of the risk assessment process. No material from the GM wheat plants will be used for human food or animal feed.

In accordance with the provisions of section 185 of the Act, DPI Victoria sought and received approval for some details of the application, including the names and classes 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, to be declared Confidential Commercial Information (CCI). The CCI was made available to the various prescribed experts and agencies that are consulted on the preparation of all RARMPs for DIR applications.

Risk assessment

The risk assessment considered information contained in the application, 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 application (one submission) and from consultation on the RARMP (15 submissions), and how it was considered, is summarised in Appendices C and D, respectively.

A reference document, *The Biology and Ecology of Bread Wheat (Triticum aestivum L. em Thell.) in Australia*, 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 hazard identification process considered the circumstances or events 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.

A hazard (source of potential harm) may be an event, substance or organism. A risk is identified when a hazard is considered to have some chance of causing harm. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

Fifteen events were identified and assessed whereby the release of the GM wheat lines might give rise to harm to people or the environment.

These 15 events included consideration of whether 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. In addition, consideration was given to the opportunity for gene flow to other organisms, and its effects if this occurred.

All events were characterised in relation to both the magnitude and probability of harm in the context of the controls proposed by the applicant to limit the spread and persistence of the GMOs in both time and space. This detailed consideration concluded that none of the 15 events gave rise to an identified risk that required further assessment. The principal reasons comprise:

- the scale of the trial is limited in both area and duration
- containment, monitoring and disposal measures proposed by the applicant to limit the spread and persistence of the GM wheat plants
- 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 the introduced genes in the environment and lack of known toxicity or allergenicity from these proteins
- limited capacity of the GM wheat lines to spread and persist outside the release sites
- limited ability and opportunity for the GM wheat lines to transfer the introduced genes to commercial wheat crops or other sexually related species.

Therefore, as no risks to the health and safety of people or the environment were identified from the limited and controlled release of the GM wheat lines into the environment, the level of risk is considered to be **negligible**.

Risk management

A risk management plan builds upon the risk assessment to consider whether any action is required to mitigate the identified risks, and what can be done to protect the health and safety of people and the environment.

As none of the 15 events that were characterised in the risk assessment process are considered to give rise to an identified risk that requires further assessment, the level of risk to human health and safety and the environment from the release of the GM wheat lines 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. However, containment measures have

been imposed to restrict the trial to the size, duration and locations requested by the applicant, as these were important parameters in establishing the context for assessing the risks.

Licence conditions to manage this limited and controlled release

A number of licence conditions have been imposed to limit and control the release, based on the risk management plan and measures proposed by the applicant. These include requirements to:

- maintain a 10 m monitoring zone around each release site free of any related species and with reduced plant cover to limit rodent refuges
- maintain an isolation zone of at least 490 m (not including the 10 m monitoring zone) around each release site free of any sexually compatible species
- enclose each site with a 1.2 m high fence with lockable gates
- locate the release sites at least 50 m away from natural waterways
- harvest the GM wheat plant material by hand 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
- following cleaning of sites, monitor for and destroy any GM wheat that may grow for at least 24 months and until the site is clear of volunteers 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, June 2001; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have also been imposed 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. The Regulator sought input on the preparation of the RARMP from 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), National Health and Medical Research Council (NHMRC) and Australian Quarantine Inspection Service (AQIS). Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies⁴.

FSANZ is responsible for human food safety assessment, including GM food. As the trial involves early stage research, the applicant does not intend any material from the GM wheat lines 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.

⁴ 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/pdf/public/raffinal2.2.pdf>>.

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.

Identification of issues to be addressed for future releases

The risk assessment identified additional information that may be required to assess an application for a large scale trial, reduced containment conditions or a commercial release of any of these GM wheat lines. This would include:

- molecular characterisation of GM wheat lines selected for possible future releases
- additional data on the potential toxicity and allergenicity of proteins encoded by the introduced genes for drought tolerance, and of plant materials from the GM wheat lines selected for possible future releases
- physiological and agronomic characteristics of the GM wheat lines indicative of weediness including measurement of altered reproductive capacity; tolerance to drought and other environmental stresses, including salinity; and disease susceptibility.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 30 GM wheat lines into the local government areas of Horsham and Mildura in Victoria poses **negligible** risks to the health and safety of people and the environment posed by, or 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 contain the release to the size, duration and locations requested by the applicant, as these were important parameters in establishing the context for assessing the risks.

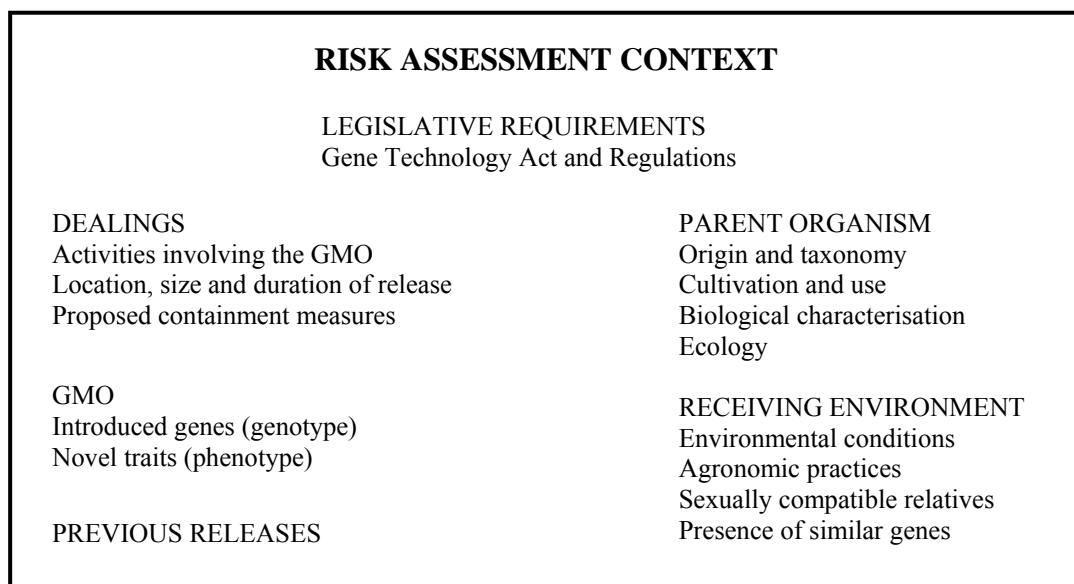
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Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people and the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation⁵, 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. Sections 49 to 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Regulator must take into account, and who she must consult with, in preparing the RARMPs that form the basis of her decision on licence applications.

3. For this application, establishing the risk assessment context includes consideration of:

- the size, duration and locations of the trial proposed by the applicant
- proposed dealings
- containment measures for the GMOs proposed by the applicant
- characteristics of the parent organism
- the nature and effect of the genetic modification
- the environmental conditions in the location(s) where the release may occur
- relevant agricultural practices
- presence of related plants in the environment
- presence of the introduced or similar genes in the environment
- any previous releases of these or other GMOs relevant to this application.

⁵ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <http://www.ogtr.gov.au/ir/process.htm> and in the *Risk Analysis Framework* (OGTR 2005a) <http://www.gov.au/pdf/raffinal2.2pdf>.

4. Initial consideration of the application under section 49 of the Act determined that public consultation was not required for the preparation of the consultation version of the RARMP. In accordance with section 50 of the Act, the Gene Technology Technical Advisory Committee (GTTAC), State and Territory governments, prescribed Australian Government agencies, the Minister for Environment and Water Resources and the local councils where the release may take place were consulted on matters relevant to the preparation of the consultation RARMP. This advice, and where it was taken into account, is summarised in Appendix B. A submission from the public was also received. This advice and its consideration is summarised in Appendix C.

5. In accordance with section 52 of the Act, the Regulator notified the public when the consultation version of the RARMP had been prepared and invited written submissions. Fifteen submissions from the public and how they were considered are summarised in Appendix D. Advice on the RARMP was also sought from the same experts, agencies and authorities as before. None of the latter raised any new issues relating to risks to human health and safety and the environment that required further consideration. However, feedback on the consideration of some previously raised issues enabled their clarification in the final RARMP.

Section 2 The application

6. The Victorian Department of Primary Industries (DPI Victoria) proposes to release up to 30 genetically modified (GM) wheat lines that have been modified to enhance drought tolerance into the environment under limited and controlled conditions.

2.1 The proposed size, duration and location

7. The trial is proposed to take place at two sites in Victoria, one on an area of 0.24 ha⁶ in the local government area of Horsham and the other on an area of 0.075 ha in the local government area of Mildura, over one season (May 2007 – March 2008).

2.2 The proposed dealings

8. 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 rain-fed, drought prone conditions. Seed would also be collected and retained for analysis and possible future trials of lines that may be selected for further development (subject to additional approvals). No material from the release would be used for human food or animal feed.

2.3 The proposed measures to limit the spread and persistence of the GMOs

9. The applicant proposes measures to limit the spread and persistence of the GM wheat lines into the environment. These measures are taken into account in the risk assessment context (this chapter) and their suitability for limiting the release to the size, duration and locations proposed by the applicant is evaluated in Chapter 2.

10. The applicant proposes the following containment measures:

- maintain a 10 m monitoring zone around each release site free of any related species and with reduced plant cover to limit rodent refuges
- maintain an isolation zone of 500 m (including the 10 m monitoring zone) around each release site free of any sexually compatible species
- enclose each site with a 1.2 m high fence with lockable gates

⁶ In its initial application, DPI-Victoria proposed a trial area of 0.15 ha in the local government area of Horsham. The applicant subsequently clarified their experimental design and as a consequence the area of the proposed site at Horsham has increased slightly to 0.24 ha.

- locate the release sites at least 50 m away from natural waterways
- harvest the GM wheat plant material by hand 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
- following cleaning of sites, monitor for and destroy any GM wheat that may grow for at least 24 months
- transportation of GM seed and plant materials in accordance with OGTR guidelines.

Section 3 The parent organism

11. 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. More detailed information on bread wheat can be found in the document *The Biology and Ecology of Bread Wheat* (*Triticum aestivum* L. *em* Thell.) in Australia (OGTR 2005b), which was produced in order to inform the risk assessment process for licence applications involving GM wheat plants. This document is available at <www.ogtr.gov.au>.

12. 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 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 4 The GMOs, nature and effect of the genetic modification

4.1 Introduction to the GMOs

13. Some details of the application, including the names and classes 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 experts and agencies that were consulted on this application.

14. Up to 30 GM wheat lines are proposed for release. Each line contains one of six different introduced genes derived from the plants *Arabidopsis thaliana* (thale cress) and *Zea mays* (maize), the moss *Physcomitrella patens* and the yeast *Saccharomyces cerevisiae*. 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.

15. 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 beta-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 and the *bla* gene does not function in plants.

16. 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.

4.2 Introduction to plant molecular responses to drought stress

17. Drought stress is an abiotic stress; a nonliving factor that causes harmful effects to plants. Other types of primary abiotic stresses include salinity, cold, heat and chemical pollution. 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 biochemical and physiological processes (Figure 2).

18. At a molecular level there are three broad categories into which plant genes can be classified depending on their role in the response to drought stress (Wang et al. 2003; Vinocur & Altman 2005). These are:

- Signal sensing, perception and transduction
- Transcriptional control
- Stress tolerance response mechanisms in terms of end functions including detoxification, osmoprotection, chaperone functions, and water and ion movement.

19. Little is known about how plants directly sense and perceive drought stress but it is thought specific receptors are involved (Xiong & Zhu 2001; Zhu 2002). Once sensed, a drought stress signal may be transmitted via a signalling cascade to eventually regulate the transcription of numerous genes involved in the stress response.

20. The transduction of the stress signal may be accomplished through molecular switches such as small GTPases, various signalling molecules such as Ca^{2+} , phospholipids, reactive oxygen species (ROS), sugars and plant hormones, or via protein messengers such as mitogen activated protein kinases and serine/threonine phosphatases (Xiong & Zhu 2001; Ramanjulu & Bartels 2002; Zhu 2002; Chaves & Oliveira 2004; Chinnusamy et al. 2004).

21. Drought stress signals activate genes that control the transcription of genes involved in the stress tolerance response mechanisms for cell protection. Some of these activated genes are controlled by abscisic acid (ABA), which itself accumulates during drought stress and controls stomatal closure, while others are ABA independent. The majority can be categorised as transcription factors (Wang et al. 2003; Shinozaki et al. 2003), but an example of other genes involved in transcription control include those encoding proteins involved in chromatin remodelling (Singh 1998). Certain regulatory genes can be induced by more than one type of abiotic stress (eg drought, cold and salinity; Seki et al. 2002).

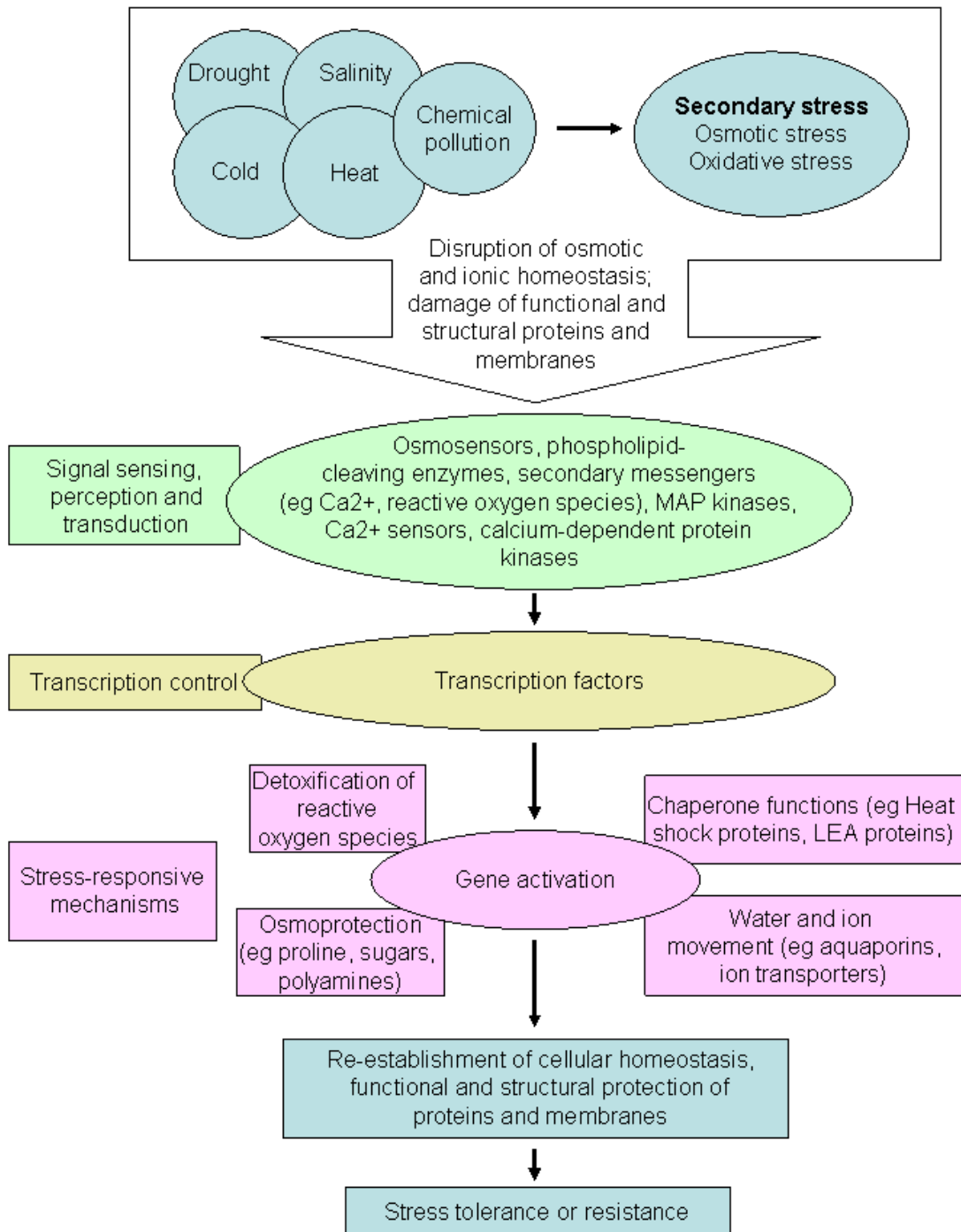


Figure 2 The abiotic stress tolerance response process in plants. Redrawn and modified from Current Opinion in Biotechnology, Volume 16, Vinocur B. and Altman A., Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations, Page no. 124, Copyright (2005), with permission from Elsevier. Abbreviations: MAP, mitogen activated protein; LEA, late embryogenesis abundant.

