



Australian Government

Department of Health and Ageing

Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 077/2007**

**Limited and controlled release of wheat and barley
genetically modified for enhanced tolerance to
abiotic stresses or increased beta glucan**

Applicant: The University of Adelaide

June 2008

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Executive Summary

Introduction

The Acting Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of up to 30 wheat and barley lines modified for enhanced tolerance to certain abiotic stresses or for increased levels of beta glucan into the environment in respect of application DIR 077/2007 from The University of Adelaide.

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

The application

The University of Adelaide applied for a licence for dealings involving the intentional release of up to 30 lines of genetically modified (GM) wheat and barley on a limited scale and under controlled conditions. The GM wheat and barley lines have been modified for enhanced tolerance to different environmental stressors, such as high soil boron levels or drought, or for increased dietary fibre. The trial is proposed to take place at one site in the local government area of Marion, South Australia on a maximum total area of 400 m² between June 2008 and June 2009.

Up to four of the GM wheat lines and up to four of the GM barley lines contain one of two genes that are expected to confer increased drought tolerance. These genes are derived from wheat.

Up to three of the GM barley lines contain a gene that is expected to confer increased tolerance to toxic levels of boron in the soil. This gene is derived from barley.

Up to four of the GM barley lines contain a gene that is expected to confer increased resistance to environmental stressors. This gene is derived from wheat.

Up to fifteen lines of the GM barley contain one of three genes that are expected to increase the levels of dietary fibre in the leaves and grains of the mature barley plants. These genes are derived from barley.

All of the GM wheat and barley lines contain an antibiotic resistance selectable marker gene. The GM barley lines that contain the gene expected to confer increased tolerance to boron also contain an additional selectable marker gene. These genes were used as selective markers during the initial development of the GM plants in the laboratory.

The purpose of the trial is to conduct proof of concept research involving experiments with the GM wheat and barley lines to assess their agronomic performance under field conditions,

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

and to obtain tissue samples for subsequent analysis of characteristics such as gene and protein expression levels, and metabolite profiles. Some seed will be stored for possible future trials of promising lines, subject to further approval(s). The GM wheat and barley will not be used for human food or animal feed.

The University of Adelaide proposed a number of controls to restrict the dissemination or persistence of the GM wheat and barley lines into the environment that have been considered during the evaluation of the application.

Confidential Commercial Information

Some details, including the name of an introduced gene expected to enhance abiotic stress tolerance, the precise function of the gene product and its application, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge, advice received from a wide range of experts, agencies and authorities consulted on the RARMP, and submissions from the public².

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

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

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

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

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

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the eight events characterised in the risk assessment are considered to give rise to an identified risk that

² The Executive Summary dated June 2008 referred to “a submission from the public” this was a typographical error which was rectified in September 2008.

requires further assessment, the level of risk from the proposed dealings is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, a range of measures have been imposed to limit the release to the size, location and duration requested by the applicant, as these were an important part of establishing the context for assessing the risks.

The licence conditions require The University of Adelaide to **limit** the duration of the release to between June 2008 and June 2009 on a maximum total area of 400 m² at one site. The **control** measures to restrict the spread and persistence of the GMOs include preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with OGTR transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed³.

Conclusions of the RARMP

The risk assessment concludes that this proposed limited and controlled release of up to 30 GM wheat and barley lines on a total area of up to 400 m² over one growing season in the South Australian local government area of Marion poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

³ The licence for DIR 077/2007 is available on the OGTR website (<http://www.ogtr.gov.au/gmorec/ir.htm#table>) via the link to DIR 077/2007

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
<i>Bot1</i>	the gene encoding the boron transporter protein
CCI	Confidential Commercial Information as declared under section 185 of the <i>Gene Technology Act 2000</i>
cm	centimetre
cDNA	complementary Deoxyribonucleic Acid
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration (USA)
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GRAS	Generally Recognized As Safe
ha	Hectare
<i>hpt</i>	the gene encoding the protein hygromycin phosphotransferase
<i>HvCslF</i>	the gene from the <i>Hordeum vulgare</i> cellulose synthase-like F gene family
kb	kilobase
km	kilometre
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment level 2
RARMP	Risk Assessment and Management Plan
RNA	Ribonucleic Acid
<i>SacB</i>	the gene encoding the protein levansucrase
<i>TaDREB</i>	gene encoding a <i>Triticum aestivum</i> dehydration responsive element binding protein
TGA	Therapeutic Goods Administration

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Technical Summary

Introduction

The Acting Gene Technology Regulator (the Regulator) has made a decision to issue a licence (DIR 077/2007) to The University of Adelaide for dealings involving the intentional release of genetically modified (GM) wheat and barley lines into the Australian environment.

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

The application

The University of Adelaide applied for a licence for dealings involving the intentional release of up to 30 lines⁵ of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) on a limited scale and under controlled conditions. The GM wheat and barley lines have been genetically modified for increased abiotic stress tolerance or dietary fibre. The trial is authorised to take place at one site in the local government area of Marion, South Australia on a maximum total area of up to 400 m² over one season between June 2008 and June 2009.

The GM wheat and barley lines contain one of four genes derived from either wheat or barley which encode proteins that are expected to enhance their tolerance to different abiotic stressors.

Up to four of the GM wheat lines and up to four of the GM barley lines contain one of two drought responsive transcription factors (*TaDREB2* and *TaDREB3*) derived from wheat. These genes have been introduced into both wheat (cv. Bob White) and barley (cv. Golden Promise). GM plants from these lines are expected to have enhanced drought tolerance.

Up to three of the GM barley lines contain a boron tolerance gene (*Bot1*) derived from barley that has been introduced into barley (cv. Flagship). Plants from these lines are expected to display enhanced soil boron tolerance.

Up to four of the GM barley lines contain an abiotic stress tolerance transcription factor derived from wheat that has been introduced into barley (cv. Golden Promise), which is subject to a commercial confidential information declaration (see below). Plants from these lines are expected to display enhanced tolerance to abiotic stressors.

Up to fifteen of the GM barley lines contain one of three cellulose synthase-like F genes (*HvCslF4*, *HvCslF6* and *HvCslF8*) derived from barley that have been introduced into barley

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

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

(cv. Golden Promise). In glass house trials, plants from these lines displayed increased levels of beta glucan⁶ in the leaves and grain.

The GM wheat and barley lines also contain an antibiotic resistance marker gene, *hpt*, from the bacterium, *Escherichia coli*. The *hpt* gene encodes hygromycin phosphotransferase. Additionally, the three GM barley lines containing the *Bot1* gene also contain the selective marker gene, *SacB*, from the bacterium *Bacillus amyloliquefaciens* which encodes an enzyme (levansucrase) involved in sucrose metabolism. These genes were used as selective markers during the initial development of the GM plants in the laboratory.

The purpose of the trial is to conduct proof of concept research involving experiments with the GM wheat and barley lines to assess the agronomic performance of the lines under field conditions, and to obtain tissue samples for subsequent analysis of characteristics such as gene and protein expression levels, and metabolite profiles. Some seed will be saved for possible future trials of promising lines, subject to further approvals. The GM wheat and barley will not be used for human food or animal feed.

The University of Adelaide proposed a number of controls to restrict the dissemination or persistence of the GM wheat and barley lines and their genetic material into the environment. These controls were considered during the evaluation of the application.

Confidential Commercial Information

Some details, including the name of an introduced gene expected to enhance abiotic stress tolerance, the precise function of the gene product and its application, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment took into account information contained in the application, relevant previous approvals, current scientific knowledge, and advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP.

Reference documents on the parent organisms, ‘*The Biology of Triticum aestivum L. (bread wheat)*’ and ‘*The Biology of Hordeum vulgare L. (barley)*’ were produced to inform the risk assessment process for licence applications involving GM wheat and barley plants. These documents are available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

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

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

⁶ Beta glucan is a plant polysaccharide (carbohydrate) which forms part of the soluble fibre in cereal grains.

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

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

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

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM wheat and barley lines into the environment are considered to be **negligible**. Hence the Acting Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to the health and safety of people or to the environment⁷.

Risk management

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

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, a range of measures have been imposed to limit the release to the size, location and duration requested by the applicant, as these were an important part of establishing the context for assessing the risks.

Licence conditions to manage this limited and controlled release

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

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

- ♦ conduct the release at one site of up to 400 m² in the local government area of Marion, South Australia, between June 2008 and June 2009
- ♦ establish a 10 m monitoring zone around the trial site that is free of any related species and maintained in a manner that does not attract or harbour rodents
- ♦ maintain an isolation zone of at least 200 m around each trial site free of any sexually compatible species
- ♦ enclose the trial site within a 1 m high fence with lockable gates and placing rodent baits within the fenced area
- ♦ locate the trial site at least 50 m away from natural waterways
- ♦ harvest the GM wheat and barley 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
- ♦ clean all equipment, including clothing, used on the site
- ♦ after harvest, apply measures to promote germination of any wheat and/or barley seeds that may be present in the soil
- ♦ monitor the site for at least 24 months and destroy any wheat and/or barley plants that may grow until no volunteers are detected for a continuous 6 month period.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs, July 2007; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have also been proposed to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

Other regulatory considerations

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

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

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

Identification of issues to be addressed for future releases

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

- ♦ characterisation of the introduced genetic material in the plants, including gene copy number and genotypic stability, and additional information on the 50 kb insert present in some of the GM barley lines;
- ♦ additional data on the potential toxicity of plant materials from the GM wheat and barley lines;
- ♦ additional data on the allergenicity of proteins encoded by the introduced genes for enhanced tolerance to abiotic stresses or increased levels of beta glucan; and
- ♦ characteristics indicative of weediness including measurement of altered reproductive capacity; tolerance to environmental stresses; and disease susceptibility.