22. Plants use four main classes of cellular response to drought stress in terms of end function. They are as follows:

- Molecular chaperones, such as heat-shock proteins, and late embryogenesis abundant proteins (Wang et al. 2003; Chaves & Oliveira 2004; Vinocur & Altman 2005). These proteins can provide protection and ensure proper folding and function of macromolecules such as enzymes during periods of stress.
- Metabolite production such as certain amino acids (eg proline), glycine-betaines, polyamines (eg spermine and spermidine), sugars and sugar alcohols (eg raffinose trehalose; Wang et al. 2003; Chaves & Oliveira 2004; Vinocur & Altman 2005). These metabolites are thought to act primarily as osmoprotectants but may also have other secondary functions such as gene regulation, which may serve to provide further protection from drought stress.
- Detoxification enzymes, such as superoxide dismutases and catalases, for the production of antioxidants and deactivation of ROS (eg hydrogen peroxide and hydroxyl radicals; Wang et al. 2003; Chaves & Oliveira 2004; Vinocur & Altman 2005). ROS are generated as a result of various abiotic stresses and can irreversibly damage cellular components such as membranes and photosynthetic machinery. As ROS also serve as signalling molecules, their levels are tightly controlled in the cell.
- Adjustment of cellular water and ion content through regulation of membrane-intrinsic proteins. Examples of these proteins include water channel proteins and ion transporters (Ramanjulu & Bartels 2002; Wang et al. 2003; Chaves & Oliveira 2004). The regulation may be in the form of control of the synthesis of the proteins and/or via modifications (eg phosphorylation) and interactions with other proteins (eg 14-3-3 protein binding).

4.3 The introduced genes and regulatory sequences, and the gene products

4.3.1 Source organisms for the introduced genes and regulatory sequences

23. Genetic material from various sources was introduced into wheat. The genetic elements, their sources and function in the source organisms are listed below (see Table 1). Some details have been declared CCI.

Table 1 Source organisms for the introduced genes and regulatory sequences

Genetic element	Source organism	Function in the source organism	Hazardous characteristics of the source organism	Comments
Promoter 1	CCI	Transcription initiation	-	
Promoter 2	CCI	Transcription initiation	-	
Gene 1	<i>Zea mays</i>	CCI	-	
Gene 2	<i>A. thaliana</i>	CCI	-	
Gene 3	<i>Saccharomyces cerevisiae</i>	CCI	Opportunistic human pathogen	Gene 3 is unlikely to cause ill effects in humans
Gene 4	<i>Physcomitrella patens</i>	CCI	-	
Gene 5	<i>P. patens</i>	CCI	-	
Gene 6	<i>S. cerevisiae</i>	CCI	Opportunistic human pathogen	Gene 6 is unlikely to cause ill effects in humans
35st terminator	Cauliflower mosaic virus	Transcription termination	Plant pathogen on Brassicaceae	This regulatory sequence does not cause disease symptoms
<i>Ubi1</i> promoter	<i>Z. mays</i>	Transcription initiation	-	

Genetic element	Source organism	Function in the source organism	Hazardous characteristics of the source organism	Comments
<i>bar</i> gene	<i>Streptomyces hygroscopicus</i>	Antibiotic resistance	-	
<i>nos</i> terminator	<i>Agrobacterium tumefaciens</i>	Transcription termination	Plant pathogen causing crown gall disease	This regulatory sequence does not cause disease symptoms
<i>bla</i> gene	<i>E. coli</i>	Antibiotic resistance	Opportunistic human pathogen	β lactamase does not cause ill effects in humans

4.3.2 The introduced genes for drought tolerance

24. Each GM wheat line contains one of six different introduced genes for drought tolerance. For each gene there are two constructs, one driven by a stress inducible promoter and one by a constitutive promoter, giving a total of 12 different gene constructs that were used to produce the GM wheat lines proposed for release.

25. 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 CaMV *35st* terminator.

26. The applicant states that the introduced genes have demonstrated the capacity to produce a water efficient phenotype in *A. thaliana* and *Z. mays*. Some of the introduced genes, or their homologs, are also known to confer other agronomic traits.

4.3.3 Toxicity of the introduced genes for drought tolerance

27. To determine if the proteins encoded by the introduced genes for drought tolerance may potentially be toxins a bioinformatics assessment was performed by the applicant. Each of the predicted proteins in their entirety was searched against the GENBANK non-redundant protein database <<http://www.ncbi.nlm.nih.gov/blast>> using the Basic Local Alignment Search Tool (BLAST). An E score of 1×10^{-10} was set as a high cut-off value for alignment significance, and the top 100 results of all BLAST search results within this threshold for each protein were searched for keywords indicating a toxin concern. The keyword list was derived from the EPA list of toxins⁷. None of the BLAST search results for any of the proteins contained a keyword on the list of toxins.

28. An extensive literature search of Pubmed through the National Center for Biotechnology Information <<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed>>, and both BIOSIS Previews and Cab Abstracts through the DataStar web site <<http://www.datastarweb.com>>, did not reveal any reports that imply the proteins encoded by the genes for drought tolerance may be toxins.

4.3.4 Allergenicity of the introduced genes for drought tolerance

29. Known food allergens generally share a number of characteristics including amino acid sequence homology, a molecular weight (Mr) of 15-70 kDa, high expression, stability (resistant to digestion in the gastrointestinal tract) and are derived from a food or biological source known to be allergenic (Metcalf et al. 1996; FAO 2001). In addition, many protein allergens are N-glycosylated (Herouet et al. 2005) leading to the concern that glycosylation may contribute to the allergenicity (Buchanan 2001).

30. All the introduced genes for altered plant architecture are in the molecular weight range identified as being typical of allergens (15-70 kDa). A motif search

⁷ <<http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=f9b0d6a758f9ede984f01da77958c2ac&rgn=div8&view=text&node=40:30.0.1.1.12.7.1.3&idno=40>>

<<http://www.expasy.org/prosite/>> with the amino acid sequences derived from the introduced gene sequences identified that three of the proteins contained putative N-glycosylation sites (Asp-X-Ser/Thr).

31. Food and Agriculture Organization of the United Nations (FAO) and World Health Organisation (WHO) guidelines propose that cross-reactivity between an unknown protein and a known allergen has to be considered when there is more than 35% identity in the amino acid sequence over a sliding window of 80 amino acids or a match of six contiguous amino acids (FAO 2001; Codex Alimentarius Commission 2003). Matches of six amino acids with any allergen have been shown to occur frequently by chance which limits the utility of this criteria for predicting allergenicity (Hileman et al. 2002; Stadler & Stadler 2003; Thomas et al. 2005; Silvanovich et al. 2006). The International Food Biotechnology Council/International Life Sciences Institute have suggested that an optimal length of between eight and twelve amino acids is required for binding to T-cells and that an immunological significant sequence identity requires a match of at least eight contiguous amino acids (Metcalf et al. 1996).

32. The predicted protein sequences for each of the six introduced genes for drought tolerance were compared by the applicant to a database of known allergens, the Food Allergy Research and Resource Program allergen protein database <<http://www.allergenonline.com>>. None of the predicted proteins contained a region of 80 amino acids with greater than 35% sequence identity to any known allergens, and none contained eight or more consecutive amino acids in common with any member of the database.

33. Furthermore, an extensive literature search of Pubmed through the National Center for Biotechnology Information <<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed>>, and both BIOSIS Previews and Cab Abstracts through the DataStar web site <<http://www.datastarweb.com>>, did not reveal any reports that imply the proteins encoded by the genes for drought tolerance may be allergens.

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

34. All GM wheat lines proposed for release also contain the *bar* herbicide tolerance marker gene. The *bar* gene was isolated from *S. hygroscopicus*, 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.

35. The active constituent of glufosinate ammonium is the amino acid phosphinothricin (PPT), an analogue of glutamate. PPT is found naturally as a component of bialaphos and phosalacine, tripeptide antibiotics produced by members of the bacterial genera *Streptomyces* and *Kitasatosporia* (Omura et al. 1984; Wehrmann et al. 1996). Bialaphos (phosphinothricyl-L-alanyl-L-alanine) consists of PPT and two L-alanine residues, while phosalacine (phosphinothricyl-L-alanyl-L-leucine) consists of PPT, an alanine residue and a leucine residue.

36. 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.

37. Glufosinate ammonium is an inhibitor of the enzyme glutamine synthetase, a key enzyme in the nitrogen metabolism of plants. Inhibition of glutamine synthetase by glufosinate ammonium causes rapid accumulation of ammonia, as well as inhibition of photosynthesis, leading to cell death (Droge-Laser et al. 1994). In GM plants containing the *bar* gene, the addition of an acetyl group to PPT by the PAT protein prevents this inhibition of glutamine synthetase and as a result detoxifies the herbicide.

38. 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 (see Section 4.3.8). In addition, a number of GM crops, including food crops, containing the *bar* gene have been approved for commercial release both in Australia (DIR 021/2003, DIR 062/2005) and overseas. No adverse effects on humans, animals or the environment have been reported from any releases.

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

39. Expression of the *bar* gene in the GM wheat lines is controlled by the promoter and first intron of the *Ubi1* ubiquitin gene from maize (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).

40. The termination and polyadenylation signals that are also responsible for controlling *bar* gene expression are derived from the nopaline synthase (*nos*) gene of *A. tumefaciens* (Bevan 1984).

4.3.7 Toxicity of PAT

41. 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 021/2003) 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 to humans. Detailed descriptions of the results of these studies are available in the RARMPs for DIR 021/2003 and DIR 062/2005. Key results are summarised below.

42. In an acute oral toxicity study, mice given a single dose of the recombinant PAT protein (2500 mg/kg bodyweight) by oral gavage showed no significant, treatment-related toxic effects over 14 days (Merriman 1996). Similarly, rats fed over 14 days with purified PAT protein at dietary levels of 0, 0.5% or 5% (equivalent to 0, 707 or 7792 mg/kg/day) showed no adverse effects (Pfister et al. 1996, as cited in Bremmer & Leist 1996). In addition, no toxic effects were observed in mice after acute intravenous administration of 10 mg/kg body weight (Kennel 2002; Herouet et al. 2005).

43. Digestibility studies have shown that the PAT protein is rapidly degraded when digested or subjected to typical mammalian stomach conditions (Wehrmann et al. 1996; European Commission 1996; Esdaile 2002a; Esdaile 2002b; Bayer CropScience 2003; Herouet et al. 2005). The PAT protein is also readily inactivated by heat or low pH (Canadian Food Inspection Agency 1995; Wehrmann et al. 1996; Canadian Food Inspection Agency 1998).

44. The amino acid sequences of the PAT protein encoded by the *bar* gene was compared for homology with amino acid sequences of known toxic proteins using seven different databases (SwissProt, trEMBL, GeneSeq-Prot, PIR, PDB, DAD and GenPept). No significant

homology to any known toxic proteins was detected (Herouet 2002b). This conclusion is supported by others (Van den Bulcke 1997; ANZFA 2001b; Bayer CropScience 2003).

45. The toxicity of the PAT protein expressed in GM plants has been assessed as low by a number of regulatory bodies in Australia, USA, Canada, and Europe (FDA 1995; FDA 1997; European Scientific Committee on Plants 1998a; European Scientific Committee on Plants 1998b; ANZFA 2001b).

46. 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; ANZFA 2001b; ANZFA 2001c; ANZFA 2002; FSANZ 2003; FSANZ 2004a; FSANZ 2004b; FSANZ 2005a; FSANZ 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.

4.3.8 Allergenicity of PAT

47. The molecular weight of the PAT protein is 22-23 kD which is in the typical range for allergenic proteins. However, it does not possess potential glycosylation sites, nor is it stable in the mammalian digestive system, decreasing the probability that it is allergenic (Bayer CropScience 2003; Herouet et al. 2005).

48. The PAT protein expressed in the GM wheat lines is not derived from a known allergen source. The protein shows no significant sequence homology to allergens assembled in the SwissProt, trEMBL, GeneSeq-Prot, PIR, PDB, DAD and GenPept protein databases (Herouet 2002a; Herouet 2002b; Herouet et al. 2005). A search for homology with known allergens of the PAT protein was conducted based on detecting identities of eight contiguous amino acids and no sequence homologies were detected (Van den Bulcke 1997; Herouet et al. 2005). In addition, no identities with known IgE epitopes were found confirming the previous results.

49. A number of GM plants containing the *pat* or *bar* gene have been trialled or commercially released in Australia (for details see Section 5.4) as well as overseas and no adverse effects on humans, animals or the environment have been reported.

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

50. 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.

51. 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.

52. 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.

53. 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 and 070/2006) and overseas. No adverse effects on humans, animals or the environment have been reported from these releases.

4.4 Method of genetic modification

54. 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 PPT (5 mg/ml).

55. 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.

4.5 Characterisation of the GMOs

4.5.1 Stability and molecular characterisation

56. 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 is not known and has not been determined. The inserted genes have been inherited as dominant Mendelian traits over at least two generations of plants grown in the glasshouse.

57. 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.

58. 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 copy numbers of between 1-7. 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.

59. 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.

4.5.2 Characterisation of the phenotype of the GMOs

60. One of the aims of the proposed trial is to compare agronomic performance of GM wheat lines and 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.

61. All of the six introduced genes for drought tolerance have been shown in *A. thaliana* and maize to confer increased water use efficiency (WUE). 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 limit the spread and persistence of the GM wheat lines, and the trial is limited in size, duration and location.

4.5.3 Toxicity and allergenicity of the drought tolerant GM wheat

62. Data on the potential toxicity and allergenicity of the GM wheat lines 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 limit the spread and persistence of the GM wheat lines and prevent products from the GM wheat lines being used in human food or animal feed, and because the trial is limited in size, duration and location.

Section 5 The receiving environment

63. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the size, duration and locations of the dealings, any relevant biotic/abiotic properties of the regions where the release would occur; 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 2005a).

5.1 Relevant abiotic factors

64. Wheat is grown across a wide range of environments around the world with the broadest adaptation of all the cereal crops species. It is a cool season crop requiring a minimum temperature for growth of 3°C to 4°C, with optimal growth occurring around 25°C and tolerance of temperatures to a maximum of about 32°C. Wheat flourishes in many different agro-climatic zones with production concentrated between latitudes 30°N and 60°N and 27°S and 40°S, but there are examples of wheat production beyond these limits. Wheat grows best on well drained soils anywhere from sea level up to heights of about 4500 m above sea level. It will grow in areas receiving 250 to 1750 mm annual precipitation, but most wheat production occurs in areas receiving 375 to 875 mm annually (Briggle & Curtis 1987).

65. Most wheat in Australia is grown as a dryland crop, with irrigated wheat contributing only a very small proportion to total production (Turner 2004). The wheat growing areas in Australia generally have a climate that is considered Mediterranean, in that there is a concentration of rainfall during the winter months while summer is drier. The summers tend to be warm to hot with high solar radiation and the winter mild. In Western Australia (WA), the climate tends to more extreme Mediterranean and crop growth is highly dependent upon winter rains (Simmonds 1989). The winter-dominant rainfall of WA differs from the generally higher and evenly distributed rainfall of Victoria and southern New South Wales (NSW), and the summer-dominant rainfall of the northern wheat growing areas (Cramb et al. 2000). Soil types in the Australian wheat growing areas vary from heavy, deep clays in northern NSW and southern Queensland to very light and sandy soils in WA (Simmonds 1989). Wheat growing areas of Australia are shown in Figure 3.