Suitability of the applicant

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

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 30 GM wheat and barley lines on a total area of up to 400 m² over one season in the South Australian local government area of Marion poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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Chapter 1 Risk assessment context

Section 1 Background

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

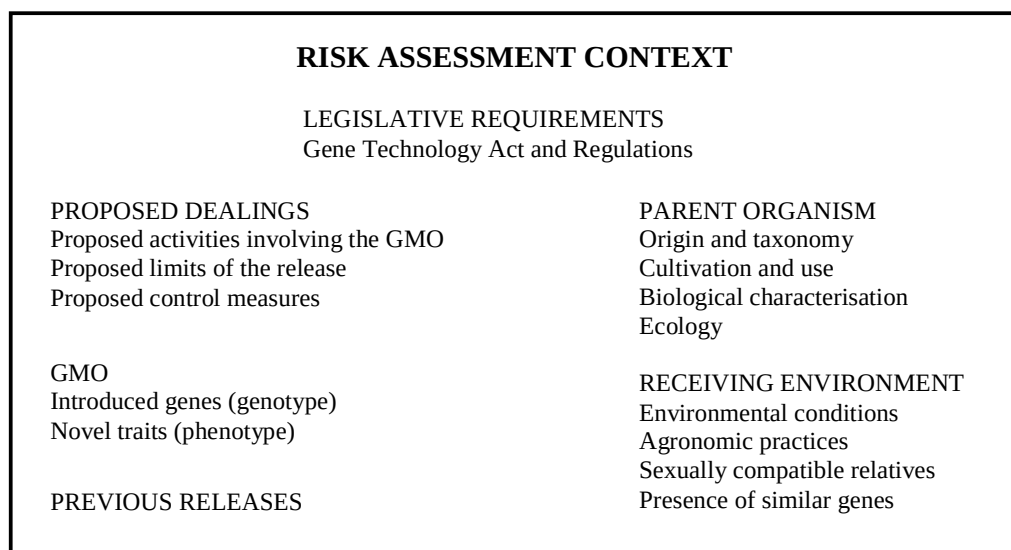


Figure 1 Components of the context considered during the preparation of the risk assessment

2. For this application, establishing the risk assessment context includes consideration of:
- the proposed dealings (Section 3.1)
 - the limits proposed by the applicant (Section 3.2)
 - the controls proposed by the applicant (Section 3.3)
 - characteristics of the parent organisms (Section 4)
 - the nature and effect of the genetic modification (Section 5)
 - the environmental conditions in the location where the release would occur (Sections 6.1 and 6.2)
 - relevant agricultural practices (Section 6.3)
 - the presence of related plants in the environment (Section 6.4)
 - the presence of the introduced or similar genes in the environment (Section 6.5)
 - any previous releases of these or other GMOs relevant to this application (Section 7).

⁹ 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 2007) <<http://www.ogtr.gov.au/pubform/riskassessments.htm>>.

Section 2 The legislative requirements

3. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Regulator must take into account, and with whom she must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions on licence applications. In addition, the *Gene Technology Regulations 2001* (the Regulations) outline matters the Regulator must consider when preparing a RARMP.
4. In accordance with section 50A of the Act, the Regulator has considered information provided in the application and is satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits on the size, location and duration of the release and controls have been proposed by the applicant to restrict the dissemination or persistence of the GMOs and their genetic material in the environment. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application qualifies as a limited and controlled release and the Regulator has prepared a RARMP for this application.
5. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Gene Technology Regulations (the Regulations), the Minister for the Environment, local council(s) where the release is proposed to take place, and the public. The advice from the prescribed experts, agencies and authorities and where it was taken into account, is summarised in Appendix B. Submissions received from members of the public and their consideration are summarised in Appendix C.
6. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a ‘significant risk’ to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d).

Section 3 The proposed dealings

7. The University of Adelaide proposes to release up to 30 genetically modified (GM) wheat and barley lines¹⁰ that have been modified for increased drought, boron or abiotic stress tolerance, or increased levels of beta glucan. The release would be conducted under limited and controlled conditions.

3.1 The proposed activities

8. The applicant has stated that the principal purpose of the proposed release is to conduct proof of concept research involving experiments to measure and assess agronomic factors such as establishment, plant height and yield under normal growing conditions; and to measure other characteristics in leaf and flower spike samples such as expression levels of the introduced genes and metabolite profiling for some plants. The GM wheat and barley plants will not be used for human food or animal feed.

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

9. The release is proposed to take place at one site on a farm owned by The University of Adelaide in the local government area of Marion, South Australia. The total area of the

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

release would be 400 m² and is proposed to take place during one growing season between June 2008 and June 2009. The trial site does not directly abut any public roads.

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

10. Only trained and authorised staff will be permitted access to the proposed location.

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

- locating the proposed trial site over 1000 m from the closest wheat or barley breeding sites and 50 km from the closest commercial wheat or barley crop
- surrounding the 400 m² trial site with a 1 m border of non-GM barley (Figure 2), a 5 m plant free zone and a 200 m zone free of wheat and barley plants
- surrounding the site with a 1 m high, 50 m by 50 m, cyclone fence and placing rodent baits within the fence
- keeping a 10 m zone surrounding the 1 m high cyclone fence and the remaining area within the fence (apart from the 400 m² trial site) mown to below 10 cm to reduce cover for rodents
- hand harvest plants to minimise GM seed spillage
- removing any GM plants that show a delay in flowering of more than one month over that of the non-GM parent plants
- analysing GM plant materials from the trial in a certified PC2 facility and then destroying the materials (except saved seed)
- destroying all (GM and non-GM) plant materials from the field trial by ploughing, irrigating the site one month after harvest and killing any volunteers with herbicide
- post harvest monitoring of the trial site for 24 months or until the site has been clear of volunteers for one growing season and destroying any volunteers
- transporting GM plant materials to and from the proposed trial site in accordance with OGTR transportation guidelines

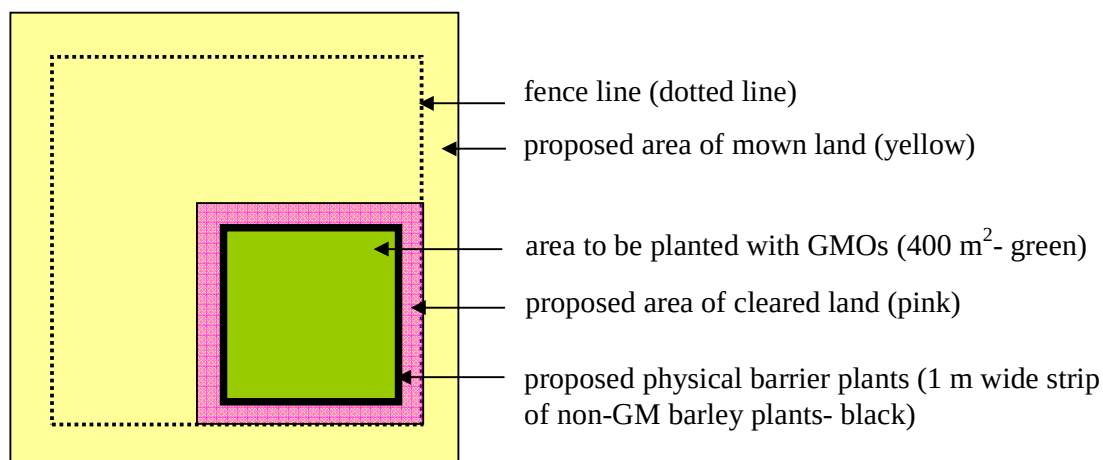


Figure 2 Schematic diagram of the proposed trial location including some proposed containment measures.

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

Section 4 The parent organism

13. The parent organisms are bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), both exotic to Australia. Commercial wheat cultivation occurs in the wheat belt from south eastern Queensland through New South Wales, Victoria, southern South Australia and southern Western Australia (OGTR 2008b). Commercial barley cultivation in Australia occurs in the wheat production areas in New South Wales, Victoria, Queensland, Western Australia and South Australia. A small amount of barley is also grown in Tasmania (OGTR 2008a).

14. The parental wheat cultivar is ‘Bob White’. This wheat cultivar is commonly used in genetic modification work because it is relatively easy to transform. It has also been used in conventional (non-GM) wheat breeding programs, as stated in DIR 071/2006. However, Bob White is not favoured as a commercial bread wheat as it is considered to be of lower quality than most of the current commercial cultivars (Bhalla et al. 2006).

15. The parental barley cultivars are ‘Golden Promise’ and ‘Flagship’. ‘Golden Promise’ is not grown commercially in Australia but is commonly used in genetic modification work. The barley cultivar ‘Flagship’ was a new commercial malting cultivar released in Australia in 2006 (ABB Grain 2006).

16. The barley cultivar ‘Golden Promise’ was derived from the ‘Maythorpe’ cultivar following modification by the use of gamma-ray irradiation. It is a semi-dwarf, malting cultivar that has been found to have greater tolerance to soil salinity than ‘Maythorpe’ (Forster 2001). While the precise genetic changes are not known, salt tolerance in ‘Golden Promise’ is a consequence of the plants’ ability to limit the uptake of salt from the soil and results in this cultivar having a higher grain yield than its parental cultivar. ‘Golden Promise’ is also reported to have some tolerance to drought as well (Forster 2001).

17. Further detailed information about the parent organisms is contained in the reference documents, *The Biology of Triticum aestivum L. (bread wheat)* and *The Biology of Hordeum vulgare L. (barley)*, that were produced to inform the risk assessment process for licence applications involving GM wheat and barley plants (OGTR 2008a; OGTR 2008b). The documents are available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

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

5.1 Introduction to the GMOs

18. Some details, including the name of an introduced gene expected to enhance abiotic stress tolerance, the precise function of the gene product and its application, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

19. The GM wheat and barley lines contain one of seven genes encoding products expected to enhance abiotic stress tolerance, including to drought and soil boron, or increase beta glucan levels in the leaves and grains. The genes introduced into the GM plants are derived from either wheat or barley, with the exception of the selectable marker genes (Table 1).

Table 1 The genes used to genetically modify wheat and barley

Gene	Accession No (GenBank)	Protein produced	Protein involved in	Source	Intended purpose
<i>TADREB2</i>	DC353852	Dehydration responsive element binding protein 2	Drought stress response	<i>T. aestivum</i>	Increased drought tolerance
<i>TADREB3</i>	DQ353853	Dehydration responsive element binding protein 3	Drought stress response	<i>T. aestivum</i>	Increased drought tolerance
<i>Bot1</i>	EF660436	Boron transporter	Boron transporter	<i>H. vulgare</i>	Increased boron tolerance
<i>transcription factor gene</i>	Not Available	Transcription factor	Abiotic stress tolerance	<i>T. aestivum</i>	Increased abiotic stress tolerance
<i>CsIF4</i>	EU267180	Cellulose synthase-like protein F4	Beta glucan synthesis	<i>H. vulgare</i>	Increased beta glucan synthesis
<i>CsIF6</i>	EU267181	Cellulose synthase-like protein F6	Beta glucan synthesis	<i>H. vulgare</i>	Increased beta glucan synthesis
<i>CsIF8</i>	EU267183	Cellulose synthase-like protein F8	Beta glucan synthesis	<i>H. vulgare</i>	Increased beta glucan synthesis
<i>hpt</i>	AAA92252	Hygromycin phosphotransferase	Antibiotic resistance	<i>Escherichia coli</i>	Selectable marker
<i>SacB</i>	X52988	Levansucrase	Sucrose metabolism	<i>Bacillus amylolique-faciens</i>	Selectable marker

20. Up to 30 GM wheat and barley lines are proposed for release. Each line was generated using a construct containing one of the seven different genes of interest (see Table 2). All GM wheat plants were generated using the biolistics transformation method and all GM barley plants were generated using the *Agrobacterium* mediated transformation method (see Chapter 1, Section 5.4).