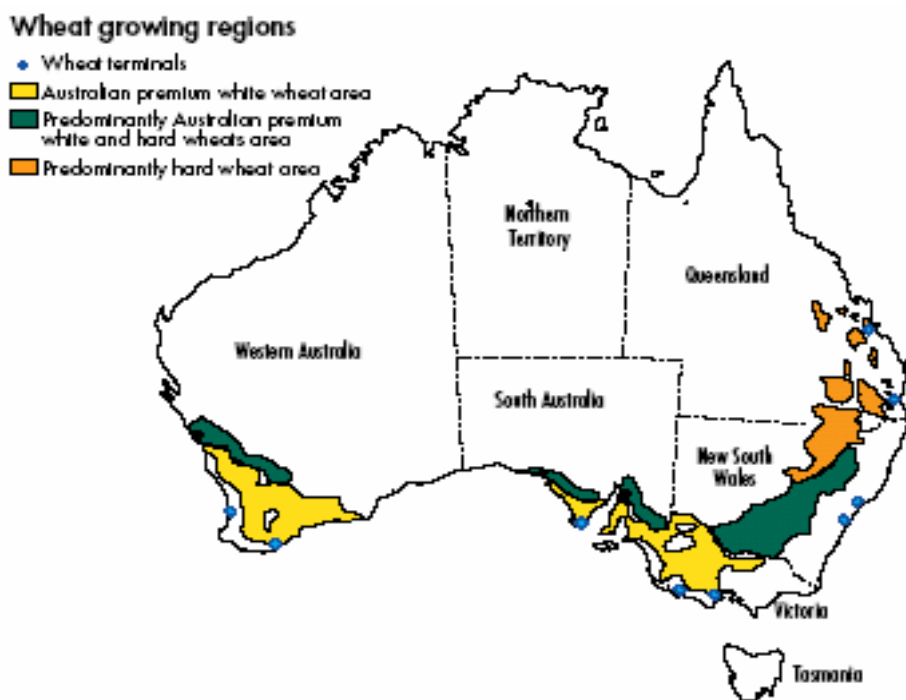


Figure 3 Wheat growing areas in Australia (ABARE 2003).

66. WUE in wheat can be defined as the grain yield per hectare (kg/ha) divided by the crop water use (mm; Tennant 2000). For wheat, WUE varies from 5 to 20 kg/ha/mm (Grains Research and Development Cooperation 1998), with a daily water use of about 2 mm (Wachsmann et al. 2003).

67. Water deficit is common in dryland wheat farming, and is the major abiotic stress limiting crop productivity in Australia. This is demonstrated in a recent research report by ABARE in which it was found that moisture availability is a dominant factor affecting total productivity in Australia's grains industry (Kokic et al. 2006). The current drought in Australia, ongoing since 2001, means that the wheat crop is forecast to be 15.5 million tonnes less in 2006-07 than the previous season (ABARE 2006).

68. The size, duration and locations of the proposed limited and controlled release of drought tolerant GM wheat lines are outlined in Section 2.1. The proposed release would occur at two sites, both of which are typical of rain-fed, drought prone wheat production environments in Victoria. The locations chosen also have much wider relevance to other areas prone to drought 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 to 350 mm. Selected climatic data for both proposed sites are given in Table 2.

Table 2 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.

5.2 Relevant agricultural practices

69. Wheat varieties are usually classed as winter or spring wheats. In the northern Hemisphere, winter wheat is planted in the autumn and it requires a period of below-freezing temperatures (vernalisation) before it can form an inflorescence. Spring wheats are planted in spring and do not require cold weather to form inflorescences or spikes. In Australia, spring wheat varieties are commonly grown as a winter crop and are usually planted May and June.

70. It was once common to cultivate the field prior to seeding, but more farmers are opting for no-till or low-till practices which can help improve soil structure, reduce erosion, increase yields and, in some cases, decrease disease. There are a number of tillage systems in use and no one system is ideal for all soils and situations (Jarvis et al. 2000).

71. There are a number of pests and diseases of wheat (see OGTR 2005b 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.

72. Harvest of the mature wheat generally occurs from mid-November to late December. Wheat can be harvested when the moisture content of the grain is between 10 to 20% (Setter & Carlton 2000). Post harvest, the wheat stubble may be left standing to reduce erosion, incorporated into the soil or even burnt prior to seeding, depending upon the management system in place (Jarvis et al. 2000).

73. 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; Yitpi, an elite variety from SA; and AU29597, a synthetic derivative line from Victoria. 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. 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, with the exception that the applicant proposes to harvest by hand to minimise seed spillage.

5.3 Presence of related plants in the receiving environment

74. 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.

75. 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 assessment is provided in Chapter 2.

76. The applicant has indicated that if planted, rye (*Secale cereale*) will be at least 500 m from the GM wheat. Barley is not known to hybridise with wheat under natural conditions (see OGTR 2005b) and may be grown within the 490 m isolation zone.

5.4 Presence of the introduced genes or similar genes in the environment

77. Two 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 two genes for drought tolerance are derived from a moss, *P. patens*, which is widespread in the northern hemisphere (Tan 1978) as well as being another common experimental organism.

78. 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 and their products or homologs.

79. 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. hygroscopicus*, 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 in Australia (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).

80. 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 6 Australian and international approvals

6.1 Australian approvals of the GM wheat lines

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

81. There has been no release of these GM wheat lines in Australia.

6.1.2 Approvals by other Australian government agencies

82. 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) and FSANZ. This is discussed further in Chapter 3.

83. 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.

84. 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.

6.2 International approvals

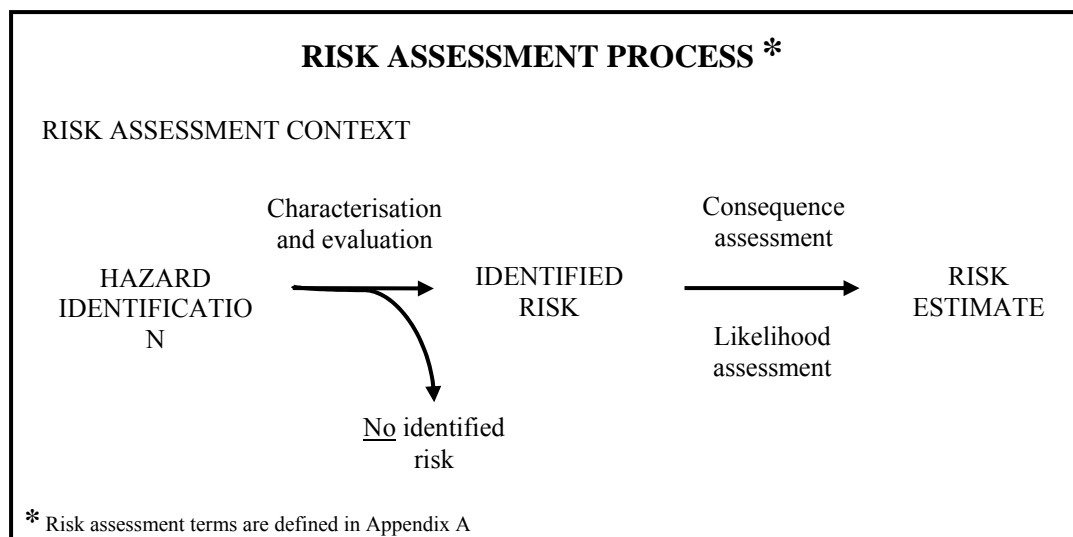
85. There has been no international release of these GM wheat lines overseas. However, all of the genes for drought tolerance contained in the GM wheat lines proposed for release have been authorised for field release in the U.S. or Canada in other crops (*Z. mays* or *Brassica napus*).

Chapter 2 Risk assessment

Section 1 Introduction

86. 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 3) 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.

Figure 3 The risk assessment process.



87. 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.

88. 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 2005a).

89. 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.

90. 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 2005a). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

91. 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

92. The list of events compiled during hazard identification are 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.

93. A risk is identified only when there is some chance that harm will occur. Those events that do not lead to an adverse outcome or could not reasonably occur do not represent an identified risk and will not advance in the risk assessment process. Risks associated with the remaining events are assessed further to determine the seriousness of harm (consequence) and chance of harm (likelihood). The identified risks must be posed by or result from gene technology.

94. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2005a). Harm is assessed in comparison to the parent organism and in the context of the proposed dealings and the receiving environment. The risk assessment process focuses on measurable criteria for determining harm.

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

- the proposed dealings, which may include experimentation, development, production, breeding, propagation, and possession, use, supply, transport or disposal of the GMOs during the course of these dealings
- the size, duration and locations of the release
- containment measures proposed by the applicant
- comparisons with 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
- properties of the biotic and abiotic environment at the sites of release
- agronomic management practices for the GMOs.

96. There have been no releases of these GMOs in Australia.

97. Events considered during the risk assessment for this application that are discussed in detail later in this Section are summarised below in Table 3. Events that share a number of common features have been grouped together in broader hazard categories. Fifteen events were characterised, none of which were considered to lead to an identified risk that required further assessment.

98. The GM wheat lines differ from non-GM wheat in that they contain three introduced genes; one encoding a protein intended to confer drought tolerance; the *bar* gene encoding the PAT protein, which confers tolerance to herbicides containing glufosinate ammonium; and the *bla* gene, which is under the control of a bacterial promoter and, therefore, is not expressed in the GM wheat plants.

99. The prevalence of the *bar* and *bla* genes in the environment and the lack of evidence for toxicity or allergenicity of the PAT and β -lactamase proteins are discussed in Chapter 1, Sections 4.3.5 to 4.3.9. In addition, the potential effects of the *bar* or *bla* genes and their

products were considered in detail in previous assessments for DIRs 021/2003, 062/2005 (*bar* gene; commercial releases); 019/2002, 026/2002, 028/2002, 051/2004, 052/2004, 070/2006 (*bla* gene; limited and controlled releases). RARMPs for those DIR licences are available from the OGTR or from the website <<http://www.ogtr.gov.au>>. Risks arising from the expression of *bar* or *bla* genes in GM wheat are considered to be negligible. Therefore, the potential effects of *bar* and *bla* genes will not be further assessed for this application, unless they are specific for the current licence application.

Table 3 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 to people	1. Ingestion of GM plant materials produced by the wheat lines containing the introduced genes.	Toxicity for people	No	<ul style="list-style-type: none"> The introduced or similar genes are widespread in the environment. There are no reports of toxicity associated with the encoded drought tolerance proteins. None of the GM plant materials from the proposed trial would be used as human food.
	2. Contact with, or inhalation of, GM plant materials produced by the wheat lines containing the introduced genes.	Toxicity for people	No	<ul style="list-style-type: none"> The introduced or similar genes are widespread in the environment. There are no reports of toxicity associated with the encoded drought tolerance proteins. Contact with, or inhalation of GM plant materials would be limited to workers associated with the field trial. Contact would be further limited by the small size and short duration of the proposed field trial, and by the containment measures proposed by the applicant.
Section 2.2 Production of a substance allergenic to people	3. Ingestion of GM plant materials produced by the wheat lines containing the introduced genes.	Allergic reactions in people	No	<ul style="list-style-type: none"> The introduced or similar genes are widespread in the environment. None of the proteins encoded by the introduced genes have any significant homology with known allergens. An extensive search of the literature did not identify any reports of allergenicity associated with the proteins encoded by the introduced genes. None of the GM plant materials from the proposed trial would be used as human food.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	4. Contact with, or inhalation of, GM plant materials produced by the wheat lines containing the introduced genes.	Allergic reactions in people	No	<ul style="list-style-type: none"> The introduced or similar genes are widespread in the environment. None of the proteins encoded by the introduced genes have any significant homology with known allergens. Non-GM wheat pollen is known to cause allergies (hay fever), but it is unlikely that the proteins encoded by the introduced genes will increase the allergenic potential of the GM wheat lines. Contact with, or inhalation of, GM plant materials would be limited to workers associated with the trial. Contact would be further limited by the small size and short duration of the proposed field trial, and by the containment measures proposed by the applicant.
Section 2.3 Production of a substance toxic to organisms other than people	5. Direct or indirect ingestion of GM plant materials produced by the wheat lines containing the introduced genes, by vertebrates, invertebrates or microorganisms.	Toxicity for vertebrates, invertebrates or microorganisms	No	<ul style="list-style-type: none"> Vertebrates, invertebrates and microorganisms are already widely exposed to the same or similar proteins. The proteins encoded by the introduced genes are not known to be toxic to any organism. None of the GM plant materials from the proposed trial would be used as animal feed. Exposure of vertebrates, invertebrates and microorganisms to the GM wheat lines would be limited by the small size and short duration of the proposed trial, and by the containment measures proposed by the applicant.
Section 2.4 Spread and persistence of the GM wheat in the environment	6. Expression of the introduced genes improving the survival of the GM wheat through enhanced drought tolerance.	Weediness	No	<ul style="list-style-type: none"> Many factors other than water availability limit the spread and persistence of wheat in the areas proposed for release, including temperature, low intrinsic competitive ability, nutrient availability, and pests and diseases. Wheat lacks most characteristics common to weeds, such as prolonged seed dormancy, seed dispersal mechanisms and rapid vegetative growth. Current cultivated non-GM wheat cultivars perform well under low rainfall conditions. The proposed trial is of small size and short duration, and the applicant proposes measures to limit the spread and persistence of the GM wheat lines.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	<p>7. Expression of the introduced genes improving the survival of the GM wheat through enhancement of:</p> <ul style="list-style-type: none"> • abiotic stress tolerances other than drought tolerance • biotic stress tolerance. 	Weediness	No	<ul style="list-style-type: none"> • Many factors limit the spread and persistence of wheat in the areas of the proposed field trial including temperature, low intrinsic competitive ability, nutrient availability, and pests and diseases. • Wheat lacks most characteristics common to weeds, such as prolonged seed dormancy, seed dispersal mechanisms and rapid vegetative growth. • Glufosinate ammonium is not registered for use in wheat plantings and it is not used as the main method to control wheat in other crops. • The proposed trial is of small size and short duration, and the applicant proposes measures to limit the spread and persistence of the GM wheat.
	<p>8. Dispersal of GM plant materials, including seed, by various means, including animals, extreme weather conditions or spillage of seed during transport or storage.</p>	Weediness	No	<ul style="list-style-type: none"> • Wheat lacks seed dispersal characteristics such as stickiness, burrs or hooks. • 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. • Proposed containment measures would limit dispersal of GM plant material. The applicant proposes to transport and store all GM plant materials including seeds according to OGTR guidelines and to incorporate all plant materials remaining on the sites into the soil to promote decomposition at the end of the growing season. • White wheats are prone to pre-harvest sprouting therefore have little seed dormancy.
	<p>9. Exposure of vertebrates (including people), invertebrates or microorganisms to materials produced from the GM wheat lines containing the introduced genes.</p>	Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates or microorganisms	No	<ul style="list-style-type: none"> • The potential effects on people are discussed in Sections 2.1 and 2.2. • The potential effects on organisms other than people are discussed in Section 2.3. • The release would be of small size and short duration, and the applicant proposes measures to limit the spread and persistence of the GM wheat lines.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.5 Gene flow by vertical gene transfer (ie gene transfer to sexually compatible plants)	10. Expression of the introduced genes in other wheat plants.	Weediness	No	<ul style="list-style-type: none"> Wheat is predominantly self-pollinating. Wheat pollen is relatively heavy and only remains viable for limited periods (<30 min). Most studies suggest that 90% falls to the ground within 3 m of the source. Gene transfer between bread wheats has not been reported over 300 m. Factors limiting the spread and persistence of wheat are discussed in Section 2.4. Outcrossing to other wheat plants would be limited due to the small size, short duration of the trial and containment measures proposed by the applicant.
	11. Expression of the proteins encoded by the introduced genes in other sexually compatible plant species as a result of gene transfer.	Weediness	No	<ul style="list-style-type: none"> Wheat is predominantly self-pollinating. Wheat pollen is relatively heavy and only remains viable for limited periods (<30 min). Most studies suggest that 90% falls to the ground within 3 m of the source. Gene transfer between bread wheat and other sexually compatible species has not been reported over 40 m. Outcrossing to sexually compatible species is limited by genome compatibility and reduced hybrid fitness. Outcrossing to sexually compatible species would be limited due to the small size, short duration and containment measures proposed by the applicant.
	12. Presence of the introduced regulatory sequences in other wheat plants or sexually compatible plant species as a result of gene transfer.	Weediness, Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms	No	<ul style="list-style-type: none"> The introduced regulatory sequences are not known to behave any differently than endogenous regulatory sequences in plants. Outcrossing to sexually compatible species would be limited due to the small size, short duration and containment measures proposed by the applicant.
Section 2.6 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	13. Presence of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer.	Weediness, Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms, Increased pathogenicity	No	<ul style="list-style-type: none"> The introduced genes or similar genes and the introduced regulatory genes are already present in the environment and are available for transfer via demonstrated natural mechanisms. Gene transfer from plants to unrelated species has not been demonstrated under natural conditions.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.7 Unintended changes in biochemistry, physiology or ecology	14. Altered biochemistry (including innate toxic or allergenic compounds), physiology or ecology of the GM wheat lines resulting from expression, or random insertion, of the introduced genes.	Weediness, Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms	No	<ul style="list-style-type: none"> Compositional analysis of the GM wheat lines has not been undertaken as the proposed trial represents early stage research involving phenotypic screening of multiple transformation events and selection of lines for possible further development. The toxicity, allergenicity and weediness potential of the GM wheat lines were assessed in Sections 2.1 to 2.4. Unintended changes typically result in deleterious effects in the plant, so are unlikely to proceed in the selection process. Unintended adverse effects, if any, would be limited by the small scale and short duration of the proposed release. None of the GM plant materials from the proposed trial would be used as human food or animal feed.
Section 2.8 Unauthorised activities	15. Use of the GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.7	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 to people

100. 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). In order for toxicity to occur, people must be exposed to the substance and at a level necessary to cause an adverse effect on health. The hallmark of toxicity is the concept of a dose-response relationship, that is, the severity of the toxic effect is proportional to dose. Exposure can occur via the oral, dermal or inhalational routes (or a combination of these) and the rate and degree of absorption, tissue distribution, metabolism and elimination of the substance all impact on its toxicity.

101. Toxicity may result from a single exposure (acute toxicity) or due to repeated long-term exposure (chronic toxicity). The adverse health effect(s) may be reversible or irreversible and there is likely to be variability within the population with regard to how people are affected by a substance; some people may be relatively sensitive while others may be refractory. Factors contributing to such variability include genetic polymorphisms, age, gender and pre-existing diseases (Aldridge et al. 2003).

102. There are various *in vitro* and *in vivo* experimental testing methods used to assess toxicity, with laboratory animals commonly used as models for people. For chemicals and drugs, a toxicological assessment typically covers acute toxicity, eye and skin irritation, skin sensitisation, repeat-dose toxicity, reproductive toxicity, developmental toxicity, genotoxicity, carcinogenicity and neurotoxicity. Human data from clinical trials, occupational exposure studies and poisoning incidents add further to the toxicological database of a substance.

103. For the risk assessment of naturally-occurring agents (ie those that are endemic in the environment) consideration needs to be given to the background level of exposure to people

(in addition to the intrinsic toxicity of the substance). Therefore, the risk will actually be incremental to this background level of exposure and must take into consideration the range (variability) of the concentration/amount of the substance in the environment.

104. For the purposes of the risk assessment of GMOs, an assessment of toxicity would take into consideration the encoded protein from the introduced gene(s), any end-product(s) produced by the biological activity of the protein(s), the toxicity of the GMO(s) and the source organisms of the genes.

105. Baseline information on the toxicity of non-GM wheat to people is given in the document *The Biology and Ecology of Bread Wheat* (*Triticum aestivum L. em Thell.*) in *Australia* (OGTR 2005b), which was produced in order to inform the risk assessment process for licence applications involving GM wheat plants. This document is available at <www.ogtr.gov.au>.

106. As the proposal is a 'proof of concept' field trial, no toxicity studies have been carried out using the proteins encoded using the introduced genes or the GM wheat lines.

107. The possibility that exposure of people to proteins encoded by the introduced genes for drought tolerance in the GM wheat plants may result in a toxic reaction is considered. Routes of exposure to the proteins encoded by the introduced genes could include ingestion or contact with GM plant materials such as wheat grain, or inhalation of plant materials from wheat plants such as pollen.

Event 1: Ingestion of GM plant materials produced by the wheat lines containing the introduced genes

108. The introduced genes for drought tolerance present in the GM wheat lines are the same or similar to those present in other plants, such as corn, or microorganisms, eg *Streptomyces hygroscopicus* or *Saccharomyces cerevisiae*. People are exposed to the proteins encoded by the introduced genes for drought tolerance, or similar proteins, through normal diet or the environment (see Chapter 1, Section 5.4). There are no reports of toxicity associated with the proteins encoded by the introduced genes (see Chapter 1, Section 4.3.4).

109. FSANZ is responsible for human food safety assessment and FSANZ approval would be required before products from the GM wheat lines could be used in human food. Ingestion of materials from the GM wheat lines containing the introduced proteins is not expected to occur from the proposed release. The applicant does not intend to use GM plant materials (including wheat grains or their products) from the proposed release in human food or as animal feed. Information on the toxicity of the GM wheat plants may be needed for a future large scale or commercial release.

110. Therefore, **no risk is identified** and the potential for toxicity for people as a result of ingestion of GM plant materials produced by the wheat lines containing the introduced genes will not be assessed further.

Event 2: Contact with, or inhalation of, GM plant materials produced by the wheat lines containing the introduced genes

111. The proteins encoded by the introduced genes for drought tolerance, or similar proteins, are present in common plant species or microorganisms and are not known to be toxic to people (see Chapter 1, Sections 4.3.4 and 5.4). Therefore they are expected to be of low acute dermal and inhalation toxicity.

112. People working with the GM wheat plants would be exposed primarily to the outer waxy cuticle layer at the plant surface, which is essentially free of protein. However, dermal and inhalation exposure to proteins (including the proteins encoded by the introduced genes

for drought tolerance) may occur via pollen or when the plant cells have been damaged or broken. Therefore, workers at the trial site may come into contact with the proteins encoded by the introduced genes during handling and/or processing the GM wheat.

113. The applicant has proposed containment measures to limit the spread and persistence of the GM wheat, including a 10 m monitoring zone and a 490 m isolation zone. Although wheat pollen is wind dispersed, it is relatively heavy and falls rapidly, at approximately 60 cm per second from a plant height of 1 m (Lelley 1966), which restricts its dispersal distance (de Vries 1971; Anand & Sharma 1974). A majority of studies suggest that more than 90% of wheat pollen falls within 3 m of the source (reviewed by Hedge & Waines 2004). In addition, compared to other grasses, the production of pollen by wheat is limited, approximately 10 and 2.5% compared to that of rye and maize, respectively (de Vries 1971). Therefore, exposure to GM pollen would be limited to workers associated with the field trial.

114. Since the proposed release is of small size and short duration, the frequency and duration of contact with the proteins encoded by the introduced genes is expected to be limited. The applicant does not intend to use GM plant materials (including wheat grain or plant products) from the proposed release in human food or animal feed, but to incorporate any plant material remaining on the trial sites after harvest into the soil to promote decomposition.

115. Therefore, **no risk is identified** and the potential for toxicity for people as a result of contact with, or inhalation of, GM plant materials produced by the wheat lines containing the introduced genes will not be assessed further.

2.2 Production of a substance allergenic to people

116. Allergenicity is the potential of a specific substance 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). During the induction or sensitisation phase of an allergic response, a person is exposed for the first time to the substance, which results in a primary immune response and a persistent state of heightened immune responsiveness to the substance. Subsequent challenges (ie exposures) result in a powerful and exaggerated immune response, which can lead to adverse health effects in people. For the purposes of the risk assessment of GM plants, a potential allergen may be a protein or an end-product generated through the biological activity of the protein.

117. Allergic reactions following ingestion or inhalation of a substance are mediated by IgE antibodies (ie they are an immediate-type hypersensitivity reaction). In contrast, allergic reactions following dermal exposure are associated with a cell-mediated immune response (type IV hypersensitivity). However, such a reaction following dermal exposure, particularly to high molecular weight compounds such as proteins, is uncommon as the skin is an effective barrier to penetration (Arts et al. 2006; Holsapple et al. 2006).

118. There continues to be considerable discussion in the scientific literature on the characteristics of protein allergens and whether one can predict allergenic potential based on these characteristics. For the assessment of transgenic food crops, the source of the candidate gene and whether it is derived from a source known to be allergenic is an important characteristic as is its reactivity to sera from patients known to be allergic to the source material (Kimber et al. 1999). Other characteristics commonly used to predict a protein's allergenicity include amino acid sequence homology with known human allergens, the stability of the protein, resistance to digestion in the gastrointestinal tract and post-translational glycosylation (Metcalf et al. 1996; Huby et al. 2000).

119. Baseline information on the allergenicity of non-GM wheat to people is given in the document *The Biology and Ecology of Bread Wheat (Triticum aestivum L. em Thell.) in Australia* (OGTR 2005b), which was produced in order to inform the risk assessment process for licence applications involving GM wheat plants. This document is available at <www.ogtr.gov.au>.

120. As the proposal is a 'proof of concept' field trial, no allergenicity studies have been carried out on the proteins encoded by the introduced genes or the GM wheat lines.

121. The possibility that exposure of people to proteins encoded by the introduced genes for drought tolerance in the GM wheat plants may result in an allergic reaction is considered. Routes of exposure to the proteins encoded by the introduced genes could include consumption of food containing wheat grain or products (such as pollen in honey) or dermal contact with materials from wheat plants such as straw or pollen.

Event 3: Ingestion of GM plant materials produced by the wheat lines containing the introduced genes

122. Some of the introduced genes have some of the characteristics of allergens (as discussed in Chapter 1 Section 4.3.5). All of the proteins predicted to be encoded by the introduced genes are in the molecular weight range identified as being typical of allergens (15-70 kDa) and three of the six predicted proteins possess at least one putative N-glycosylation site (Asp-X-Ser/Thr) typical of allergens.

123. However, the proteins encoded by the introduced genes for drought tolerance have no significant homology with known allergens listed in a public allergen database and an extensive search of the literature did not identify any reports of allergenicity associated with these or similar proteins (see Chapter 1, Section 4.3.5). People are already exposed to the same or similar proteins through normal diet or the environment. Taken together, the weight of available evidence suggests that it is unlikely that ingestion of GM plant materials would cause allergic reactions in people due to the presence of proteins encoded by the genes for drought tolerance.

124. The applicant does not intend to use GM plant materials (including wheat grain or plant products) from the proposed release in human food or as animal feed. FSANZ approval would be required before products from the GM wheat lines could be used in human food. Information on the allergenicity of the GM wheat plants may be needed for a future large scale or commercial release.

125. Therefore, **no risk is identified** and the potential for allergic reactions in people as a result of ingestion of GM plant materials produced by wheat lines containing the introduced genes will not be further assessed.

Event 4: Contact with, or inhalation of, GM plant materials produced by the wheat lines containing the introduced genes

126. Non-GM wheat pollen is known to cause allergies (hay fever), however, as discussed in Event 3, it is unlikely that the proteins encoded by the introduced genes will increase the allergenic potential of the GM wheat lines.

127. As mentioned in Event 2, there would be some contact between a small number of people and the GM plant materials containing the proteins encoded by the introduced genes during the release. However, human contact would be limited because wheat pollen dispersal distances are minimal, and containment measures including the use of an isolation zone have been proposed (see Event 2, Paragraph 113).

128. In addition, the proposed release is of small size and short duration, and the applicant does not intend to use GM plant materials (including wheat grain or plant products) from the proposed release in human food or animal feed, but to incorporate any plant material remaining on the trial sites after harvest into the soil to promote decomposition.

129. Therefore, **no risk is identified** and the potential for allergic reactions in people, resulting from contact with GM plant materials (including pollen) produced by wheat lines containing the introduced genes, will not be further assessed.

2.3 Production of a substance toxic to organisms other than people

130. General information on toxicity mechanisms and toxicity testing methods has been provided in Section 2.1.

131. Baseline information on the toxicity of non-GM wheat is given in the document *The Biology and Ecology of Bread Wheat* (*Triticum aestivum L. em Thell.*) in Australia (OGTR 2005b), which was produced in order to inform the risk assessment process for licence applications involving GM wheat plants. This document is available at <www.ogtr.gov.au>.

132. As the proposal is a 'proof of concept' field trial, no toxicity studies have been carried out using the proteins encoded by the introduced genes or the GM wheat lines.

133. A range of organisms may be exposed directly or indirectly to the proteins (and enzymatic products) encoded by the introduced genes for drought tolerance. 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 or degrade it (vertebrates, insects, fungi and/or bacteria).

Event 5: Direct or indirect ingestion of GM plant materials produced by the wheat lines containing the introduced genes, by vertebrates, invertebrates or microorganisms

134. There are no reports of toxicity associated with the proteins encoded by the introduced genes (see Chapter 1, Section 4.3.4). Furthermore, vertebrates, invertebrates and microorganisms would already be exposed to the introduced proteins or very similar proteins in the environment (see Chapter 1, Section 5.4). Information on the toxicity of the GM wheat plants may be needed for a release of a future large scale or commercial release.

135. Livestock, wildlife (including mammals), birds and fish may be exposed through direct feeding on the GM wheat or indirectly through consumption of other organisms which have fed on the GM wheat plants.

136. Although wheat pollen is wind dispersed, it is relatively heavy and falls rapidly (Lelley 1966), which restricts its dispersal distance (de Vries 1971; Anand & Sharma 1974). In addition, compared to other grasses, the production of pollen by wheat is limited, approximately 10 and 2.5% compared to that of rye and maize, respectively (de Vries 1971). Similarly, the wheat grains are heavy and not easily dispersed by wind. Therefore, exposure of animals to wheat grain or pollen as a result of dispersal by wind would be limited.

137. The applicant has proposed containment measures to limit the spread and persistence of the GM wheat, including a distance of at least 50 m between the trial and the nearest natural waterway, further limiting the amount of GM wheat materials entering aquatic habitats. The level of exposure of aquatic species to the GM wheat lines is expected to be very low.

138. Vertebrate pests of wheat include kangaroos, rabbits, mice, rats and birds (including emus). The applicant has indicated that sightings of kangaroos or emus at the proposed site in the local government area of Horsham are rare. Sightings of emus at the proposed site in the local government area of Mildura are also rare, although kangaroos may be present.

139. Exposure of larger animals including livestock would be limited by the use of a 1.2 m high fence around each trial site, as proposed by the applicant. Exposure of rodents would be limited by the use of a 10 m monitoring zone of reduced plant cover around the trials. Reduced plant cover has been reported to be a deterrent to the movement of mice (AGRI-FACTS 2002).

140. Invertebrates, including beneficial insects, could be exposed directly to the GM wheat lines containing the proteins encoded by the introduced genes through feeding on the plants, seeds or pollen, or via the soil when GM wheat tissues decompose. Exposure of soil invertebrates to the proteins encoded by the introduced genes could also occur as a result of root exudations. Exposure could also occur indirectly, through consumption of other organisms that have fed on the GM wheat plants.

141. Microorganisms, particularly soil micro-organisms, would be exposed to the GM wheat lines containing the proteins encoded by the introduced genes during growth and decomposition of plant material, possibly as a result of root exudations. Root exudation has been observed in GM corn (Saxena et al. 1999; Stotzky 2000) and GM cotton (Gupta et al. 2002). Root breakage could also lead to the release of the introduced proteins into the soil.

142. The applicant does not intend to use GM plant materials (including wheat grain or plant products) from the proposed release in animal feed, but to incorporate any remaining plant material remaining on the trial sites after harvest into the soil to promote decomposition. Although this may prolong exposure of soil organisms to GM wheat material, the proposed release would be of small size and short duration and therefore the frequency and duration of contact with, or ingestion by, vertebrates, invertebrates and microorganisms, of the proteins encoded by the introduced genes and the GM wheat plant materials is expected to be limited.

143. Therefore, **no risk is identified** and the potential for toxicity for organisms other than people as a result of direct or indirect ingestion of the GM plant material containing the proteins encoded by the introduced genes will not be assessed further.

2.4 Spread and persistence of the GM wheat in the environment

144. Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens. Weediness in Australia is often correlated with weediness of the plant, or a close relative, elsewhere in the world (Panetta 1993; Pheloung et al. 1999). The likelihood of weediness is increased by repeated intentional introductions of plants outside their natural geographic range that increase the opportunity for plants to establish and spread into new environments, eg escapes of commonly used garden plants (Groves et al. 2005).

145. Characteristics in plants that are generally associated with weediness include prolonged seed dormancy, long persistence of seeds in the soil, germination under a broad range of environmental conditions, rapid vegetative growth, short lifecycle, very high seed output, high seed dispersal and long-distance seed dispersal (Keeler 1989; Keeler et al. 1996).

146. Baseline information on the spread and persistence of non-GM wheat is given in the document *The Biology and Ecology of Bread Wheat (Triticum aestivum L. em Thell.) in Australia* (OGTR 2005b), which was produced in order to inform the risk assessment process

for licence applications involving GM wheat plants. This document is available at www.ogtr.gov.au.

147. 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, it 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, very high seed output, high 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 therefore have little seed dormancy (OGTR 2005b). Modern wheat cultivars are not recognised as problematic weeds in Australia, and there have been no reports of bread wheat becoming an invasive pest in Australia or overseas.

148. The applicant states that this is a 'proof of concept' field trial. Therefore, the ability of the GM wheat plants to withstand drought stress or other environmental stresses in the field throughout different stages of their lifecycle, as compared to commercially available wheat cultivars including those with high drought tolerance, is unknown.