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

Promoter	Gene of Interest	Marker Gene	Terminator	Species	Max lines per construct	Total no. of lines
dual 35S promoter (2 x 35S)	<i>TaDREB2</i>	<i>hpt</i>	<i>nos</i>	wheat	2	8
				barley	2	
2 x 35S	<i>TaDREB3</i>	<i>hpt</i>	<i>nos</i>	wheat	2	
				barley	2	
Endogenous promoter	<i>Bot1</i>	<i>hpt/SacB</i>	Endogenous terminator	barley	3	3
Ubi	<i>transcription factor gene</i>	<i>hpt</i>	<i>nos</i>	barley	4	4
2 x 35S	<i>CsIF4</i>	<i>hpt</i>	<i>nos</i>	barley	5	15
2 x 35S	<i>CsIF6</i>	<i>hpt</i>	<i>nos</i>	barley	5	
2 x 35S	<i>CsIF8</i>	<i>hpt</i>	<i>nos</i>	barley	5	
						30

5.2 The introduced genes, encoded proteins and end products

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

21. Up to four lines of GM wheat and four lines of GM barley contain one of two drought responsive transcription factors isolated from wheat.

22. The *TaDREB2* and *TaDREB3* genes are expected to increase the plants' tolerance to drought. The introduced genes encode drought responsive transcription factor proteins that contain an APETALA2 DNA binding domain (AP2) (Lopato et al. 2006). The AP2 domain was identified in a number of genes from *Arabidopsis* involved in gene expression regulation during vegetative growth and reproduction (Okamuro et al. 1997). *TaDREB3* has 77.7% amino acid sequence identity to a cold induced CBF (C-repeat binding factor) transcription factor (Lopato et al. 2006).

23. Both the *TaDREB2* and *TaDREB3* genes are expressed only at low levels in wheat plants grown under normal culture conditions, with a higher level of *TaDREB2* expression in seedlings. However, the *TaDREB2* gene was expressed at the highest levels in wounded leaves (Lopato et al. 2006). *Arabidopsis* plants have been modified to over express a constitutively active form of their *DREB2A* gene and the resulting GM plants were drought tolerant (unpublished data of Y. Sakuma, cited in Yamaguchi-Shinozaki & Shinozaki 2006).

5.2.2 Gene expected to enhance response to boron toxicity, and the encoded protein

24. Up to three lines of GM barley contain a gene involved in soil boron tolerance, *Bot1*. The gene was isolated from a landrace of barley, Sahara 3771, which is tolerant to high levels of boron in the soil. Cultivated barley also encodes a copy of this gene. However, there are several differences in the coding and protein sequences to the gene found in cultivated barley and Sahara 3771.

25. The *Bot1* gene, which codes for a boron efflux transporter, was identified using the results from a quantitative trait locus study of doubled-haploid lines of Sahara 3771 barley crossed with a boron intolerant cultivar (Jefferies et al. 1999). The *Bot1* gene in the boron tolerant cultivar encodes a protein of 666 amino acids which has two amino acid differences when compared to the intolerant cultivar. The Sahara 3771 cultivar has approximately four times more copies of the gene and approximately 160-180 times greater steady-state RNA levels in the leaf blades and root tissue than the intolerant barley cultivar (Sutton et al. 2007). It is suggested that the expression of the gene in the leaf blades indicates that the protein has a role in the removal of excess boron through the guttation fluid (fluid extruded through the leaf stomata) (Sutton et al. 2007).

26. *Bot1* is an ortholog of another boron transporter, *BOR1*, an efflux type borate transporter (Sutton et al. 2007). The *BOR1* protein transports boron from the roots to the shoots under conditions of low boron but under high boron concentrations the protein is degraded (Miwa et al. 2007). The protein product of the *Arabidopsis thaliana* ortholog of the *BOR1* gene, *AtBOR4*, was not degraded under high boron concentrations. In GM *A. thaliana* high levels of the *AtBOR4* protein were positively correlated with boron tolerance. At boron concentrations which killed most control plants the modified plants showed healthy root and shoot growth (Miwa et al. 2007). An alignment of the amino acid sequences of *Bot1* orthologs indicates that the Sahara 3771 *Bot1* gene shares similarity with the *A. thaliana* *BOR4* and *BOR1* genes.

5.2.3 Gene expected to enhance response to abiotic stresses, and the encoded protein

27. Up to four lines of GM barley contain a transcription factor gene isolated from wheat. This gene is expected to enhance tolerance to abiotic stresses.

5.2.4 Genes expected to enhance beta glucan synthesis, and their encoded proteins

28. Up to fifteen lines of GM barley contain one of three *CsIF* genes isolated from barley, *HvCsIF4*, *HvCsIF6* and *HvCsIF8*. In glasshouse trials, GM plants expressing these genes have been shown to have increased levels of beta glucan in their leaves and grains.

29. The *CsIF* gene family has only been found in the monocotyledons, consistent with their proposed role as beta glucan synthases. Six rice genes belonging to the cellulose synthase like-F (*CsIF*) gene family have been identified in rice. When single rice *CsIF* genes were expressed in GM *Arabidopsis* plants, some of the T₁ plants examined had detectable beta glucans in the epidermal cell walls (Burton et al. 2006). The results from the rice study have been used to identify the seven members of the barley *CsIF* gene family (Burton et al. 2008). Four *HvCsIF* genes are located on chromosome 2H, including *HvCsIF4* and *HvCsIF8*, and one single gene is located on each of chromosomes 7H (*HvCsIF6*), 5H and 1H. The position of the *HvCsIF6* gene is reported to correspond to previously identified QTLs associated with grain beta glucan content. The levels of *HvCsIF6* transcript in the developing grain are also relatively high when compared to other *HvCsIF* genes measured (Burton et al. 2008).

5.2.5 Toxicity/allergenicity of the proteins/end products encoded by the introduced genes to enhance response to drought stress, boron toxicity, abiotic stresses and beta glucan synthesis

30. The genes introduced into the GM plants were isolated from wheat or barley plants. Although, wheat and barley contain a number of anti-nutritional factors and allergens that, in extreme cases, may have a toxic effect (OGTR 2008a; OGTR 2008b), the proteins encoded by the introduced genes are not expected to have any toxic or allergenic effects as they are widely consumed as both human food and animal feed without any reported adverse effects.

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

32. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). No analysis has been performed on the encoded proteins associated with this early stage experimental trial.

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

5.2.6 The selectable marker genes and the encoded proteins

34. Two selectable marker genes have been used in constructing the 30 GM wheat and barley lines proposed for release.

35. The *hpt* gene encodes the HPT enzyme which catalyses the phosphorylation of the 4-hydroxy group on the hyosamine moiety, thereby inactivating hygromycin (Rao et al. 1983) and preventing it from killing cells producing HPT. The *hpt* gene was used as a selectable marker gene in the early laboratory stages of development of the plants to enable selection of plant cells containing the desired genetic modification. The *hpt* gene sequence was also used as a probe in the Southern blot analysis of some of the modified plant lines.

36. The *hpt* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in the RARMP for DIR 073/2007 the use of *hpt*, or other hygromycin B phosphotransferase encoding genes, as marker genes in GM plants has been assessed as not posing a risk to human health and safety or the environment. HPT is easily digested by simulated gastric juices and the amino acid sequence contains no similarities to known allergens (Lu et al. 2007). An assessment of antibiotic resistance genes used in GM plants by the European Food Safety Authority concluded that inclusion of the *hpt*

gene in GM plants would not significantly affect the health of humans or animals (EFSA 2004).

37. The *SacB* gene was isolated from the bacterium *Bacillus amyloliquefaciens* and encodes an enzyme (levansucrase) involved in sucrose metabolism (Gay et al. 1983). This gene was used to select for bacteria containing plasmids with the *Bot1* gene in the laboratory, prior to the production of the GM plants. The production of levansucrase in *E. coli* containing a functional *SacB* gene on a plasmid is lethal to the cells when they are grown on medium containing sucrose. When a gene of interest is introduced into the plasmid between the *SacB* gene and its promoter, the *SacB* gene is no longer expressed and the *E. coli* cells containing the plasmid with the gene of interest survive. This allows researchers to select for bacteria containing the plasmid which encodes the gene of interest.

38. Levansucrase is widespread in soil bacteria. The enzyme catalyses the transfer of fructose units from sucrose to other acceptors, which results in the degradation of sucrose and the production of levan. Levan is a polysaccharide that is produced naturally by a number of microorganisms but when artificially produced can be lethal to some microorganisms, such as *E. coli* and *Agrobacterium tumefaciens*. Expression of the *SacB* gene in GM plants has resulted in the accumulation of fructans in tissues and tissue damage to the plants (Caimi et al. 1996; Caimi et al. 1997). However, the *SacB* gene is not expected to be expressed in the GM barley lines as its bacterial promoter is not known to function in GM plants and furthermore, it is prevented from functioning by the 50 kb *Bot1* insert. The effect that the 50 kb of inserted DNA may have on the expression of the *SacB* gene is unknown, for example it is not known whether there are any active promoter sequences present in this 50 kb region.

5.2.7 The end products/effects associated with the introduced genes

Drought and other abiotic stress tolerances

39. Drought stress is an abiotic stress; a nonliving factor that causes harmful effects to plants. Other types of abiotic stresses include salinity, temperature and nutrient deficiency or toxicity. Plants respond to different abiotic stresses often through an interconnecting series of signalling and transcription controls that ultimately aim to increase the plant's ability to tolerate the initial stress through different response mechanisms that include a range of biochemical and physiological processes. Certain regulatory genes can be induced by more than one type of abiotic stress (eg drought, cold and salinity; Seki et al. 2002). The majority of these can be categorised as transcription factors (Wang et al. 2003; Shinozaki et al. 2003). Plant molecular responses to drought stress have been discussed in detail in the RAMP for DIR 071/2006 (<http://www.ogtr.gov.au/ir/dir071.htm>).

40. Evidence of cross tolerance to different abiotic stresses has led researchers to conclude that the signalling pathways for abiotic stress tolerance are not strictly isolated (Yamaguchi-Shinozaki & Shinozaki 2006). This is supported by the finding that transcript levels of some genes are altered by several different abiotic stressors, with some transcript levels altered by three different abiotic stressors (drought, cold and salinity) (Mantri et al. 2007). Cross tolerance to drought and highly saline soils has been reported to be greater than cross tolerance to cold and highly saline soils (Seki et al. 2002). The DREB2 family of proteins provides tolerance to both saline soils and drought (Vij & Tyagi 2007).

41. The enhancement of tolerance in plants to other abiotic stresses as a result of increased tolerance to one abiotic stress has also been discussed in detail in the RAMP for DIR 071/2006 (Chapter 2, Event 7). It was noted in the discussion of this event that a gene expressed in a different species may not necessarily produce the same phenotype as in the parent species.

Boron tolerance

42. Boron is an essential nutrient in plant growth. However, when boron occurs in elevated levels in the soil it can cause stress symptoms and yield reduction in plants. The phenotypic effects of boron toxicity on plants can be mistaken for symptoms of net blotch disease (DAFWA 2006). Boron toxicity initially affects the oldest leaves with symptoms becoming visible in successive leaves (Cartwright et al. 1986). Brown lesions are found initially at the leaf margins while chlorosis and necrosis begin at the leaf tips (Cartwright et al. 1986). In a study of barley displaying boron toxicity symptoms, the upper vegetative parts of the plants contained concentrations of boron greater than 50 mg/kg (Cartwright et al. 1986).

43. Boron toxicity has been observed in barley crops in the Australian states of Victoria, South Australian and Western Australia (Cartwright et al. 1986; Brennan & Adcock 2004). It is suggested that high levels of boron are found in weathered soils of marine origins (Cartwright et al. 1986). A 17% yield reduction in barley was observed at a site in South Australia with a mean soil boron concentration of 62.4 mg/kg (Cartwright et al. 1984). It has been shown that there is some interaction between boron toxicity and terminal drought (lack of water during reproductive growth) producing reduced straw yield and root growth (Yau 2002).

44. Barley cultivars display variable tolerance to high levels of soil boron with soil type also affecting boron toxicity. In Western Australia, barley cultivars are currently rated for boron susceptibility or tolerance and several cultivars of feed and malting varieties are rated as being moderately to highly tolerant to boron toxicity (Garlinge 2005). Crops of the cultivar Stirling were uniformly affected by boron toxicity when grown on red-brown clays in the lower rainfall zones of eastern and south-eastern Western Australia. However, across different soil types boron toxicity was patchy (Brennan & Adcock 2004). In this study, approximately 70% of the concentration of boron in whole shoots could be explained by the concentration of extractable boron in the 50-80 cm soil profile (Brennan & Adcock 2004).

45. The barley cultivar Sahara which displays high tolerance to boron toxicity is able to maintain a boron concentration in root tissue of approximately 50% below the boron concentration in the external medium (Hayes & Reid 2004). It was determined that this maintenance of root boron concentrations within an acceptable level was not the result of transpiration moving boron out of the roots but an active efflux of boron from the roots to the external environment. It was also shown that in the tolerant cultivar Sahara this mechanism is constitutively active (Hayes & Reid 2004). In barley cultivars susceptible to boron toxicity putrescine expression was detected in leaves under conditions of stress; putrescine is expressed under conditions of biotic and abiotic stress (Roessner et al. 2006). The lack of putrescine expression in the boron tolerant cultivar, under boron stress conditions, was suggested to be a result of the ability of the Sahara cultivars to actively exclude toxic concentrations of boron from the roots (Roessner et al. 2006).

Beta glucan synthesis

46. Beta glucan is defined as a soluble fibre. Beta glucans in the grains of commercially produced cereals are of interest due to a number of health benefits that have been linked to beta glucan consumption. These include a lowering of blood serum cholesterol, a lowering of both the postprandial blood glucose and insulin response, an increase in postprandial satiety, and an increase in stool bulk which relieves constipation (reviewed in Brennan & Cleary 2005; and Wood 2007). The benefits of consuming beta glucans have been correlated with the amount of soluble beta glucan in the gastro-intestinal tract and its molecular weight (Lazaridou & Biliaderis 2007). The high level of beta glucan in the barley grain (5-11%) makes it a crop of interest to confer these health benefits to humans (Brennan & Cleary 2005;

Wood 2007). While beneficial to humans, beta glucan consumption by poultry reduces the digestibility of their feed and metabolisable energy and can result in sticky droppings, with similar effects being observed in pigs (Brennan & Cleary 2005).

47. (1,3;1,4)- β -D-glucan (referred to here as beta glucan) is a polysaccharide present in the walls of elongating cells and endosperm of grasses (reviewed in Buckeridge et al. 2004). Beta glucans are not thought to be present in the dicotyledons (Buckeridge et al. 2004). However, they are not specific to the grasses. A beta glucan molecule is present in the lichen *Cetraria islandica* (Buckeridge et al. 2004) and a beta glucan molecule, with structural differences to those present in the grasses, has recently been identified in the horsetail *Equisetum arvense* (Sorensen et al. 2000).

48. Beta glucan of barley is mainly composed of cellotriosyl (a chain of three (1,4)- β -D-glucosyl groups) and cellotetraosyl (a chain of four (1,4)- β -D-glucosyl groups) units connected by single (1,3)- β linkages (Staudte et al. 1983). Cellotriosyl and cellotetraosyl units make up approximately 90% of the beta glucan molecule and the remaining 10% consists of groups of four to fifteen (1,4)- β -D-glucosyl units (Staudte et al. 1983). The distribution of the two unit types is non-random and non-repeating. In barley, the ratio of cellotriosyl units to cellotetraosyl units is 2.8 to 3.3 (reviewed in Wood 2007).

49. Evidence suggests that beta glucans are synthesised by a Golgi membrane associated synthase using uridine diphosphoglucose (UDP-Glc) as the *in vitro* substrate (Buckeridge et al. 1999). A barley endosperm (1,3;1,4)- β -D-glucan synthase has been identified and there may be several isomers of the enzyme present in the endosperm as the level of beta glucan in the endosperm continues to increase toward seed maturity even though activity of the single enzyme studied had decreased (Tsuchiya et al. 2005). In maize, a model in which three UDP-Glc transferase activities are associated with the synthase has been suggested (Buckeridge et al. 1999).

50. Beta glucan synthesis begins in plant cells at the start of the cell elongation phase. The maximum abundance of beta glucan in the cell occurs during the stage of rapid cell elongation after which it begins to degrade when cell elongation ends (reviewed in Buckeridge et al. 2004). Beta glucan makes up approximately 75% of the mass of the barley endosperm cell wall with the majority of beta glucan present in the central endosperm (Chandra et al. 1999; Buckeridge et al. 2004). The amount and distribution of beta glucan in the endosperm differs between varieties of barley, seasons and cultivation areas. The variation in the amount and distribution of beta glucan in the endosperm clearly impacts on grain morphology (Chandra et al. 1999). A positive correlation exists between the beta glucan content and the protein content of barley grains; and there are negative correlations between beta glucan content and amylose content, seed yield and grain weight (Hang et al. 2007).

5.2.8 Toxicity of the end products associated with the introduced genes for increased beta glucan synthesis

51. Fifteen of the GM barley lines proposed for release are expected to produce higher levels of beta glucan in their leaf and grain tissues than the parental cultivar of barley. The levels of beta glucan in the GM barley lines was determined for plants grown in glasshouse trials and found to be 3.7 to 5.0% (w/w) for leaves and 5.7 to 6.8% (w/w) for grain. The applicants determined the beta glucan levels in the leaves and grain of the parental non-GM barley plants to be 1.0% (w/w) and 4.6 to 5.0% (w/w), respectively. An application has been made to the US Food and Drug Administration (FDA) that barley fibre, containing 70% beta glucan, be regarded as a substance generally recognised as safe (GRAS). In response to this

application the FDA had no questions in relation to the company's conclusion that the barley fibre preparation was GRAS (US FDA 2006).

5.3 The regulatory sequences

5.3.1 Regulatory sequences for the introduced genes

52. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal.

53. The expression of five of the seven introduced genes is regulated by a promoter derived from the plant pathogen Cauliflower mosaic virus (CaMV), 35S. Although this organism is a plant pathogen, the promoter is not capable of causing disease. The *Bot1* gene construct contains the endogenous gene promoter, ie the promoter naturally found in association with this gene in the plant. The expression of the gene expected to confer increased tolerance to abiotic stresses is regulated by a maize promoter, Ubi (Table 2).

54. The mRNA termination region for the introduced genes in 27 of the GM lines proposed for release is derived from the nopaline synthase gene (*nos*) of *Agrobacterium tumefaciens*. This sequence is widely used in constructs for plant genetic modification (Reiting et al. 2007). The mRNA termination region endogenous for the *Bot1* gene is used for the GM lines into which the gene was introduced.

55. While some of the regulatory sequences are derived from plant pathogens (*Agrobacterium tumefaciens*, CaMV) the sequences are not pathogenic in themselves nor do they cause any disease symptoms in the GM plants. Those regulatory sequences derived from plants that are associated with allergenic or toxic responses in humans (i.e. *Zea mays*) are not in themselves allergenic or toxic.

5.3.2 Regulatory sequences for the expression of the selectable marker genes

56. Three plasmid vectors were used in the construction of the 30 GM plant lines proposed for release. Each vector contains the bacterial *hpt* gene. In both the pMDC32 and pMDC32Ubi vectors, expression of the *hpt* gene is regulated by the CaMV 35S promoter and the A35S transcription termination sequence, both from the CaMV. The *hpt* gene in the vector pYL7AC is also regulated by the 35S promoter and the transcription termination sequence is the Tnos sequence from *Agrobacterium*. The pYL7AC vector also contains the *SacB* gene which is regulated by a synthetic *E. coli* promoter (Pierce et al. 1992).

57. Although *Agrobacterium* and the CaMV are plant pathogens, the regulatory sequences comprise only a small part of the total genome, and are not capable of causing disease.

5.4 Method of genetic modification

58. The genes were introduced into wheat by biolistic transformation, also known as particle bombardment. This technique involves coating the expression cassette containing the gene constructs onto very small gold particles that are 'shot' into wheat embryos, which are then regenerated into whole plants in tissue culture. Particle bombardment has been widely used in Australia and overseas for introducing new genes into plants and is not known to cause any adverse effects on human health and safety or the environment.

59. The genes were introduced into barley by *A. tumefaciens* mediated transformation. This technique introduces the gene and associated regulatory sequences into immature barley embryos via the vector *A. tumefaciens*. Transformed barley tissue was then grown on media containing an antibiotic to prevent the growth of *A. tumefaciens*. This was followed by

regeneration and selection of plants that contained the genes of interest. The vector is ‘disarmed’ since it lacks the genes which encode the tumorigenic functions of *A. tumefaciens*.

60. The 30 GM wheat and barley lines are generated from independent transformation events.

5.5 Characterisation of the GMOs

5.5.1 Stability and molecular characterisation

61. The inserted genes were fully sequenced before insertion into the plants. The *Bot1* gene was inserted as genomic DNA and the remaining six genes were inserted as cDNA. The exact location of the inserted genes within the wheat and barley genomes has not been determined.

62. Gene copy number, determined by Southern hybridisation analysis using probes to the coding region of the *hpt* gene or the double 35s promoter, has been determined for some lines. Copy numbers for the remaining GM lines are still being determined. Analysis to date indicates a range of copy numbers of between 1 and 6. Insertion copy number in the three barley lines containing the *Bot1* has not yet been determined.