149. The possibility that the proteins encoded by the introduced genes for drought tolerance or herbicide tolerance may result in increased spread and persistence of the GM wheat is considered. Scenarios that could lead to increased spread and persistence include expression of the introduced genes conferring drought tolerance and herbicide tolerance, tolerance to other abiotic or biotic stresses, or dispersal of GM plant materials. These events could lead to increased exposure to vertebrates (including people), invertebrates and microorganisms.

Event 6: *Expression of the introduced genes improving the survival of the GM wheat through enhanced drought tolerance*

150. The GM wheat lines contain introduced gene constructs for drought tolerance, which if successful would confer enhanced tolerance to drought stress. 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 weediness of the GM wheat lines.

151. However, the survival of the GM wheat plants would still be limited by temperature, low intrinsic competitive ability, nutrient availability, pests and diseases and other environmental factors that normally limit the spread and persistence of wheat plants in Australia (Slee 2003; Condon 2004).

152. Several commercial cultivars with tolerance to drought are already available in Australia. For example, the variety Gladius, released by Australian Grain Technologies in February 2007, produces yields 20-30% higher than the benchmark variety Yitpi, which may be grown as a non-GM variety during the proposed trial (Wheeler 2007). Wyalkatchem, which may also be grown during the proposed trial, is another variety that performs well under low rainfall conditions. The GM wheat lines in the proposed release were derived from the wheat cultivar Bobwhite 26, which is considered to be of lower quality than most commercial cultivars (Bhalla et al. 2006). The GM wheat lines are therefore unlikely to be more competitive than existing elite varieties, even if an increase in drought tolerance is achieved.

153. In addition, the release would be of limited size and short duration and the applicant proposes a number of measures to limit the spread and persistence of the GM wheat lines

proposed for release, including hand-harvesting to avoid seed spillage and monitoring of the trial sites for a period of at least two years, including irrigation to promote germination of seeds and destruction of any volunteer plants (see Chapter 1, Section 2.3).

154. Therefore, **no risk is identified** and the potential for the increased spread and persistence (weediness) of wheat as a result of the expression of the introduced genes for drought tolerance will not be further assessed.

Uncertainty

155. As this is a 'proof of concept' field trial, the effect of the introduced genes for drought tolerance on the GM wheat lines is unknown in the field. Also, the ability of the GM wheat plants to withstand drought stress throughout different stages of their lifecycle, as compared to commercially available wheat cultivars, is unknown. Data on drought tolerance relating to weediness may be required to assess possible future applications involving larger scale or commercial releases if any of these GM wheat lines are selected for further development. However, the data are not required for this release because the trial is limited in size, duration and locations and the applicant has proposed other measures to limit the spread and persistence of the GM wheat.

Event 7: Expression of the introduced genes improving the survival of the GM wheat through enhancement of abiotic stress tolerances (other than drought tolerance) or biotic stress tolerances

Abiotic stress tolerances

156. The GM wheat lines express genes that encode proteins that are expected to enhance drought tolerance. Plants respond to different abiotic stresses often through an interconnecting series of signalling and transcription controls. Therefore, the regulatory nature of the introduced genes may mean that the encoded proteins could also confer tolerances to other environmental stresses, which could lead to increased spread and persistence of the GM wheat plants. This could lead to the spread and persistence of the GM wheat lines if these environmental stresses were the main limiting factors.

157. Other enhanced abiotic stress tolerances may improve the ability of the GM wheat lines to survive, or have improved performance, at lower and higher growing temperatures. It is also possible the plants could tolerate higher soil salinity, or have increased seed dormancy, viability, and/or improved seedling germination rates under stress (other than drought stress).

158. There are examples of genes isolated from other organisms, belonging to the same gene families as two of the introduced genes, that confer enhanced abiotic stress tolerances other than drought stress to GM plants. It should be noted that when a gene is expressed in different plant species the same effect(s) on phenotype does not always eventuate (eg Oh et al. 2005). Therefore, these genes may not confer enhanced stress tolerance, other than drought stress tolerance, to the GM wheat plants.

159. Tolerance to chemicals such as herbicides is another form of abiotic stress tolerance. 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. The applicant stated that the herbicide tolerance was used during the initial selection of transformed plants in the laboratory. The applicant does not intend to apply glufosinate ammonium during the field trial.

160. The potential effects of the *bar* gene and its product was considered in detail in previous assessments, including for DIRs 021/2003 and 062/2005 (commercial releases). RARMPs for those DIR licences are available from the OGTR or from the website

<<http://www.ogtr.gov.au>>. No adverse effects on humans, animals or the environment have been reported from these releases. An extensive search of the literature did not reveal any reports that would implicate the *bar* gene or its product in any stress tolerances other than the herbicide glufosinate ammonium (CAB Abs, Current Contents, Ovid MEDLINE(R), Ovid MEDLINE(R) in-Process and other non-indexed citations; keywords: bar gene AND stress tolerance; PAT protein AND stress tolerance).

161. 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 (see Chapter 1, Section 4.3.6). Therefore, the genetic modification is not expected not confer a selective advantage to the GM wheat lines.

162. In addition, in the unlikely instance where the plants may have enhanced tolerances to several environmental stresses, 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.

Biotic stress tolerances

163. The genes for drought tolerance could potentially enhance resistance of the GM wheat lines to pests, diseases and pathogens. This could lead to a spread and persistence of the GM wheat lines if diseases and pathogens were the main limiting factors.

164. There are examples of genes isolated from other organisms, belonging to the same gene family as one of the introduced genes, that have been shown to both positively and negatively affect disease and pathogen resistance of plants when expressed as an introduced gene. However, the exact mechanism for the effects on pathogen resistance is not known.

165. The introduced genes for drought stress tolerance, or homologs other than the aforementioned, are not known to confer enhanced biotic tolerances.

Conclusion

166. As discussed above, wheat is not considered a problem weed in Australia, and its spread and persistence is limited by multiple factors. In addition, the release would be of limited size and short duration and the applicant proposes a number of measures to limit the spread and persistence of the GM wheat lines proposed for release (see Chapter 1, Section 2.3).

167. Therefore, **no risk is identified** and the potential for the spread and persistence (weediness) of wheat as a result of the expression of the introduced genes resulting in stress tolerances other than drought tolerance will not be further assessed.

Uncertainty

168. This is a 'proof of concept' field trial and, therefore, the ability of the GM wheat plants to withstand abiotic and biotic stress tolerances throughout different stages of their lifecycle as compared to commercially available wheat cultivars is unknown. Data on abiotic/biotic stress tolerances of the GM wheat lines, additional to that for enhanced drought stress tolerance, may be required to assess possible future applications involving larger scale or commercial releases if any of these GM wheat lines are selected for further development. However, the data are not required for the proposed release because the trial is limited in size, duration and locations and the applicant has proposed other measures to limit the spread and persistence of the GM wheat.

Event 8: *Dispersal of GM plant materials, including seed, by various means, including animals, extreme weather conditions or spillage of seed during transport or storage*

169. Pests of wheat may inadvertently spread GM wheat seeds or plant materials. The Western Australian Department of Agriculture (2004) has identified the pests of wheat including birds (including emus), which feed on developing seeds and seedlings and also ripening grain; kangaroos and rabbits, which graze on wheat plants; rats and mice, which eat seedlings, seed and ripening grain; and Arthropods including insects. The GM wheat could also be inadvertently spread from the release site during transport or storage of GM wheat seed, or through adverse weather resulting in flooding. If GM wheat seed were spread from the release site it could result in establishment and persistence of the GM wheat in the environment.

Dispersal of GM plant materials by birds

170. Damage to wheat crops by birds has been noted in Australia and around the world (Temby & Marshall 2003; Davies 1978; Jarman & McKenzie 1983; Jones 1987; Massam 2000; Massam 2001; Coleman & Spurr 2001). Birds are more likely to eat the GM wheat or grain on site rather than carry it elsewhere for storage or consumption.

171. In Australia, birds, such as the sulphur-crested cockatoo, ducks, tree sparrows, house sparrows, long-billed corellas, galahs and emus, are known to cause damage to cereal crops. Birds such as cockatoos damage the cereal crop most during germination in autumn, but may feed on the crop at different times including grain ripening (Temby & Marshall 2003). When feeding on seed, cockatiels appear to prefer softer, younger seed to harder, mature seed (Jones 1987).

172. Emus feed on a great variety of plant material, but prefer succulent foods, such as fleshy fruits, rather than drier items (Davies 1978). Emus have been shown to disperse seeds (Calvino-Cancela et al. 2006), however germination rates are generally very low (Rogers et al. 1993; McGrath & Bass 1999). Viable seed from *Avena sativa*, a grass from the same subfamily as wheat (Pooideae), was detected in emu droppings (Calvino-Cancela et al. 2006). It has been stated that seeds of wheat will also germinate after passage through an emu's digestive system, although no experimental evidence was provided (Davies 1978). The applicant has indicated that sightings of emus at the proposed trial sites are rare.

173. An extensive search of the literature did not identify any reports of other birds transporting and dispersing wheat seed (eg through the digestive tract or taking panicles containing viable seed) or seedlings from wheat crops, only reports of consumption (Western Australian Department of Agriculture 2003). The GM wheat lines proposed for release are in white wheat parental backgrounds, which have a thin seed coat (Hansen 1994) and are readily digested by birds (Yasar 2003).

Dispersal of GM plant materials by vertebrates other than birds

174. Dispersal of GM plant materials by vertebrates other than birds is also possible. Kangaroos, rabbits, mice and rats are known pests of wheat (Hill et al. 1988; Western Australian Department of Agriculture 2004). As with birds, it is far more likely that these animals would eat wheat seed on site rather than carry the seed to another location. Wheat lacks seed dispersal characteristics such as stickiness, burrs, and hooks, which contribute to seed dispersal via animal fur (Howe & Smallwood 1982).

175. The possession of small dormant seeds 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,

and are therefore likely to be easily broken down in the digestive system of mammals (Hansen 1994). However, viable wheat seeds have been found in deer dung (Malo & Suárez 1995), and 30% of wheat grain fed to cattle is excreted whole and undamaged (Kaiser 1999), suggesting there is the potential for livestock in the area to disperse viable wheat seed after consumption. Consumption and dispersal of the GM wheat seeds by larger animals would be limited by the use of a standard height (1.2 m) fence around each trial site.

176. Kangaroos are reported to damage grain crops by feeding on seedlings or trampling mature plants. Eastern grey kangaroos, for example, may feed on young green cereal crops when native grasses are dry and producing no new growth (Hill et al. 1988). It has been reported that kangaroo densities in the grain growing district of western Victoria were low and significant numbers on crops would not be expected (Hill et al. 1988). The applicant has indicated that sightings of kangaroos at the proposed site at Horsham are rare, but they may be present near the proposed site at Mildura.

177. Like kangaroos, rabbits prefer soft, green, lush grass (Myers & Poole 1963) and select the most succulent and nutritious plants first (Croft et al. 2002). Although viable seeds from a variety of plant species have been found in rabbit dung, viable wheat seeds were not among them (Malo & Suárez 1995). In a study that looked at the germination of seeds on dung from cattle, red deer, sheep, hare, rabbit and red grouse, the number of germinations was least on rabbit dung (Welch 1985). Similarly, a study that looked at viable grass seeds in dung from cattle, pronghorn and rabbit, found few seedling populations of any species emerged from rabbit dung (Wicklow & Zak 1983).

178. The main rodent pest in Australian wheat crops is the house mouse (*Mus domesticus*), causing average annual losses to Australian agricultural crops of US\$10 million (ACIAR 2003). Rodents are opportunistic feeders and their diet can include seeds, the pith of stems and other plant materials (Caughley et al. 1998). Rodents may eat seeds, thus destroying them, at the seed source or they may hoard seed (AGRI-FACTS 2002), thus GM wheat seeds could potentially be removed from the proposed trial site.

179. Caughley et al. (1998) indicate that the average territory size of mice varies between breeding and non-breeding seasons, from 0.015 to 0.2 hectares respectively, whereas others have suggested a much smaller territory of 3 to 10 m in diameter (AGRI-FACTS 2002). The applicant proposes to surround the GM wheat trial with a 10 m monitoring zone of reduced plant cover to reduce movement of mice into the trial site. Reduced plant cover has been reported to be a deterrent to the movement of mice (AGRI-FACTS 2002).

Dispersal of GM plant materials by invertebrates

180. A variety of insects are likely to feed on the wheat crop, however, it is unlikely that most of these would contribute to the dispersal of material from the GM wheat plants beyond the trial sites. It is possible that ants may remove seeds for underground storage, but to depths where germination is highly unlikely. Although there are differences in ant behaviour and territory size across species, seed dispersal occurs at a local scale, such that seeds are usually only moved a few metres (Cain et al. 1998; Peters et al. 2003). Maximum seed dispersal distances by ants in Australia and the rest of the world are typically less than 40 m, with a mean dispersal distance of 0.96 m (Berg 1975; Beattie 1982; Gómez & Espadaler 1998). Therefore, GM wheat seed is unlikely to be removed beyond the trial sites or monitoring zones.

Dispersal of GM plant materials through extreme weather conditions

181. Generally, wheat seeds are large and heavy and therefore are not easily dispersed long distances by wind. However, severe weather conditions (eg flooding) could lead to the

dispersal of GM wheat materials, including seed. Dispersal of wheat seed would be the only way in which the GM wheat lines could spread effectively as wheat does not generally propagate vegetatively (as reviewed by Tanzarella & Greco 1985).

182. The applicant proposes a number of containment measures to limit the spread and persistence of the GM wheat plants, including hand-harvesting all plant material to minimise seed spillage, and cleaning of trial sites within 14 days of harvest and incorporating any remaining plant material into the soil to promote decomposition. Dispersal of GM wheat seed is further reduced because the proposed release is a small scale trial of limited duration which is to be located at least 50 m away from natural waterways. Neither of the sites proposed for the trial is prone to flooding.

Dispersal of GM plant materials during transport and storage

183. In the course of the proposed dealings the applicant proposes to transport seed to and from the release sites, cultivate GM wheat plants, store all GM wheat seed harvested from the crop and collect GM plant materials for research purposes, laboratory research or possible future release of promising lines (subject to additional approvals). Accidental spillage or dispersal of GM plant materials, especially seed, in the course of these dealings could allow the GM wheat plants to spread and persist in the environment.

184. The applicant proposes a number of containment measures to limit dispersal of the GM wheat seed, including hand harvesting, destruction of GM plant materials not required for future study or release, incorporating any plant material remaining on the trial sites after harvest into the soil to promote decomposition, and transportation and storage of GM plant materials according to OGTR guidelines.

185. Therefore, any spillage of seed during transport to and from the release sites or while in storage would be rare. Any incident involving spillage of GM seed is expected to be readily controlled through cleaning and monitoring of the site of the spill.

Conclusion

186. Dispersal of GM wheat plants outside of the trial sites would be limited by the containment measures proposed by the applicant and by the small scale and short duration of the trial.

187. In addition, the opportunity for any adverse outcome from any such rare occurrence is further diminished by the need for appropriate environmental conditions for germination, survival and persistence of any few escaped seeds. The spread and persistence of the GM wheat plants outside of the trial sites would also be limited by multiple factors including temperature, low intrinsic competitive ability, nutrient availability, and pests and diseases. Therefore, dispersal of any GM wheat seed is not expected to result in the establishment of wheat plants.

188. In the highly unlikely event of plant establishment, weediness is unlikely to occur as wheat lacks most characteristics that are common to many weeds (Keeler 1989). Modern wheat cultivars are not recognised as problematic weeds in Australia, and there have been no reports of bread wheat becoming an invasive pest in Australia or overseas.

189. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of dispersal of GM seed or other GM plant materials by animals, extreme weather conditions, or during transport or storage, will not be further assessed.

Event 9: Exposure of vertebrates (including people), invertebrates or microorganisms to materials produced from the GM wheat lines containing the introduced genes

190. The potential for increased spread and persistence of the GM wheat lines in the environment was addressed in Events 6 and 7 and no risk was identified. However, in the highly unlikely instance of this occurring, spread and persistence of the GM wheat plants in the environment could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the proteins encoded by the introduced genes for drought tolerance.

191. An adverse outcome could occur if the proteins encoded by the introduced genes for drought tolerance were toxic or allergenic for people. The potential for the proteins causing toxic or allergic reactions in people was assessed in Sections 2.1 and 2.2 and no risk was identified.