63. The expression levels of the introduced *TaDREB2* and *TaDREB3* genes were determined in the modified plants by northern blot analysis in the T₁ and T₂ generations. Lines with high expression of the introduced gene were selected for use in the proposed trial.

64. A wheat transcription factor has been inserted into the plants modified for increased abiotic stress tolerance. Levels of expression of the transcription factor gene were determined using northern blot analysis over two generations. This allowed plants with high levels of expression to be selected at each generation for use in the proposed trial.

65. Expression levels of the introduced *HvCslF4*, *HvCslF6* and *HvCslF8* genes were determined by real time PCR using plant tissue from the T₀ generation and the results were compared against control plants transformed with the empty vector. The modified plants containing the *HvCslF4*, *HvCslF6* and *HvCslF8* genes were bred until either the T₂ or T₃ generation. Southern blot analysis was used to determine copy number at each generation of these lines and allowed selection of lines with a T₂ homozygous parent by determining the frequency of segregation of the marker gene.

66. The insert containing the *Bot1* gene is approximately 50 kb of genomic DNA from the barley cultivar Sahara 3771. This inserted sequence contains the full genomic sequence encoding the *Bot1* gene and its promoter as well as several repetitive sequence elements. The applicant reports that these repetitive elements are common to non-coding regions of the barley genome. Gene expression levels of the introduced *Bot1* gene have not been determined.

5.5.2 Characterisation of the phenotype of the GMOs

67. The main aim of the proposed trial is to characterise agronomic traits of the GMOs under normal field conditions. Traits that will be measured include; heading date, plant height and other growth characteristics as well as yield traits. The applicant is not proposing to induce conditions of drought stress or other abiotic stresses in this trial and the soil boron levels at the proposed trial site are believed to be below toxic levels and within levels required for normal plant growth.

68. When grown under glass house conditions, some of the GM wheat and barley lines displayed altered growth phenotypes. Several barley lines modified by the insertion of the transcription factor gene had larger spikes and seeds and delayed growth and development compared to the wild type control plants. A semi-dwarf phenotype was observed in wheat and

barley plants modified with the *TaDREB2* or *TaDREB3* gene from wheat. One of the aims of the proposed trial is to determine whether these altered growth phenotypes are observed under natural field conditions.

69. Under glass house conditions, the GM barley lines expressing the *HvCsIF* genes were determined to contain 5.7 to 6.8% (w/w) beta glucan in the grains. While the non-GM barley plants were determined to contain 4.6 to 5.0% (w/w) beta glucan in the grains (data supplied by the applicant, Chapter 1, Section 6.5).

70. While there may be changes in the levels of products produced as a result of the activity of the encoded proteins, no new products should be produced by expression of the introduced genes. However, there may be unintended effects due to random insertion of the introduced genes (see Chapter 2, Event 6).

Section 6 The receiving environment

71. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the size, location and duration of the dealings, any relevant biotic/abiotic properties of the geographic 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; OGTR 2007).

6.1 Relevant abiotic factors

72. The abiotic factors relevant to the growth and distribution of commercial wheat in Australia are discussed in the ‘The Biology of *Triticum aestivum* L. em Thell (Bread Wheat)’ (OGTR 2008b). The abiotic factors relevant to the growth and distribution of commercial barley in Australia are discussed in the ‘The Biology of *Hordeum vulgare* L. (Barley)’ (OGTR 2008a).

73. Water deficit is common in dryland wheat farming, and is the major abiotic stress limiting crop productivity in Australia, including grains. (Kokic et al. 2006). Barley consumes less water per unit weight of dry matter produced than other cereals (Queensland Department of Primary Industries and Fisheries 2007) and compared to other cereals, barley is well adapted to drought through water use efficiency.

74. Barley is not as cold hardy as wheat, and is more susceptible to frost at the early seedling stage (Gomez-Macpherson 2001). Barley is well adapted to a wide range of soils and is the most tolerant cereal to salinity. Barley is sensitive to aluminium toxicity, which is linked to acidic soils, and boron toxicity (van Gool & Vernon 2006).

75. The proposed release site is situated within a farm owned by The University of Adelaide and is typical of rain-fed, wheat production environments in South Australia. Selected climatic data for the proposed site is given in Table 3. The applicant states that the farm is not expected to have toxic levels of boron in its soil. Although the applicant has stated that the proposed release site is not experiencing exceptional drought conditions; the areas around Adelaide have been declared Exceptional Circumstance (drought) areas by the Australian Government¹¹. It also appears to be in a region that is not currently affected by dryland

¹¹ http://www.daff.gov.au/agriculture-food/drought/ec/south_australia accessed on 26 Feb. 2008.

salinity¹². There is a dam located on the farm, approximately 1 km from the proposed release site.

Table 3 Climatic data for proposed GM wheat and barley trial site

	ADELAIDE (Waite Institute)
Average daily max/min temperature (Summer*)	27.0 °C / 15.8 °C
Average daily max/min temperature (Winter*)	14.8 °C / 8.1 °C
Average monthly rainfall (Summer*)	25.6 mm
Average monthly rainfall (Winter*)	79.9 mm

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

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

6.2 Relevant biotic factors

76. The biotic factors pertaining to the growth and distribution of commercial wheat and barley in Australia are discussed in the ‘The Biology of *Triticum aestivum* L. em Thell (Bread Wheat)’ and ‘The Biology of *Hordeum vulgare* L. (Barley)’ (OGTR 2008a; OGTR 2008b). Of relevance to this proposed release are the following points:

- The farm has been used in the past, and will continue to be used, by the South Australian Barley Improvement Program.
- Two small scale farms that have previously cultivated wheat and/or barley are situated 2.5 km north east and 1.5 km west north-west of the farm.
- The nearest commercial wheat or barley crop will not be within 50 km of the trial.
- Invertebrates, vertebrates and microorganisms could be exposed to the introduced genes, their encoded proteins and end products. In particular, native birds and rodents (either introduced or native) may visit the proposed release site. This is discussed in Chapter 2.

6.3 Relevant agricultural practices

77. The size, location and duration of the proposed limited and controlled release of the GM wheat and barley lines are outlined in Chapter 1, Section 3.2.

78. It is not anticipated that the agronomic practices for the cultivation of the GM wheat and barley by the applicant will be significantly different from conventional practices for wheat and barley, with the exception that the applicant proposes to harvest by hand to minimise seed spillage. Conventional cultivation practices for wheat and barley are outlined in more detail in the relevant Biology Documents (OGTR 2008a; OGTR 2008b).

79. Non-propagative plant material at the field location (for example, stem stubble left after harvest) would be ploughed into the ground after the trial and left to decompose. Excess seed or plant material not required for experimental analysis, or future trials would be removed from the site and disposed of by steam sterilisation or autoclaving.

80. In Australia, spring wheat varieties are commonly grown as a winter crop and are usually planted in May and June. Harvest of the mature wheat generally occurs from mid-November to late December. The parental barley cultivars ‘Flagship’ and ‘Golden Promise’

¹² Figure 8. Dryland salinity risk in South Australia 2000.

http://www.anra.gov.au/topics/salinity/pubs/national/salinity_sa.html accessed on 29 Feb. 2008.

are two-row, malting, spring varieties. The parental wheat cultivar ‘Bobwhite’ is a spring wheat cultivar. If the proposed release is approved the applicant anticipates planting the trial in June 2008 and harvesting in December 2008.

6.4 Presence of related plants in the receiving environment

81. The GM wheat and barley lines proposed for release will be grown together at the field trial site. Barley and wheat are not known to hybridise with each other under natural conditions (see OGTR 2005b).

82. The applicant has indicated that for the 2008-2009 growing season there will be approximately 1000 m between the GM barley and other barley plants which will be used for breeding trials, with the exception of any non-GM barley plants grown as part of the trial.

83. The applicant has indicated that for the 2008-2009 growing season there will be approximately 1000 m between the GM wheat and other wheat plants which may be planted in barley breeding trial plots where there is insufficient barley to fill all the required area. Any wheat plants used for this purpose would be destroyed after harvest¹³. The applicant also indicated that there will be no wheat breeding trials planted in the 2008-2009 growing season.

84. Apart from commercially cultivated bread and durum wheat, other *Triticum* species are not known to be present in Australia. However, the applicant has indicated that the related plants *Secale cereale* and *Hordeum leporinum* may be found in the area of the proposed release. Wild barley, *H. vulgare* ssp. *spontaneum* is not known to be present in Australia (reviewed in OGTR 2008a).

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

85. All of the introduced genes were isolated from cultivars of wheat or barley, or in one case from a barley landrace. The isolated genes have not been modified by the applicant and have been reintroduced into the wheat and barley plants in their native form. Therefore, it is expected that humans, herbivores/omnivores and microorganisms routinely encounter the introduced genes and their gene products, or their homologues, through contact with plants and food derived from plants. This information forms the baseline data for assessing the risks from exposure to these proteins as a result of the trial of the GM wheat and barley lines.

86. The *Bot1* gene that is expected to confer increased boron tolerance was isolated from the Algerian landrace Sahara 3771. It encodes a protein of 666 amino acids that has two amino acid differences compared to Australian commercial cultivars and is therefore very similar to what exists in the Australian environment.

87. The *CsIF* genes are expected to increase the level of beta glucan in the leaves and grain of the GM barley. Humans regularly come into contact with beta glucan through the consumption of cereal grains. The applicants determined the beta glucan level in the grain of non-GM barley plants to be 4.6 to 5.0% (w/w). The levels in the grain of GM barley lines was determined for plants grown in glasshouse trials and found to be 5.7 to 6.8% (w/w). A preparation of barley fibre containing 70% beta glucan has been added to the FDA’s list of GRAS notices with no questions from the FDA (discussed in paragraph Chapter 1 Section 5.2.8; (US FDA 2006)). Domestic animals also come into contact with beta glucan through

¹³ The applicant supplied more information, during the consultation period, regarding the breeding trials that are to occur on the farm, where the release site is located. This information has been updated to reflect the planned activities for the 2008-2009 grain growing season.

consumption of cereal grains which, while not toxic, consumption by poultry and pigs can have antinutritional effects (Section 5.2.4).

88. The *hpt* gene is derived from *E. coli*, which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997). As such, it is expected that humans, animals and microorganisms routinely encounter the encoded protein.

89. The *SacB* gene is derived from the soil bacterium *B. amyloliquefaciens* and therefore humans and other organisms are commonly exposed to this gene and its encoded protein.

Section 7 Australian and international approvals

7.1 Australian approvals of the GM wheat and barley lines

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

90. There has been no release of these GM wheat and barley lines in Australia.

91. The Regulator has issued licences for the conduct of three field trials involving other GM wheat lines under limited and controlled conditions: DIR 053/2004 was issued to Grain Biotech for GM salt tolerant wheat on an area of 0.45 ha in Western Australia, DIR 054/2004 was issued to CSIRO for GM wheat with altered starch content on 0.25 ha in the Australian Capital Territory and DIR 071/2006 was issued to Department of Primary Industries – Victoria for GM drought tolerant wheat on 0.315 ha in Victoria.

92. Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been five field trials of different GM wheat ranging in size from 325–1500 plants: PR65 (1996) for herbicide tolerance, PR66 (1996) for altered starch levels in the grain, PR102 (1998) for modified grain qualities, PR102X (2000) for modified grain qualities, and PR107 (1999) for herbicide tolerance. Five field trials of different GM barley also occurred under GMAC. They ranged in size from 400–2940 plants: PR88 (1998) for resistance to yellow dwarf virus, PR92 (1998) for improved malting qualities, PR106 (1998) for herbicide tolerance, PR88X (1999) for resistance to yellow dwarf virus, and PR139 (2000) for malting and brewing qualities.

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

7.1.2 Approvals by other Australian government agencies

94. 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 Food Standards Australia New Zealand (FSANZ). This is discussed further in Chapter 3.

95. 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 and barley 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 and barley lines could be used in food.

7.2 International approvals

96. There have been no releases of these GM wheat and barley lines internationally.

97. However, there have been releases of other different GM wheat and barley plants. The traits which have been modified include; novel protein production, disease resistance, altered grain properties and herbicide tolerance¹⁴.

¹⁴< <http://www.aphis.usda.gov/brs/status/relday.html>>, <http://gmoinfo.jrc.it/gmp_browse.aspx> accessed 13 March 2008.

Chapter 2 Risk assessment

Section 1 Introduction

98. 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. It takes into account information in the application, relevant previous approvals and current scientific knowledge.

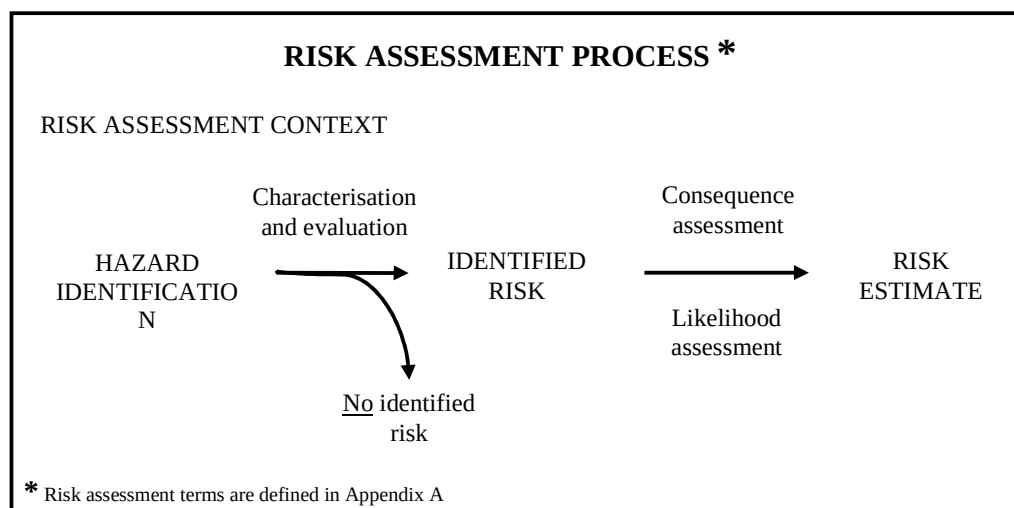


Figure 3 The risk assessment process

99. 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.

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

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

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

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

Section 2 Hazard characterisation and the identification of risk

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

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

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

- ♦ the proposed dealings, which may be for the purpose of experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- ♦ the proposed limits
- ♦ the proposed controls
- ♦ characteristics of the non-GM parents
- ♦ routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- ♦ potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- ♦ potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- ♦ the biotic and abiotic factors at the site of release
- ♦ agronomic management practices for the GMOs.

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

108. The GM wheat and barley lines contain one or two selectable marker genes; the *hpt* gene encoding the HPT protein which confers tolerance to the antibiotic hygromycin; and the *SacB* gene encoding levansucrase.

109. The prevalence of the *hpt* and *SacB* genes in the environment and the lack of evidence for toxicity or allergenicity of the HPT and levansucrase proteins, to humans and animals are discussed in Chapter 1, Section 5.2.6. Therefore, the potential effects of the *hpt* and *SacB* genes will not be further assessed for this application.

Table 4 Summary of events that may give rise to an adverse outcome through the expression of the introduced genes for increased drought, boron or abiotic stress tolerance, or increased beta glucan.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Ingestion of, contact with, or inhalation of GM plant material containing proteins encoded by the introduced genes or their end products.	Allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The encoded proteins and their end products are widespread in the environment and are unlikely to be toxic/allergenic to people or toxic to other organisms. The limited scale, short duration and other proposed limits and controls, further reduce exposure of people and other organisms to products of the introduced genes.
Section 2.2 Spread and persistence of the GM wheat and/or barley lines in the environment	2. Expression of the introduced genes improving the survival of GM wheat or barley plants.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Cultivated wheat and barley are not considered to be weedy and the genetic modifications are not expected to change the weediness characteristic of the GMOs. The limits and controls proposed for the release would minimise persistence.
	3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including, animals and extreme weather conditions.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> As discussed in Event 1 the encoded proteins and their end products are already widespread in the environment. Wheat and barley seeds have limited dispersal characteristics, which are not expected to be changed in the GMOs. The proposed limits and controls would minimise dispersal.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced genes or regulatory sequences in other wheat or barley plants or in other sexually compatible plants	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Wheat and barley are predominately self-pollinating and outcrossing is limited. Limited fertile hybrid production has been reported for sexually compatible species known to be present at the site. The applicant proposed a number of controls, including 200 m zone around the release site in which no other wheat or barley plants are grown which would limit the potential for vertical gene flow.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence and expression of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The introduced genes or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms. Events 1 – 3 did not constitute identified risks for people or the environment associated with expression of the introduced genes.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.5 Unintended changes in biochemistry, physiology or ecology	6. Changes to biochemistry, physiology or ecology of the GM wheat and barley lines resulting from expression, or random insertion, of the introduced genes.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The expression of the introduced genes is not expected to result in the production of proteins/end products that are not already found in the non-GM plant. Unintended, adverse effects, if any, would be minimised by the proposed limits and controls. Unexpected alterations are likely to be detected and eliminated during the selection process.
	7. Altered soil boron/beta glucan levels and increased persistence of beta glucan in the soil as a result of growing the GM wheat and barley lines.	Altered soil ecology or toxicity to other organisms	No	<ul style="list-style-type: none"> Boron is already present in the soil. Beta glucans are already present in cereal stubble. The proposed limits and controls would minimise any adverse effects.
Section 2.6 Unauthorised activities	8. Use of the GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	<ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

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

110. 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).

111. Allergenicity is the potential of a protein to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

112. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced genes for increased drought, boron or abiotic stress tolerance, or increased beta glucan levels. Workers cultivating the GM wheat and barley would be exposed to all plant parts. Organisms may be exposed directly to the proteins through biotic interactions with GM wheat and barley 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 and barley plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

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

113. People may come into contact with, or inhale GM wheat and barley or GM plant material through working either within the proposed trial site or a nearby area. Animals, such as rodents and birds, may also come into contact with, inhale or ingest GM plant material.

114. Non-GM wheat and barley flour can both produce allergic responses in susceptible individuals on inhalation or ingestion. Ingestion of wheat and barley flour by coeliac disease

sufferers will trigger a sensitivity response caused by the prolamin fraction of the storage protein complex, gluten (reviewed in OGTR 2008a; OGTR 2008b). There are no known significant toxic properties of non-GM wheat or barley (OGTR 2008a; OGTR 2008b). These properties are not expected to be altered in the GM wheat and barley lines proposed for release.

115. Although no toxicity studies on the GM wheat and barley plant material or encoded proteins have been performed all of the genes introduced into the GM wheat and barley lines, with the exception of the two marker genes, were isolated from either wheat or barley. Therefore, the encoded proteins or end products are widespread in the environment and the likelihood that the GM wheat or barley plants would cause allergenicity or toxicity to humans or other organisms is no greater than for non-GM wheat or barley (see Chapter 1, Section 6.5).

116. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. The short duration and small size of the trial will limit the potential for exposure of humans and animals to the GM plant tissues. Contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff associated with the field trial. Although preliminary glasshouse studies suggest that some plants may show delayed flowering, the applicant proposes that any which show a delay in flowering of more than one month over that of the non-GM parent plants will be removed from the field, thereby limiting the potential of increased exposure to pollen that could result from prolonged flowering.

117. The proposed trial site will be surrounded by a 1 m high fence with access to the trial site being via a locked gate and access to the research farm is also restricted, which limits exposure of the public and larger animals to the GM plant material. There is limited potential for exposure of the public to plant materials via ingestion, skin contact or inhalation as no plant material will be used as human food, animal feed or plant products. The short duration (one growing season) and small size (400 m²) of the proposed trial would also limit the potential for exposure to the GM plant material.

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

2.2 Spread and persistence of the GM wheat and/or barley lines in the environment

119. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM wheat and barley plants in particular, is provided in the review documents '*The biology of Triticum aestivum L. em Thell. (bread wheat)*' and '*The biology of Hordeum vulgare L. (barley)*' (OGTR 2008a; OGTR 2008b). In summary, wheat and barley share some characteristics with known weeds, such as wind-pollination (although both species are predominantly self-pollinating) and the ability to germinate and/or to produce some seed in a range of environmental conditions. However, both species lack 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, very high seed output, high seed dispersal and long-distance seed dispersal.

120. Scenarios that could lead to increased spread and persistence of the GM wheat and barley lines include expression of the introduced genes conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These events could

lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins and their end products.

Event 2: Expression of the introduced genes improving the survival of the GM wheat and/or barley plants

121. Expression of the introduced plant genes for enhanced tolerance to abiotic stress tolerance, including to drought and soil boron, or increased beta glucan could potentially improve the survival of the GM wheat and/or barley lines. These GM lines have not previously been grown in the field and therefore the impact of the genetic modifications on survival of the GM wheat and barley lines is uncharacterised under field conditions. However, a number of predictions can be made based on knowledge of the gene functions and their predicted effect when expressed in the modified plants. Predictions can also be made based on the observed phenotypes of the GM wheat and barley lines grown under glasshouse conditions and comparing them to the phenotype of the non-GM wheat and barley plants. These predictions are summarised in Chapter 1, Section 5.5.2.