192. Organisms other than people may be exposed directly, through feeding on the GM wheat plants or indirectly through eating organisms that have fed on or degrade the GM wheat plants as a result of spread and persistence of the GM wheat in the environment. These organisms include vertebrates, invertebrates and microorganisms. The potential for toxicity of proteins encoded by the drought tolerance genes to organisms other than people was considered in Section 2.3 and no risk was identified.

193. In addition, the chain of events that would lead to increased exposure of vertebrates, invertebrates and microorganisms depends on the least likely event to occur, the spread and persistence of the GM wheat lines outside of the proposed trial sites (discussed in Section 2.4). The release would be of limited size and short duration and the applicant proposes a number of measures to limit the spread and persistence of the GM wheat lines in the environment (see Chapter 1, Section 2.3).

194. Therefore, **no risk is identified** and the potential for toxicity or allergic reactions in people or other organisms as a result of spread and persistence of the GM wheat lines in the environment will not be further assessed

2.5 Gene flow by vertical gene transfer

195. 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).

196. Baseline information on vertical gene transfer of non-GM wheat is given in the document *The Biology and Ecology of Bread Wheat (Triticum aestivum L. em Thell.) in Australia* (OGTR 2005b), which was produced in order to inform the risk assessment process for licence applications involving GM wheat plants. This document is available at www.ogtr.gov.au.

197. As the proposal is a 'proof of concept' field trial, no gene transfer studies have been carried out on the GM wheat lines.

198. Expression of the introduced proteins as a result of gene transfer could confer tolerance to drought or other environmental stresses, which may result in increased spread and persistence of the recipient plant compared with the non-GM parent. Gene transfer through cross pollination could occur to other wheat plants, or other sexually compatible plants, that are in the areas proposed for release.

Event 10: Expression of the introduced genes in other wheat plants

199. Both bread wheat and durum wheat (*Triticum turgidum* ssp. durum) are grown in Australia, while other species of *Triticum* are not known to be present. Inter- and intraspecific hybrids between durum wheat and bread wheat can occur naturally under field conditions (Matus-Cadiz et al. 2004). In Victoria, durum wheat is grown only on a small scale.

200. Wheat is largely cleistogamous, which means that pollen is shed before the flower opens (Frankl & Galun 1977) and thus wheat is primarily self-pollinating with low rates of out-crossing. Any out-crossing that may occur is facilitated by wind dispersal. Generally, wheat flowers lack nectaries to attract insects and produce relatively small amounts of pollen (Eastham & Sweet 2002) and the role of insects in cross-pollination is considered to be minimal (Glover 2002).

201. Gene transfer from the GM wheat lines to other wheat plants (eg volunteer wheat plants or commercial wheat crop) can only occur if they are in very close proximity and flower synchronously. Compared to other grasses, the production of pollen by wheat is limited, approximately 10 and 2.5% compared to that of rye and maize, respectively (de Vries 1971). In addition, wheat pollen does not remain viable for long periods. Under field conditions, wheat pollen has a viable lifespan of less than 30 minutes (OECD 1999b). Field conditions including temperature, relative humidity and wind intensity have a great influence on pollen viability and pollen movement.

202. There have been no reports of intraspecific pollen-mediated gene flow in bread wheat beyond 300 m, and no reports of pollen-mediated gene flow between bread wheat and durum wheat beyond 40 m (Matus-Cadiz et al. 2004). A majority of studies suggest that more than 90% of wheat pollen falls within 3 m of the source (reviewed by Hedge & Waines 2004). Under Australian conditions, pollen-mediated gene flow from GM wheat was observed only at low frequencies (0.012% and 0.0037%) over short distances (less than 12 m; Gatford et al. 2006).

203. The applicant proposes a 490 m isolation zone between the GM wheat trial and the nearest planting of wheat. This isolation zone will be monitored for *Triticum* species during the release and any found will be destroyed. Thus, the potential for gene flow from the GM wheat lines to other wheat plants will be severely limited.

204. If hybridization between the GM wheat and other bread or durum wheats did occur, the resulting hybrid GM wheat would contain the same introduced genes as the GM wheat lines currently proposed for release. The expression of these introduced genes may provide the hybrid GM wheat with an advantage in some environmental conditions relative to non-GM wheat. However, as discussed in Section 2.4, the introduction of these genes is unlikely to either alter the many other characteristics associated with weediness or overcome the other environmental conditions which normally limit the spread and persistence of wheat in Australia.

Conclusions

205. As discussed above, multiple factors would limit the transfer of genes from the GM wheat lines to other wheat plants and the potential for weediness of these plants.

206. In addition, the applicant proposed a release of limited size and short duration and a number of measures to limit the spread and persistence of the GM wheat lines. These include post harvest monitoring of the trial sites for at least 24 months following the final harvest and the destruction of any volunteers.

207. Therefore, **no risk is identified** and the potential of expression of the introduced genes for drought tolerance in other wheat plants leading to weediness will not be assessed further.

Event 11: Expression of the proteins encoded by the introduced genes in other sexually compatible plant species as a result of gene transfer

208. Transfer and expression of the introduced genes in sexually compatible species could confer a selective advantage under drought or other environmental stress conditions. This could result in spread and persistence of sexually compatible species in the environment.

209. There are few species outside the *Triticum* genus (eg *Aegilops cylindrica*, *A. ovata*, *A. biuncialis*, *Hordeum marinum*, and *Secale cereale*) that are both sexually compatible with wheat and known to form hybrids under natural conditions. Generally, crosses with wheat as the female parent are easier than the reverse. A number of natural barriers exist that preclude hybrid formation between wheat and wild relatives. These natural barriers include: asynchronous flowering, gametic, zygotic or endosperm incompatibility, and reduced hybrid fitness or hybrid sterility. The barriers arise mainly from the fact that wheat and its wild relatives have distinctly different sets of genomes. The chromosomes of these different genomes do not pair during gamete formation in the F1 hybrids or may result in developmental instability, which reduces the hybrid's ability to survive in parental habitats (Hedge & Waines 2004). Most reported hybrid formation between wheat and other species resulted from human intervention using artificial conditions such as the application of hormones or embryo rescue (Knobloch 1968; Maan 1987; Ellstrand et al. 1999; Eastham & Sweet 2002). Further discussion regarding hybridisation between bread wheat and other species can be found in *The Biology and Ecology of Bread Wheat (Triticum aestivum L. em Thell.) in Australia* (OGTR 2005b).

210. Of the species that might hybridise with bread wheat under natural conditions, few are known to be present in Australia. *Aegilops* spp are recognised as quarantine weeds in Australia and are not known to be present. Rye (*Secale cereale* L.) and sea barley (*Hordeum marinum* Huds.) are the only two sexually compatible species capable of hybridising with wheat under natural conditions which may be present at, or near, the proposed release site.

Gene transfer to Rye (*Secale cereale* L.)

211. Rye is considered weedy, particularly under some cropping systems in some areas of the USA (Stump & Westra 2000). However, Groves et al. (2003) rated rye lower than wheat as a potential weed in agricultural areas of Australia. Rye, like wheat, is a domesticated crop species and as such does not possess many of the characteristics associated with weediness, such as seed dormancy or long distance seed dispersal mechanisms. It is unlikely that expression of the introduced genes in rye will alter the intrinsic characteristics which currently limit the spread and persistence of rye. Furthermore, expression of the *bar* gene is not expected to confer any selective advantage in rye for the reasons outlined in event 2.4.6, and because glufosinate ammonium is not registered for use on rye.

212. A number of conditions need to be met for successful hybridisation between rye and the GM wheat to occur. First, the rye and the GM wheat would have to be in close proximity and flower synchronously for pollination to occur. Second, to be fully fertile, there would have to be spontaneous doubling of the chromosome number in the hybrid plant (the chemical colchicine is used to double chromosome number in artificial crosses between rye and wheat). Third, for introgression of the introduced genes into the rye genome, back crossing needs to occur, so the hybrid would have to grow in close proximity to and flower synchronously with rye in subsequent years. Fourth, each backcross needs to result in offspring with some level of fertility to allow for subsequent backcrossing to occur.

213. There are non-peer reviewed reports of natural hybrids forming between bread wheat and rye in Canada (Hedge & Waines 2004). In contrast, there have been no natural hybrids between these species reported in peer-reviewed studies in Europe (Eastham & Sweet 2002) or the USA (Hedge & Waines 2004). If hybridisation between the GM wheat and rye did occur it is unlikely that the hybrid would be fertile (Hedge & Waines 2004). Hybrid sterility may explain why hybridisation between wheat and other sexually compatible species appears to be restricted to the F1 generation with little evidence of subsequent introgression (Eastham & Sweet 2002). Crosses with wheat as the female parent are easier to obtain than the reverse, and usually result in hybrids that are completely male sterile (Hedge & Waines 2004).

Gene transfer to Sea Barley (*Hordeum marinum* Huds.)

214. *H. marinum* is an annual weed in southern Australia including Victoria (Walsh & Entwisle 1994) but it is not considered a noxious weed in Australia (Parsons & Cuthbertson 2001). Hybridisation would require synchronicity of flowering with the GM wheat lines to enable cross-pollination and gene flow to occur. The applicant has indicated that *H. marinum* has not been observed near the trial sites.

215. There is one report of possible hybridisation in nature between bread wheat and *H. marinum*. Guadagnuolo et al. (2001) used RAPD (random amplified polymorphic DNA) markers to search for evidence of introgression from cultivated bread wheat to adjacent naturalised populations of sea barley. Seed was harvested from the sea barley population, but no F1 hybrids were found among germinated seedlings, suggesting there had been no hybridisation during the growing season. However, one of the *H. marinum* plants in the naturalised population (from which seed was collected) generated a few RAPD markers which were specific to bread wheat. This particular plant had nine of the possible 40 RAPD markers specific to bread wheat and was morphologically indistinguishable from the other *H. marinum* plants in the area. All other *H. marinum* plants analysed had RAPD markers specific to sea barley.

216. The authors suggested that hybridization between the two species had occurred at some previous time and that subsequent back crossing between the hybrid and pure *H. marinum* could lead to the introgression of wheat DNA into sea barley (Guadagnuolo et al. 2001). However, bread wheat (*T. aestivum*) is a hexaploid (AABBDD) containing the A, B, and D genomes and these genomes are shared with many other *Triticum* or related species (OGTR 2005b). It is probable that a portion of the RAPD markers are specific to each of the three different genomes of bread wheat. Thus, for example, hybridisation between *H. marinum* and *T. boeoticum* (AA), *T. turgidum* (AABB), *Aegilops tauschii* (DD), *Ae. cylindrica* (CCDD), or others, may account for the results obtained by Guadagnuolo et al. (2001). The authors could find no evidence for further introgression of the wheat DNA into the *H. marinum* population, suggesting that a difference in ploidy levels is likely to have rendered the putative hybrid sterile.

217. If hybridisation did occur between the GM wheat lines and sea barley, it is unlikely that introgression of the introduced genes from the GM wheat into the genome of sea barley would occur. Sea barley, a diploid, (genome formula XX) does not share homologous chromosomes with bread wheat, a hexaploid, (genome formula AABBDD), thus regular pairing of homologous chromosomes would not occur and recombination (ie introgression) would be highly unlikely. Similarly, the lack of pairing and differences in ploidy would likely result in hybrid sterility. The putative sea barley x bread wheat hybrids showed limited introgression and apparent hybrid sterility (Guadagnuolo et al. 2001).

Potential for gene flow

218. As discussed in Event 10, wheat is primarily self-pollinating with low rates of outcrossing. A majority of studies suggest that more than 90% of wheat pollen falls within 3 m of the source (reviewed by Hedge & Waines 2004). There have been no reports of hybridisation between bread wheat and other sexually compatible species beyond 40 m (Matus-Cadiz et al. 2004).

219. The applicant has proposed surrounding the GM wheat trial with a 10 m monitoring zone free of any sexually compatible species. The applicant also proposes surrounding the monitoring zone with a 490 m isolation zone, in which no rye, sea barley or wheat plants could be grown. The lack of proximity between the GM wheat trial and any sea barley or potential planting of rye will further limit the restricted potential for pollen flow and gene transfer to these species.

Conclusion

220. Thus, **no risk is identified** and the potential for increased spread and persistence or weediness due gene transfer and expression of the introduced proteins in other sexually compatible plant species will not be assessed further

Event 12: Presence of the introduced regulatory sequences in other wheat plants or sexually compatible plant species as a result of gene transfer

221. All of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to wheat plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. The impacts from the introduced regulatory elements are equivalent and no greater than the endogenous regulatory elements.

222. Transfer of genetic material to wheat or other sexually compatible plant species was assessed in Events 10 and 11 and no risk was identified.

223. The applicant proposes to conduct post harvest monitoring of the GM wheat trial sites for 24 months and to destroy any volunteers found on the sites or monitoring zones.

224. Therefore, **no risk is identified** and the expression of the introduced regulatory sequences in other wheat plants or other sexually compatible plant species as a result of gene transfer will not be assessed further.

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

225. Transfer of genetic materials to unrelated species (horizontal gene transfer) that could result in the transfer of the introduced genes for water efficiency or their associated regulatory elements to other plants, animals and/or microorganisms is considered.

Event 13: Presence of the introduced genes, or the introduced regulatory sequences, in unrelated organisms as a result of gene transfer

226. Transfer of the introduced regulatory sequences and/or the introduced genes for drought tolerance, antibiotic resistance (*bla* gene) or herbicide tolerance (*bar* gene), from the GM wheat plants to sexually incompatible plants, animals or microorganisms (horizontal gene transfer) could only occur rarely without human intervention.

227. Most gene transfers have been identified through analyses of gene sequences (Worobey & Holmes 1999; Ochman et al. 2000). In general, gene transfers are detected over evolutionary time scales of millions of years (Lawrence 1999). Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman et al. 2000). In contrast, transfers

of plant genetic materials to other microorganisms such as bacteria, viruses or fungi have been exceedingly rare.

228. Transfer of the regulatory sequences to other organisms could alter the expression of endogenous genes in unpredictable ways. However, all of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to wheat plants. The transfer of either endogenous or introduced regulatory sequences could result in adverse unpredictable effects. As there is no difference between those two events, this does not represent a novel adverse outcome as a result of the genetic modification.

229. Horizontal gene transfer has been examined 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 other sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. Reports of horizontal gene transfer from plants to bacteria occurring during laboratory experiments have not only relied 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). Horizontal gene transfer is not expected to produce any adverse outcomes during this proposed limited release.

230. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of horizontal gene transfer will not be further assessed.

2.7 Unintended changes in biochemistry, physiology or ecology

231. A single plant gene can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Single genes inserted into a plant by genetic modification can also result in pleiotropy. Certain types of genes have a higher likelihood of causing pleiotropic effects. These genes include those that encode proteins that are involved in the regulation of the transcription of a number of genes (eg transcription factors, signalling compounds). It is therefore necessary to evaluate GM plants for unintended pleiotropic effects, such as changes in agronomic characteristics, which may be a consequence of the gene insertion.

232. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropic effects (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. Therefore, unintended changes in phenotype, as well as mutations, can occur upon transformation that are similar to those in conventional breeding and mutation breeding (Bradford et al. 2005a; Cellini et al. 2004). Recent results using proteomics have indicated that, in potatoes, there are fewer changes between GM and non-GM potatoes than between different conventionally bred varieties (Catchpole et al. 2005; Lehesranta et al. 2005).

233. Possible 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.

234. Unintended pleiotropic effects might result in adverse outcomes such as toxicity or allergenicity; weediness, 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. 2005a). Additionally, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

Event 14: Altered biochemistry, physiology or ecology of the GM wheat lines resulting from expression of the introduced genes

235. Biochemical, physiological or ecological changes to the GM wheat lines proposed for release could occur either as a result of the expression of the introduced genes for drought tolerance or of the transformation process itself.

236. Due to the predicted higher regulatory functions of proteins encoded by some of the introduced genes, various biochemical pathways of the GM wheat plants could be affected, 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 and Ecology of Bread Wheat* (*Triticum aestivum L. em Thell.*) in *Australia* (OGTR 2005b).

237. Thus far, exposure to plant materials from the GM wheat lines has been limited to a few workers maintaining these plants in the glasshouse. The applicant has reported no adverse outcomes from exposure to the GM wheat plant materials.

238. Accumulated experience with genetic modification of plants indicates that the process has little potential for unexpected outcomes that are not detected and eliminated during the routine process of selecting plants that are morphologically similar to the conventional plant except for the new properties (Bradford et al. 2005b). Additionally, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

239. The applicant has observed no unintended or secondary effects in the GM wheat lines grown under glasshouse conditions, and has stated that the growth characteristics of the GM wheat lines are similar to conventional non-GM wheat. During this limited and controlled release, the applicant proposes to measure the agronomic performance of the GM wheat lines, and adverse effects, if any, would most likely be detected during the trial and the lines eliminated from further development. Furthermore, the trial is limited in size, duration and locations, none of the GM wheat plant materials are intended for use in human food or animal feed, and the applicant proposes other measures to limit the spread and persistence of the GM wheat.

240. Therefore, **no risk is identified** and the potential for weediness and/or toxicity and/or allergenicity to people and other organisms, as a result of unintended changes in biochemistry, physiology or ecology will not be further assessed.

Uncertainty

241. Insertion of new genes and traits by conventional breeding or genetic modification can result in unintended and unexpected changes. Data on the potential pleiotropic effects of the

genetic modifications on GM wheat lines selected for further development, and how these may affect potential weediness, toxicity and allergenicity, would be required to assess any future applications for a larger scale or commercial release of any of these GMOs.

2.8 Unauthorised activities

Event 15: Use of GMOs outside the proposed licence conditions (non-compliance)

242. 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.

243. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of unauthorised activities will not be further assessed.

Section 3 Risk estimate process for identified risks

244. The hazard identification process considered 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.

245. Fifteen events were identified and assessed whereby the proposed release of the GM wheat lines might give rise to harm to people or the environment.

246. These 15 events 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; produce unintended changes in the biochemistry, physiology or ecology of the GM wheat lines; or alter characteristics that may impact on spread and persistence of the GMOs. In addition, consideration was given to the opportunity for gene flow to other organisms, and its effects if this occurred.

247. All events were characterised in relation to both the magnitude and probability of harm in the context of proposed controls to limit the spread and persistence of the GMOs. Detailed consideration of the fifteen events for this particular field trial demonstrated that none gave rise to an identified risk that required further assessment. The principal reasons include:

- the scale of the trial is limited in both area and duration
- containment, monitoring and disposal measures proposed by the applicant to limit the spread and persistence of the GM wheat plants
- 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 the introduced genes in the environment and lack of known toxicity or allergenicity from these proteins
- limited capacity of the GM wheat lines to spread and persist outside the release sites
- limited ability and opportunity for the GM wheat lines to transfer the introduced genes to commercial wheat crops or other sexually related species.

248. Therefore, as no risks to the health and safety of people or the environment were identified from the proposed limited and controlled release of the GM wheat lines, the level of risk is considered to be **negligible**.

Chapter 3 Risk management

249. 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 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 are to 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

250. 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.

251. Section 63 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.

252. 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

253. Australia's gene technology regulatory system operates as part of an integrated legislative framework. 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 agencies⁸.

254. 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.

255. FSANZ is responsible for human food safety assessment, including GM food. As the trial involves early stage 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

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. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>>.

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.

256. 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. Glufosinate ammonium is not registered for use on wheat and APVMA approval would be required to apply the herbicide to the GM wheat lines.

257. No other approvals are required.

Section 3 Risk treatment measures for identified risks

258. 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*, which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

259. These events were considered in the context of the scale of the release (a maximum total area of 0.315 hectares over one growing season (2007/08) on two sites in the Victorian local government areas of Horsham and Mildura), the containment measures and agricultural practices proposed by the applicant and the receiving environment (see Chapter 1, Section 5).

Section 4 General risk management

260. Containment measures consistent with the risk assessment context have been imposed to limit the trial to the size, duration and locations requested by the applicant, which are summarised below (Section 4.1).

4.1 Summary of proposed licence conditions

4.1.1 Measures to limit and control the proposed trial

261. A number of licence conditions have been imposed to limit and control the trial, including requirements to:

- maintain a 10 m monitoring zone around each release site free of any related species and with reduced plant cover to limit rodent refuges
- maintain an isolation zone of at least 490 m (not including the 10 m monitoring zone) around each release site free of any sexually compatible species
- enclose each site with a 1.2 m high fence with lockable gates
- locate the release sites at least 50 m away from natural waterways
- harvest the GM wheat plant material by hand 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
- following cleaning of sites, monitor for and destroy any GM wheat that may grow for at least 24 months and until the site is clear of volunteers for a continuous 6 month period.

262. DPI Victoria proposed to include rabbit proofing as part of the 1.2 m high fence surrounding each trial site, as an additional containment measure to prevent the possibility of dispersal of GM plant materials, including seed. However, seed dispersal by rabbits is not expected because rabbits prefer soft, green, lush grass (Myers & Poole 1963), and evidence

suggests that viable wheat seeds are unlikely to pass through the digestive system of rabbits (see Event 8). Therefore, rabbit proofing has not been imposed as a licence condition.

4.1.2 Measures to control other activities associated with the trial

263. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, June 2001; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have been imposed 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.

4.2 Other risk management considerations

264. 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
- monitoring for compliance.

4.2.1 Applicant suitability

265. 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.

266. Before making the decision to issue a licence for this application (DIR 071/2006), the Regulator considered the suitability of DPI Victoria to hold a licence.

267. Conditions in the licence 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.

268. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Compliance and contingency plans

269. The licence requires DPI Victoria to submit a plan detailing how it intends to ensure compliance with the licence conditions and document that compliance. This plan is required before the planting of the GM wheat lines commences.

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

271. 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 instrument 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

272. 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 this licence.

4.2.4 Reporting structures

273. 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.

274. 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.

275. 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 final destroying and cleaning at the end of the trial.

4.2.5 Monitoring for Compliance

276. 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.

277. 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.

278. In cases of non-compliance with licence conditions, the Regulator may also 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

279. The proposed trial involves early stage research intended to determine whether the introduced genes for drought tolerance enable normal plant growth with reduced amounts of

water without adversely affecting agronomic performance. The risk assessment identified additional information that may be required to assess an application for a larger scale trial, reduced containment conditions or a commercial release of any GM wheat lines that may be selected for further development. This would include:

- molecular characterisation of the GM wheat lines selected for possible future releases
- additional data on the potential toxicity and allergenicity of proteins encoded by the introduced genes for drought tolerance, and of plant materials from the GM wheat lines selected for possible future releases
- physiological and agronomic characteristics of the GM wheat lines indicative of weediness including measurement of altered reproductive capacity; tolerance to drought and other environmental stresses, including salinity; and disease susceptibility.

Section 6 Conclusions of the RARMP

280. The risk assessment concludes that this limited and controlled release of up to 30 GM wheat lines on a maximum area of 0.315 ha over one growing season in the Victorian local government areas of Horsham and Mildura poses **negligible** risks to the health and safety of people and the environment.

281. The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to contain the trial to the size, duration and locations requested by the applicant.

Section 7 DIR 071/2006 Licence

282. The licence DIR 071/2006 is available on the OGTR website <http://www.ogtr.gov.au/gmorec/ir.htm#table>, following the path to DIR 071/2006.

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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 authorities⁹ on application DIR 071/2006

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 the preparation of the consultation RARMP.

Issues raised relating to the Risk Assessment (considered in Chapter 2):

- Enhanced spread and persistence (weediness) of the GM wheat (Events 6, 7 and 8).
- Gene flow to other commercial wheat crops, related species or unrelated species (Events 10 to 13).
- Toxicity and/or allergenicity of the proteins (and enzymatic products) encoded by the introduced genes (Events 1 to 5).
- Dissemination of the GM wheat material beyond the intended areas for the proposed field trial (Events 8, 10 to 13, and 15).
- Potential unintended effects of the introduced genes (Event 14).

Issues raised relating to the Risk Management Plan (considered in Chapters 3 and 4):

- Containment measures
- Storage and transport procedures for the GM wheat
- Post-harvest monitoring and practices
- Disposal of GM plant materials not required for further research or approved plantings.

⁹ GTTAC, State and Territory governments, Australian Government agencies, the Minister for the Environment and Water Resources and Local councils where the release may occur.

Appendix C Summary of issues raised in submissions received from the public on application DIR 071/2006

One submission received from a member of the public is summarised below.

All issues relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence.

Issues raised relating to the Risk Assessment (considered in Chapter 2):

- Human health effects (Events 1 to 4)
- Gene flow to other commercial wheat crops (Event 10).

Issues that were outside the scope of assessments conducted under the Gene Technology Act 2000:

- Agricultural production benefits
- Marketing issue.

Appendix D Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 071/2006

The Regulator received 15 submissions from the public on the consultation RARMP, which are summarised in the table below in order of receipt. Six submissions supported the release while seven raised issues relating to risks to human health and safety and the environment that 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.

Issues: **AG:** agricultural production; **AL:** allergenicity; **AR:** antibiotic resistance; **B:** benefits of gene technology; **C:** consultation; **CCI:** confidential commercial information; **CP:** contingency plan; **EC:** economic issues **EN:** environmental risks; **GT:** gene transfer; **H:** human health and safety; **HT:** herbicide tolerance; **HU:** herbicide use; **L:** labelling; **M:** marketing concerns; **Mor:** moratoria; **Res:** further research; **RM:** risk management; **S:** segregation; **T:** toxicity; **UE:** unintended effects; **UP:** unintended presence; **W:** weediness

Other Abbreviations: **APVMA:** Australian Pesticides and Veterinary Management Authority; **FSANZ:** Food Standards Australia New Zealand; **GM:** genetically modified; **GMO:** genetically modified organism.

^a Submission from: **A:** agricultural/industry organisation; **IG:** Interest Group; **I:** Individual.

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
1	A	States that Australian wheat breeders have been breeding for drought tolerance for a long time and GM technology may be able to fast track this with novel genes. Providing it goes through the usual testing procedures, this could be worth millions of dollars and should be allowed to proceed.	AG, EC	Noted. Agricultural production benefits and economic issues are outside the scope of assessments required by the <i>Gene Technology Act 2000</i> (The Act).
2	IG	Supports the application for the following reasons: <ul style="list-style-type: none"> • Risks of this trial are small, applicant is a government department and trial will be conducted under controlled and rigorous conditions on government research facilities • Drought is a recurring phenomenon in the Australian wheat belt and is likely to increase • Limited techniques are available to farmers for risk management in response to drought • Better adapted varieties are a major factor in maintaining farm income, but Australian gene pool is limited and the use of GM opens up new possibilities and may reduce breeding times • Inspection, quarantine and segregation methods exist to allow GM and non-GM crops to coexist • To not support this application could result in reduced funding into this research • We need to maintain market competitiveness 	B, S, M	Noted. Agricultural production benefits, and segregation and marketing issues are outside the scope of assessments required by the Act.

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
3	A	Supports the application and accepts that the controlled trial poses negligible risks.	None	Noted.
		States that wheat is the most commonly used raw material in certain animal feeds. Recognises that the use of GM plant material in animal feeds presents no direct safety risk to the human food supply chain as consumed GM material is not transferred to meat, milk or eggs.	H	No GM plant material from this trial is permitted to be used in human food or animal feed.
		States that the Australian stockfeed and livestock industries are presently under pressure due to drought conditions and long term grain production capacity is a major limitation to industry growth.	AG	Noted. Agricultural production benefits are outside the scope of required by the Act.
4	I	Claims the organic industry can not afford to have gene constructs in the environment. States that there are other ways of breeding new plant varieties with improved water utilisation that don't involve potential dispersal of GM pollen. Claims that Tasmania regularly receives topsoil blown in from Victoria and SA so a 490 m isolation zone is not enough.	GT, UP	Wheat is predominantly self pollinating. Wheat pollen is relatively heavy and only remains viable for limited periods (<30 min). Most studies suggest that 90% falls to the ground within 3 m of the source. There have been no reports of pollen mediated gene flow in bread wheat beyond 300 m (Event 10). The RARMP concludes that risks to human health and safety or the environment from this release are negligible.
		Questions the timing of the assessment, with submissions closing 25 May but applicant proposing to plant in May. There is not enough time to adequately consider submissions.	C	The Act stipulates a statutory timeframe of 170 days for decision on DIR licence applications, which expired on 20 June 2007. The Regulator issued a licence for DIR 071/2006 on 13 June 2007. DPI Victoria could not start planting until after that time. All issues relating to the health and safety of people and the protection of the environment raised during the consultation process were thoroughly considered in the context of current scientific knowledge before finalising the RARMP and making the decision to issue the licence.
		Concerned that the trial has inadequate safeguards and the fact that no adverse impacts on the environment have been recorded from similar trials is no proof that there were none or that there won't be any.	EN	Each application for a DIR licence is assessed on a 'case by case' basis. The RARMP concludes that risks to human health and safety or the environment from this release are negligible. Licence conditions have been imposed to limit the release to the size, duration and locations proposed by the applicant.
5	I	States that Australian farmers need to stay at the technological edge. Claims resistance to GM is diminishing worldwide, markets are not differentiating and there is no premium for non-GM. States that drought is significant in Australia and may become more so with global warming, therefore drought tolerant wheats are an important tool.	M, AG	Noted. Agricultural production benefits and marketing issues are outside the scope of assessments required by the Act.

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
		Considers GM wheat is of low risk, chances of outcrossing are minimal and the consequences of releasing a drought tolerant plant low.	GT, W	Noted. The RARMP concludes that risks associated with gene flow are negligible, and that the overall risk to human health and safety or the environment from this release is negligible.
6	I	Claims consumers do not want GM in general and GM wheat specifically as it can't be segregated. Concerned that wheat is in everything in the supermarkets and consumers don't know what is GM.	S, L	No GM plant material from this trial is permitted to be used in human food or animal feed. Segregation at a commercial level is outside the scope of assessments required by the Act. However, licence conditions stipulate that plant material from the trial, including seed, must be harvested by hand and separately from other crops, and harvested material must be either destroyed or stored in a certified facility or other facility approved by the Regulator and transported according to OGTR Guidelines. Labelling of food is the responsibility of FSANZ.
		Concerned we are being manipulated by American advertising to believe that GM is wonderful, but we don't know if GM is safe yet. Claims consumers don't want to be guinea pigs in an American experiment but will have no choice. Claims scientists are concerned about the safety of GM foods and articles state more testing needs to be done. Asks why rats have to be force fed GM grain.	H	Each application for a DIR licence is assessed on a 'case by case' basis. The RARMP concludes that risks to human health and safety or the environment from this release are negligible. The risk assessment identified additional information that may be required to assess future applications to trial lines selected for further development. This information includes data on potential toxicity and allergenicity of proteins encoded by the introduced genes and of plant materials from the GM wheat lines (Ch 3, Section 5). No GM plant material from this trial is permitted to be used in human food or animal feed. FSANZ approval would be required before products from the GM wheat lines could be used in human food.
		Claims GM technology has no long term benefits and that after the first 2-3 years it costs more and will be controlled by a huge chemical company that is benefiting from increased herbicide use.	B, EC	Noted. Benefits of gene technology and economic issues are outside the scope of assessments required by the Act. The APVMA is responsible for the regulation of agricultural chemical use.

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
		This has not been advertised in the community.	C	The Act requires extensive consultation with a wide range of experts, agencies and authorities, and with the public via posting on the OGTR website and advertisements in the Commonwealth Gazette and a national newspaper. The Regulator consistently exceeds these requirements. Notifications of receipt of applications are sent to over 800 people and organisations that have registered on the OGTR mailing list. In addition, for this application, an invitation for public comment on the consultation RARMP was published in two national and several regional newspapers, including <i>The Weekend Australian</i> , <i>The Age</i> , <i>The Weekly Times</i> , <i>The Weekly Advertiser</i> , <i>The Wimmera Mail-Times</i> , the <i>Sunraysia Daily</i> , the <i>Mildura Midweek</i> , the <i>Mildura Independent Sunday Star</i> and the <i>Mildura Weekly</i> , as well as being again posted on the OGTR website and sent to people and organisations on the OGTR mailing list.
7	A	Supports application for the following reasons: <ul style="list-style-type: none"> • Risks inherent in this trial are small as it is being conducted under controlled and rigorous conditions on government facilities. Supports conclusion of RARMP that risks are negligible. • Wheat is grown in drought prone environment that may become more so under climate change scenario. • Better adapted varieties are important in maintaining farm incomes. • To not support this application would disadvantage farmers against competitors and would send a signal to investors to reduce funding in this area. • To not support this application would come at an unfortunate time in terms of review of State moratoria. 	AG, M, EC, Mor	Noted. Agricultural production benefits, marketing and economic issues are outside the scope of assessments required by the Act.
8	A	Notes the thorough approach taken by the OGTR and supports this application as the risks are negligible and Victorian research must be fostered. States that research into variety improvement is important, particularly this specific trial of drought resistance. Believes farmers should have the right to choose their technology.	AG, B	Noted.
		Acknowledges the consultation that was carried out on the application is important for open and informed debate and as an educational process.	C	Noted.
9	IG	Concerned about the health risk of GM products entering the human food chain.	H	No GM plant material from this trial is permitted to be used in human food or animal feed.
		Concerned about the commercial wisdom of GM products.	M	Marketing issues are outside the scope of assessments required by the Act.