122. Delayed development, including a delay in flowering time, has been seen in several of the GM lines proposed for release. Several lines expressing the transcription factor gene displayed delays in growth and development which may alter the survival of the GM plants. For example by altering, under field conditions, seed dormancy, disease pressures or outcrossing opportunities. The applicant has proposed a number of conditions to limit and control the release, including the removal of plants that display a prolonged delay in flowering, post harvest monitoring and a 200 m zone in which no wheat or barley is grown.

123. The majority of the GM wheat and barley lines contain genes that are expected to increase their ability to survive under conditions of abiotic stress, such as drought and high soil boron levels. There are some factors relevant to the proposed trial that would limit the potential effects of these introduced genes. The applicant states that the area proposed for the release is believed to be free of soils with high levels of boron and does not appear to be affected by dryland salinity (Chapter 1, Section 5.2.7). The applicant also states that the area proposed for the release is not a drought prone area; although it is in an area for which a declaration of Exceptional Circumstances has recently been made (see Chapter 1, Section 6.1). The degree of drought stress experienced by a crop will differ between growing seasons, across local regions and can be affected by soil type. As discussed in the RARMP for DIR 071/2006 and in Chapter 1, Section 5.2.7, tolerance to one abiotic stress can lead to enhanced tolerance to environmental stressors other than those for which they have been modified, and therefore the expression of the introduced genes may confer additional tolerances.

124. However, the survival of the GM wheat and barley 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 and barley plants in Australia (Slee 2003; Condon 2004). Furthermore, in the unlikely instance that cross tolerance is conferred, the GM plants will most likely be less fit as compared to other commercially available wheat and barley varieties because of the potential metabolic/physiological burdens (eg as discussed in Pretty 2001). For example, the wheat or barley may have stunted growth, produce less seeds, and have a decreased ability to tolerate competition from other plants.

125. The applicant has proposed limits and controls to minimise the possibility of persistence of the GM wheat and barley lines. They include a post harvest irrigation of the site to promote germination of any GM seed that may be present and post harvest monitoring of the proposed

site and destruction of any volunteers until no wheat or barley plants have been found on the site for two years or until the site has been clear of volunteers for one growing season.

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

127. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials, the encoded proteins or end products has been considered in Event 1 and was not considered an identified risk.

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

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

129. Dispersal of reproductive GM plant materials, for example viable grain, could occur through endozoochory, the activity of rodents or through extremes of weather such as flooding or high winds. Dispersal of viable plant material could result in increased allergic reactions in people and toxicity to people or other organisms, and persistence of the GM wheat and barley lines in the environment. The effects of contact, inhalation or ingestion of the GM wheat or barley lines have been assessed in Event 1 and were not an identified risk. The introduced genes improving survival of the GM wheat and barley lines in the environment was assessed in Event 2 and was also found not to be an identified risk.

130. Seed production and dispersal characteristics are not expected to be altered in the GM lines compared to non-GM parental cultivars.

131. Cultivated wheat is generally considered to no longer have seed dispersal mechanisms (OGTR 2008b). However, barley seeds have special bristles on the spikelets structures and seeds could potentially adhere to animals and the clothing of people (OGTR 2008a). The proposed release site will be surrounded by a 1 m cyclone fence with access through a locked gate limiting the possibility of seed dispersal by any large animals which may be present on the farm or by unauthorised people. Dispersal by authorised people entering the proposed trial site would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing.

132. Endozoochory has not been identified as a form of seed dispersal for barley or wheat in the literature although viable wheat and barley seeds have been detected in cattle dung. The possibility of dispersal of GM plant materials by birds was considered in detail in the previous assessment for RARMP for DIR 071/2006 and barley seed dispersal by birds has been considered in the 'The Biology of *Hordeum vulgare* L. (Barley)' (OGTR 2008a). To briefly summarise, bird damage has been reported for wheat and barley crops although birds appear to prefer softer plant parts, and are more likely to eat the GM plants or grain on site rather than carry them elsewhere for storage or consumption (as reviewed in OGTR 2008a; OGTR 2008b).

133. Mice are known pests of grain crops (OGTR 2008a; OGTR 2008b). Different territory sizes and movement between refuge areas and wheat fields have been estimated for mice (Newsome 1969; reviewed in OGTR 2008a). Habitat modification (for example, cultivation and maintenance of grassland vegetation below 10 cm) has been shown to reduce rodent numbers in irrigated farming systems and macadamia orchards in Australia, and in and around

farm buildings (White et al. 1998; Central Science Laboratory 2001; Brown et al. 2004). Reduced plant cover has also been reported to be a deterrent to the movement of mice (Agri-Facts 2002). The applicant has proposed several measures to limit and control rodent numbers at the proposed release site. These include an area of cleared land of 5 m wide from the edge of the proposed release and a 10 m wide mown zone, adjacent to the cleared zone, in which plant cover would be kept at or below 10 cm; the applicant has also proposed placing rodent baits inside the fence line.

134. Extremes of weather may cause dispersal of plant parts. However, control measures have been proposed by the applicant to minimise dispersal (see Chapter 1 Sections 3.2 and 3.3). These include locating the proposed release site 50 m away from natural water ways in the event of flooding, and having a 200 m zone in which there are no other wheat or barley plants in the event of strong winds dispersing pollen.

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

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

136. 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).

137. Baseline information on vertical gene transfer associated with non-GM wheat and barley plants is provided in the ‘The Biology of *Triticum aestivum* L. em Thell (Bread Wheat)’ and ‘The Biology of *Hordeum vulgare* L. (Barley)’ (OGTR 2008a; OGTR 2008b). In summary, wheat and barley are predominantly self-pollinating and the chances of natural hybridisation occurring with commercial crops or other sexually compatible plants are low.

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

138. Transfer and expression of the introduced genes for enhanced abiotic stress tolerance, including to drought and soil boron, in other wheat, barley or sexually compatible plants could confer a selective advantage to these plants under conditions of environmental stress. While it is unlikely that the GMOs will differ in their sexual reproduction characteristics from the parent organism, the altered flowering time observed in some lines expressing the transcription factor gene, *TaDREB2* and *TaDREB3* genes could produce an advantageous alteration in flowering synchronicity with sexually compatible plants in the area of the proposed release.

139. As discussed in Event 2, the survival of the GM wheat and barley plants proposed for release would be limited by a diverse range of environmental factors that normally limit the spread and persistence of wheat and barley plants in Australia. Therefore, expression of the introduced genes in other wheat and barley plants would also result in plants limited by these factors. The expression of the introduced genes in other sexually compatible species is also unlikely to give these plants a significant selective advantage. The conditions that limit the spread and persistence of any hybrids between non-GM wheat or barley and other sexually compatible plants would be expected to limit the spread and persistence of any hybrids between the GM wheat or barley and other sexually compatible species.

140. As discussed in Event 3 allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM wheat and barley plants by the introduced genes or regulatory sequences. This will be the same if the introduced genes are expressed in other wheat and barley plants. Similarly, if the introduced genes are expressed in other sexually compatible species, allergenicity and toxicity are not expected to be altered.

141. All of the introduced regulatory sequences are expected to operate in the same manner as regulatory elements endogenous to the wheat and barley plants. While the transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects, the impacts from the introduced regulatory elements are likely to be equivalent and no greater than the endogenous regulatory elements.

142. As discussed in the ‘The Biology of *Triticum aestivum* L. em Thell (Bread Wheat)’ and ‘The Biology of *Hordeum vulgare* L. (Barley)’, both wheat and barley are predominantly self-pollinating and any outcrossing occurs through wind pollination. Intraspecific gene flow has been detected over much shorter distances for field trial size releases compared to commercial scale, although gene flow levels are highly variable. Generally, low levels of gene flow occur up to 100 m for wheat with rare incidences occurring at greater distances; barley gene flow distances are less and gene flow has been measured up to 60 m (reviewed in OGTR 2008a; OGTR 2008b).

143. As discussed in ‘The biology of *Triticum aestivum* L. em Thell. (Bread Wheat)’ (OGTR 2008b), there are few species outside the *Triticum* genus (for example, *Aegilops cylindrica*, *A. ovata*, *Hordeum marinum* and *Secale cereale*) that are sexually compatible with wheat and which may be able form hybrids under natural conditions. However, any hybrids formed have been largely sterile (see discussion in DIR 071/2006, Chapter 2, Event 11).

144. The related species that the applicant has identified at or near the proposed release site are *S. cereale* (rye), Triticale and *Hordeum leporinum* (barley grass). The ability for wheat to cross with *S. cereale* was discussed in detail in DIR 071/2006 and it was noted that there have been non-peer-reviewed reports of naturally occurring hybrids between wheat and rye in Canada. Triticale is a forced hybrid of wheat and rye and to date there are no reports in the literature of natural hybrids occurring between triticale and wheat. A literature search did not identify any natural or artificial hybrids of wheat and *H. leporinum*. The weedy species *Hordeum marinum* (sea barley) has also been collected in South Australia around the Adelaide region¹⁵ and there is one report of possible hybridisation between wheat and *H. marinum* in nature (Guadagnuolo et al. 2001).

145. *Hordeum vulgare* ssp. *spontaneum* (known as wild barley) is the only species that can cross with cultivated barley under natural conditions. Wild barley is not found in Australia (OGTR 2008a). There are no reports of cultivated wheat forming hybrids with barley under natural conditions (OGTR 2008b).

146. Therefore, the likelihood of gene flow from wheat and barley (whether non-GM or GM) to sexually compatible species and subsequent production of fertile hybrids is low.

147. The applicant has proposed a number of measures to limit and restrict the potential for pollen flow and gene transfer to sexually compatible plants. These include a zone with minimal vegetation (in which the land closest to the proposed release is cleared of vegetation) and a 200 m zone free of wheat and barley plants, and the removal of plants that display a prolonged delay in flowering. The applicant has indicated that for the 2008-2009 growing

¹⁵ <http://www.anbg.gov.au/avh/> Public Access Map Search Interface accessed 5 Feb 08.

season there will be approximately 1000 m between the GM wheat and other wheat plants which may be planted in barley breeding trial plots where there is insufficient barley to fill all the required area. Any wheat plants used for this purpose would be destroyed after harvest. The applicant also indicated that there will be no wheat breeding trials planted in the 2008-2009 growing season. The applicant proposes to irrigate the site after harvest to encourage germination of any seedbank and to perform post harvest monitoring of the site for twenty four months or until the site has been clear of volunteers for one growing season; and destroy any volunteer plants found. These proposed controls would reduce the already low likelihood of gene flow from the GMOs to other wheat, barley or related species resulting in expression of the introduced genes.

148. **Conclusion:** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced genes and regulatory sequences in other wheat and/or barley plants or in other sexually compatible plants is **not an identified risk** and will not be assessed further.