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
		Considers OGTR assessments to be repetitive, and the bottom line of each states that since negligible risk is involved, risk management is reduced to recording location, size and duration of the trials.	RM	The Act requires that the risk assessment for every DIR licence considers the release as proposed by the applicant. The process commences with a detailed consideration of events that could lead to harm to people or the environment. Each hazard is then analysed, taking into account the risk context, which includes the location, size and duration of the trial, to determine whether a risk is identified for further assessment. The RARMP for the proposed release concludes that it poses negligible risks to people or the environment. However, a range of conditions have been imposed in the licence to ensure that the release is limited to the size, duration and locations requested by the applicant. They include measures to restrict the spread of the introduced genes; requiring that transport and storage of the GM plant materials are in accordance with OGTR guidelines; and monitoring for, and destroying, any volunteer plants on the release site for a minimum of 2 years and until the site is clear of volunteers for six months.
		Perceives little change in OGTR requirements from many years back, despite unintended presence of GM seeds in Australian commercial canola crops. Has OGTR undertaken an investigation into the source of this unintended presence? Has the role of field trials as a source of unintended presence been investigated?	UP	An investigation by the OGTR into the unintended presence of Topaz 19/2 (an event now approved by the Regulator for commercial release on human health and environmental safety grounds) in non-GM (conventional) 'Grace' canola did not identify any plausible link with field trials with GM canola containing the event conducted in Australia. The containment measures for different GM crop species are based upon knowledge of the biology and ecology of the parent organisms and a case by case assessment of the proposed dealings with the GMO. A number of licence conditions have been imposed to contain this GM wheat trial including isolation zones, fencing, hand harvesting, and monitoring for and destroying volunteers for a minimum of two years and until the site is clear of volunteers for six months.
10	IG	Considers US farmers to have had problems with GM crops due to: <ul style="list-style-type: none"> • Cross-pollination and seed drift (dispersal) 	GT	Wheat is predominantly self pollinating. Wheat pollen is heavy and only remains viable for limited periods (<30 min). Most studies suggest that 90% falls to the ground within 3 m of the source. There have been no reports of pollen mediated gene flow in bread wheat beyond 300 m (Event 10). Wheat seeds are also heavy and lack dispersal characteristics such as stickiness, burrs or hooks (Event 8). Licence conditions require a 490 m isolation zone around each trial site in which no sexually compatible species, including cultivated wheat, can be grown.

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
		<ul style="list-style-type: none"> Mix ups in seed supply resulting in unapproved GM grain being exported for human consumption. Australia has had an example of seed contamination (Grace canola) 	S	Licence conditions stipulate that plant material from the trial, including seed, must be harvested by hand and separately from other crops, and harvested material must be either destroyed or stored in a certified facility or other facility approved by the Regulator, and transported according to OGTR Guidelines.
		<ul style="list-style-type: none"> Herbicide resistance 	HT	The herbicide tolerance marker gene, <i>bar</i> , was used to select modified plants during initial research and development work in the laboratory. The applicant does not intend to apply glufosinate ammonium during the trial. Risks associated with this trait were assessed in Event 7 and no risk was identified. The use and safety of herbicides is the responsibility of the APVMA.
		Considers that GM food crops have not been proven safe for human consumption. Monsanto's MON863 corn is under review by FSANZ. Claims studies on GM soy, potatoes and field peas have resulted in adverse health effects in rats or mice. Also claims independent scientists and professional bodies have warned of health risks and point out that the consequences of consuming GM food are not known. Believes that in the absence of full labelling, the impact cannot be monitored or identified.	H, L	Each application is assessed on a case by case basis. No GM plant material from this trial is permitted to be used in human food or animal feed. FSANZ approval would be required before products from the GM wheat lines could be used in human food. Labelling of food is also the responsibility of FSANZ.
		Considers that antibiotic resistance markers may cause increased pools of antibiotic resistant bacteria.	EN, AR	The ampicillin resistance gene, <i>bla</i> , is widespread in the environment and in food. The <i>bla</i> gene was used to select for bacteria containing the desired genes in the laboratory. The <i>bla</i> gene is not expressed in the GM wheat lines as it is linked to a bacterial promoter that does not function in plants.
		Considers wheat to be an important crop and major export for Australia. Therefore, believes any risk is significant and urges OGTR to reject application.	EC	Noted. The RARMP concludes that risks to human health and safety or the environment from this release are negligible. Economic and trade issues are outside the scope of assessments required by the Act.
11	I	Dr Arden Andersen believes that GM food affects health by causing autoimmune responses. Although this trial is of a research nature, pressure for commercial release would follow if it is successful, which would probably override health concerns.	H	No GM plant material from this trial is permitted to be used in human food or animal feed. FSANZ approval would be required before products from the GM wheat lines could be used in human food. The risk assessment identified additional information, including data on toxicity and allergenicity, that may be required to assess an application for a larger scale trial, reduced containment conditions or a commercial release (Ch 3, Section 5).

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
12	IG	Concerned that the GM wheat has not been tested in any animal feeding trials or human health studies to determine toxic or allergenic events. Believes that transparent, long-term, generational animal feeding studies must be conducted before approval is granted.	H	No GM plant material from this trial is permitted to be used in human food or animal feed. The potential for toxicity and allergenicity for people was considered in Events 1-4 and no risk was identified from the proposed release. The risk assessment identified additional information that may be required to assess an application for a larger scale trial, reduced containment conditions or a commercial release, including data on toxicity and allergenicity.
		Believes that the field trial should not be permitted without evidence to support the supposition of negligible risk. Concerned that the public and the environment are offered no protection from the spread of GMOs due to wind, floods, bird and insect movement, or human error.	H, EN	Extensive scientific literature cited in the RARMP supports the conclusion that there is negligible risk to human health and safety or the environment from this GM wheat trial. The potential dispersal of the GMOs and the introduced genes via a range of mechanisms was considered in Event 8 and no risks were identified.
		Believe that buffer zones are insufficient to retain GM wheat pollen.	GT	Wheat is predominantly self pollinating. There have been no reports of pollen mediated gene flow in bread wheat beyond 300 m (Event 10). A 490 m isolation zone around each trial site in which no sexually compatible species, including cultivated wheat, can be grown has been imposed in the licence.
		Believes that there is a risk that the herbicide tolerance gene could be transferred to non-GM wheat varieties. This trial should not be approved until organisations/companies accept liability for this.	S, UP	Licence conditions have been imposed to limit the release, including a 490 m isolation zone in which no sexually compatible species, including cultivated wheat, can be grown.
		Considers that these GM plants are just as likely to succumb to insects and disease due to drought stress as non-GM varieties.	AG	Noted. The genetic modifications aim to improve drought tolerance and is not intended, or expected, to alter susceptibility to insect predation or disease compared to the parent organism.
		Believes that the herbicide should be tested for safety before approval of these GM wheat lines containing the herbicide tolerance gene.	HU	Noted. The applicant does not intend to apply glufosinate ammonium during the trial. The regulation of the use and safety of herbicides is the responsibility of the APVMA.
		Expects that farmers will have to participate in identity preservation, and therefore a field test needs to be developed to determine possible unintended presence of GM wheat in conventional crops.	S, UP	The licence requires the licence holder to submit a protocol that identifies the GMOs. Segregation issues are outside the scope of assessments required by the Act.

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
		As the OGTR is the only authority assessing the GM wheat, the following matters must be considered even though they are outside the remit of the OGTR: Australian Wheat Board does not support the commercialisation of GM wheat. There is no market for GM wheat and it should be labelled. No other country grows GM wheat commercially due to economic risk. The economic impact of allowing GM wheat to be commercialised must be assessed.	M, EC, L	The OGTR is part of an integrated regulatory framework. APVMA approval would be required before glufosinate ammonium could be applied to the GM wheat, and approval from FSANZ would be required for its use in food. Labelling of food is also the responsibility of FSANZ. Marketing and economic issues are outside the scope of assessments required by the Act.
13	I	The presence of putative N-glycosylation sites in three of the six proteins introduced for increased drought tolerance is of concern as glycosylation may contribute to allergenicity.	H, AL	Food allergens generally share a number of characteristics including amino acid sequence homology, high expression, stability, and are derived from an allergenic source. The presence of potential N-glycosylation sites may contribute to allergenicity but alone does not mean a protein will be allergenic. The proteins encoded by the introduced genes have no significant homology with known allergens. People are already exposed to the same or similar proteins through normal diet or the environment. The potential for allergic reactions in people was considered in Events 3 and 4 and no risk was identified. The risk assessment identified additional information that may be required to assess an application for a larger scale trial, reduced containment conditions or a commercial release, including data on allergenicity.
		Considers information on lack of sequence homology and extensive existing exposure via presence of the proteins in the environment to be inadequate for assessment of toxicity and allergenicity. Calls for animal feeding studies citing adverse effects observed in Irina Ermakova rat study and CSIRO GM pea study.	H, T, AL	No GM plant material from this trial is permitted to be used in human food or animal feed. The information available on toxicity and allergenicity was considered to be sufficient for an assessment of the risks that may be associated with the dealings proposed in this limited and controlled release. The risk assessment identified additional information that may be required to assess an application for a larger scale trial, reduced containment conditions or a commercial release, including data on toxicity and allergenicity.

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
		The presence of the PAT protein is of concern as there is not sufficient information given on the effect of consuming the PAT protein or the breakdown products of glufosinate ammonium. FSANZ has approved GM foods containing the <i>bar</i> or <i>pat</i> gene, but in most it is only the oil used.	H	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 to humans. Detailed descriptions of the results of these studies are available in the RARMPs for DIR 021/2003 and DIR 062/2005. Metabolism of glufosinate ammonium in plants modified with the <i>bar</i> gene has also been assessed in these RARMPs. The applicant does not intend to apply glufosinate ammonium during the trial. APVMA approval would be needed before the herbicide could be used on these plants. No GM plant material from this trial is permitted to be used in human food or animal feed.
		The RARMP states that these GM wheat lines are unlikely to be more competitive than existing elite varieties since it is agronomically inferior, so why proceed with these trials?	AG	The proposed release represents early stage, 'proof of concept' research to test whether any of the introduced genes improve drought tolerance under field conditions. The parent wheat line was used because it is relatively easy to transform. If promising results were obtained, further transformations in elite lines may be developed and trialled (subject to additional approvals).
14	A	Claims that the pet food consumer market (including export market) has poor sentiment towards GM products. Opposed to introduction of GM grains and wishes to maintain integrity of Australian pet food products.	M	Noted. Marketing issues are outside the scope of assessments required by the Act. No GM plant material from this trial is permitted to be used in human food or animal feed.
		Concerned that the creation of two classes of grain (GM and non-GM) will create unnecessary regulatory burden and cost.	S, EC	Noted. Segregation and economic issues are outside the scope of assessments required by the Act.
15	IG	Concerned that DPI may be unaware of unintended effects. If the unintended effects are long term and far away, who is responsible and liable? Suggests that ultimately the public will bear associated costs. Considers that the trial cannot be limited or controlled where living organisms are concerned in an open environment.	UE	It is a condition of all DIR licences that any unexpected or unintended effects of the GMO be reported to the Regulator. This trial is of small size and limited duration (one year only) so long term effects are extremely unlikely. Licence conditions have been imposed to restrict the release to the size, duration and locations requested by the applicant and include a requirement to monitor the trial site for at least two years and until the site is clear of volunteers for six months, to ensure that no GMOs persist.
		Considers that the RARMP has no scientific basis and offers no real protection to the public or environment.	H, EN	The Regulator's decision to issue a licence was based on a rigorous process of risk analysis with a focus on scientific evidence and extensive consultation with experts. A range of licence conditions have been imposed to limit and control the release to the size duration and locations proposed by the applicant.

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
		The CCI declaration is cause for concern. Considers that all information should be freely available.	CCI	The Act provides for commercially sensitive information to be protected. The CCI was made available to all experts and agencies prescribed for consultation under the Act.
		Considers that the contingency plan should be submitted with the application and not 30 days after the licence is issued.	CP	Details of contingency measures to rectify any unintended consequence if a hazard becomes evident during the course of the release were provided in Part 10 of the application, in addition to the requirement in the licence.
		States that the trials are proof of concept and therefore many outcomes or consequences are unknown, and there are too many unknown variables and vague statements (eg 'does not intend') in the application and RARMP. Believes that the Regulator should define these trials as high risk. Asks if they have been defined as negligible because there are no means for mitigation of unintentional events. Claims there is no definition for risk in the Risk Analysis Framework used by the Regulator.	H, EN	The purpose of the trial is to examine whether drought tolerance displayed in glasshouse experiments occurs under field conditions. Hence, the unknowns relate to the performance of the introduced genes under field conditions, not the Regulator's ability to ensure the release is limited and controlled. The Regulator's <i>Risk Analysis Framework</i> (RAF) explains the approach used to evaluate licence applications and to develop the RARMPs that form the basis of her decisions. The RAF defines risk as 'the chance of something happening that will have an undesired impact'. It contains a detailed discussion on estimation of risk. Note that impact in terms of the Act is the chance of harm to human health and safety, or the environment due to or as a result of gene technology. Risk is measured in terms of a combination of the likelihood that a hazard gives rise to an undesired outcome and the seriousness of that undesired outcome.
		Claims there is the possibility of GMOs spreading beyond the trial site, and the consequences of it entering the human food chain are unknown. Believes that the introduced genes and gene products will come in a different sequence or form than any already encountered in the environment, so risks associated with ingestion are high.	H	The RARMP concludes that the proposed release poses negligible risks to people or the environment. A range of licence conditions have been imposed to limit the release to the size, duration and locations proposed by the applicant, including measures to restrict the spread of the GMOs and introduced genes. No GM plant material from this trial is permitted to be used in human food or animal feed.
		Concerned that the parent organism is used for GM work but not human consumption.	H	The parent organism of the GM wheat lines is bread wheat, cultivar Bobwhite. Bobwhite is used for GM work because it is easily transformed. It is not generally favoured as a commercial variety as it is considered to be of lower quality.
		Considers wheat is weedy and cross pollinates (including bee pollination), therefore a 500 m isolation zone is insufficient.	W, GT	Wheat is not a problem weed in Australia. Wheat lacks most characteristics common to weeds, such as prolonged seed dormancy, seed dispersal mechanisms and rapid vegetative growth (Events 6-8). Wheat is predominantly self pollinating and there have been no reports of pollen mediated gene flow in bread wheat beyond 300 m (Event 10).

Sub. No.	Type ^a	Summary of issues raised	Issue	Comment
		Concerned about monoculture and use of chemicals, and how GMOs will affect this. Believes that any unintentional release of GMOs could destroy balance between farmer's inputs and outputs. Concerned that if an unintended presence is detected, farmers have no recourse by law or nature thereby exposing farmers to higher risks.	AG, HU	Issues of agricultural production are outside the scope of the assessments required by the Act. The use and safety of pesticides is the responsibility of the APVMA. Common law provides recourse for the recovery of damages for economic losses.
		Concerned about possible adverse effect that might only become evident in the future.	H, EN	This trial is of small size and limited duration and licence conditions have been imposed to limit the release to the size, duration and locations proposed by the applicant. The RARMP concludes that risks to human health and safety or the environment from this release are negligible.
		Concerned about effects of GMOs on soil bacteria, fungi and insects including feral bees.	T, EN	The potential for toxicity for organisms other than people, including soil organisms and insects, was considered in Event 5 and no risk was identified.
		Considers wheat to be unsuitable to plant in most of the country, experiments with wheat seeds will do nothing. Believes that GM drought tolerant wheat will not provide a quick fix to Australia's water problems, and industrialised farming, monocultures and inappropriate crops are the problem and all contribute to global warming.	AG	Agricultural production issues are outside the scope of assessments required by the Act.
		Asks how the GMO will respond to water logging in the event of a flood?	Res	This release involves 'proof of concept' research on the performance of the introduced genes in improving drought tolerance under field conditions. The risk assessment identified additional information that may be required to assess future applications to trial lines selected for further development. This information includes data on the physiological and agronomic characteristics of the GM wheat lines including tolerance to drought and other environmental stresses.