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

149. Horizontal gene transfer is the movement of genetic information (DNA) between sexually unrelated organisms (Thomson 2000). In the context of genetic modification, a major concern has been whether DNA introduced into crops could transfer into bacteria in the soil or into the cells of organisms that may eat the crops. Horizontal gene transfer has been considered in previous RARMPs (including in detail in DIR 057/2004), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. These assessments have concluded that horizontal gene transfer from plants to other sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. There are no more recent reviews that alter this conclusion.

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

150. The probability of transferring the introduced genes and regulatory sequences contained in the GM wheat and barley plants is no greater than that of transferring any of the native genes. Non-GM wheat and barley contain homologues of all of the introduced wheat and barley genes and the regulatory sequences are also widespread in the environment. Therefore these genes and regulatory sequences are already available for transfer via demonstrated natural mechanisms (Chapter 1 Section 5.3).

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

152. The safety of the protein product(s) resulting from the expression of the introduced gene(s) rather than horizontal gene transfer *per se* is a key consideration in the risk assessment process (Thomson 2000). If the protein products are not associated with any risk to humans, animals or the environment then, even in the unlikely event of horizontal transfer occurring, they should still not pose any such risk. Events 1–3 (associated with the encoded proteins or end products) were not considered identified risks.

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

2.5 Unintended changes in biochemistry, physiology or ecology

154. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy¹⁶ (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such pleiotropic effects may include:

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

155. Such 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. 2005).

Event 6: *Changes to biochemistry, physiology or ecology of the GM wheat and barley lines resulting from altered expression or random insertion of the introduced genes*

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

157. Gene silencing, and/or reduced or no expression of endogenous and introduced genes, has been observed in plants genetically modified with additional copies of endogenous genes. In gene silencing, the introduced genes may be expressed but the messenger RNA is degraded before protein translation (reviewed in Bruening 1998). As the proposed release is early stage research information on the levels of expressed protein is not available for all lines.

158. The barley lines expressing the *Bot1* gene contain a 50 kb insert of genomic DNA. This region of genomic DNA originates from the Algerian barley landrace Sahara 3771 and contains four putative repetitive elements which the applicant indicates are common to non-coding regions of the barley genome. It is not known how this region of DNA will function in the *Bot1* expressing GM barley lines. However, it is not expected that the introduction of this region of genomic DNA would behave differently to DNA introduced during traditional plant breeding.

159. The applicant has indicated that plants with a delay in flowering of more than one month, compared to parent plants, will be removed from the site and destroyed. The

¹⁶ Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

likelihood of any pleiotropic effects causing adverse effects is minimised by the limits and controls outlined in Chapter 1, Sections 3.2 and 3.3.

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

Event 7: *Altered biochemistry or ecology of the surrounding environment as a result of growing the GM wheat and barley lines*

161. Changes in the biochemistry or ecology of the surrounding environment could result as an indirect effect from the expression of the introduced genes for boron tolerance or increased beta glucan.

162. The *Bot1* gene which has been introduced into some of the barley plants acts as an active efflux pump in the roots of the plants. It is possible that, in areas of high soil boron when the efflux pump is working to maintain acceptable levels of boron in the plant, small local changes in soil boron concentration may occur. However, the applicant has indicated that the soils at the proposed release site are not high in boron and the proposed release is a small scale release with only three lines out of the thirty proposed for release containing the introduced *Bot1* gene. Boron is also commonly present in the environment and the soils of the Australian wheat belt display natural variation in boron levels (Refer to Chapter 1, Section 5.2.1).

163. After the plants are harvested it is proposed that the residual plant material be ploughed into the soil. It is possible that the expression of the introduced *CsIF4*, *CsIF6* and *CsIF8* genes may alter the time taken by the remaining plant material to decompose in the soil due to the potentially higher beta glucan levels. Beta glucan synthesis occurs in cells during the elongation phase and beta glucan in the cell walls begins to degrade when cell elongation ends (Chapter 1, Section 5.2.4). Glasshouse trials show that the GM lines have 3.7 to 5.0% (w/w) beta glucan in the leaf tissue and 5.7 to 6.8% (w/w) beta glucan in the grains, where in non-GM plant tissues it is 1% (w/w) and 4.6 to 5.0% (w/w), respectively (information provided by the applicant). After hand harvest of the plants, the applicant proposes to plough no more than 10 cm of stem material into the ground. Mature stem stubble would be expected to have lower levels of beta glucan than actively growing plant tissues as the beta glucan levels in the cells of mature stems would be expected to be largely degraded. Beta glucan is not toxic as discussed in Event 1.

164. The likelihood of any adverse effects resulting in changes in the biochemistry or ecology of the surrounding environment as an indirect effect from the expression of the introduced genes for boron tolerance or increased beta glucan is unlikely. The likelihood will be further minimised by the limits and controls (Chapter 1, Sections 3.2 and 3.3) proposed for the release.

165. **Conclusion:** The potential for an adverse outcome as a result of unintended changes in biochemistry or ecology of the surrounding environment is **not an identified risk** will not be assessed further.

2.6 Unauthorised activities

Event 8: *Use of GMOs outside the proposed licence conditions (non-compliance)*

166. 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 and barley 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.

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

Section 3 Risk estimate process and assessment of significant risk

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

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

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

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

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

172. Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM wheat and barley lines into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed

limited and controlled release of GM wheat and barley **do not pose a significant risk** to the health and safety of people or to the environment¹⁷.

Section 4 Uncertainty

173. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (i.e. consequence and likelihood) are always uncertain to some degree.

174. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹⁸. For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily treating an identified risk.

175. For DIR 077/2007 which involves proof of concept research, uncertainty exists in relation to the characterisation of:

- ♦ Event 1, regarding potential increases in allergenicity or toxicity through contact with plant material containing proteins encoded by the introduced genes or their end products
- ♦ Event 2, associated with a potential for increased survival of the GMOs; and
- ♦ Event 6, due to incomplete information on the 50 kb of barley genomic DNA inserted in the GMOs with the *Bot1* gene.

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

177. Chapter 3, Section 5 discusses the additional data that may be required for future releases.

¹⁷ As none of the proposed dealings were considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

¹⁸ A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at <<http://www.ogtr.gov.au/pubform/riskassessments.htm>> or via Free call 1800 181 030.

Chapter 3 Risk management

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

Section 1 Background

179. 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.

180. 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.

181. 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

182. Australia's gene technology regulatory system operates as part of an integrated legislative framework avoids duplication and enhances coordinated decision making. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Health and Medical Research Council (NHMRC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹⁹.

183. 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.

¹⁹ 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>>.

184. 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 and barley lines to be used in human food. Accordingly the applicant has not applied to FSANZ for evaluation of any of the GM wheat lines for use in human food. FSANZ approval would need to be obtained before they could be used in food.

185. No other approvals are required.

Section 3 Risk treatment measures for identified risks

186. 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 and barley. The *Risk Analysis Framework* (OGTR 2007), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

187. These events were considered in the context of the scale of the proposed release (an area of 400 m² in one growing season, between June 2008 and June 2009, on one site in the South Australian local government area of Marion), the containment measures (Chapter 1, Section 3) and the receiving environment (see Chapter 1 Section 6).

Section 4 General risk management

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

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by The University of Adelaide

189. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls proposed by The University of Adelaide in their application, and discussed in the characterisation of events in Chapter 2. The appropriateness of these limits and controls are considered further below.

190. The release will be confined to one site, which occurs within a farm owned by The University of Adelaide and staffed by personnel who will receive appropriate training in practices relevant to the handling and disposal of GMOs. The release site will also be surrounded by a 1 m high fence to restrict access to the site by humans and large animals limiting both exposure to and dispersal of GM plant material outside the proposed release site (Event 3).

191. The applicant does not intend to use any of the GM plant material as human food or animal feed. This will limit the potential exposure of humans and vertebrates to the GMOs (Event 1) and the potential dispersal of the GMOs (Event 3).

192. The applicant's proposals to limit gene flow from the GM wheat and barley (Event 4) include: surrounding the proposed release site with a 1 m wide border of non-GM barley plants to act as a physical barrier; and a 200 m zone in which no wheat or barley plants are grown.

193. In wheat, differences in pollen flow have been observed between field and commercial trial size releases. For small scale trials, low rates of outcrossing occur up to 100 m with rare

occurrences up to 300 m, while at the commercial scale gene flow was found up to 2720 m from the pollen source. In barley, differences in pollen flow between field and commercial trial size releases have also been documented. However levels of gene flow are lower than those found for wheat. A small field scale barley trial has shown low levels of gene flow over a maximum distance of 10 m and at the commercial scale up to 60 m.

194. Field trial releases of GM wheat in Canada require a 30 m isolation distance between the GM plants and other wheat plants, while in the United States the isolation distance is reduced to 20 feet (approximately 6.1 m) (USDA-APHIS 1994; Canadian Food Inspection Agency 2006). In both Germany and Italy trials with GM wheat plants are required to be surrounded by a 5 m strip of plants, and in addition Germany requires a separation distance of 20 m (Directorate General for the Environment & European Commission 2004b; Directorate General for the Environment & European Commission 2004c; Directorate General for the Environment & European Commission 2006). A field trial release of GM barley in Iceland requires a separation distance of at least 300 m from other barley fields (Directorate General for the Environment & European Commission 2004a).

195. Both basic and certified wheat and barley seed in Australia is separated from other cereals by at least a two metre strip or a physical barrier such as a fence to prevent any mixture of seed during harvest (Smith & Baxter 2002). The acceptable level of off-types or other cultivars of the same species are 0.1% for basic seed and 0.3% for certified seed. Basic seed allows no contamination from other cereal species while in certified seed other cereal seeds may be present at a level of one seed in every two thousand (Smith & Baxter 2002). The OECD rules relating to the production of basic and certified seed from self-pollinated cereal state the same requirements (OECD 2008). The United States federal seed regulations do not specify an isolation distance for either wheat or barley used for seed production. However, for hybrid seed production a distance of 300 feet (approximately 100 m) is specified for both wheat and barley (Code of Federal Regulations 2006).

196. When considering the scientific literature, international containment measures for GM wheat and barley trials, and the rules for producing basic and certified seed, a 200 m isolation zone should minimise gene flow from the GM wheat and barley plants to other wheat and barley plants (Event 4).

197. The 1 m wide border of non-GM barley plants proposed by the applicant is suggested as a physical barrier. Relatively high rates of gene flow (0.8%) have been recorded for barley plants that are in physical contact with the rate of gene flow decreasing when separation distance increases (OGTR 2008a). However, the use of physical barrier plants is not expected to effectively reduce pollen dispersal by wind. Therefore, the requirement of a 1 m wide physical barrier is not imposed as a licence condition as it is considered that the 200 m isolation zone is adequate to minimise gene flow. The applicant intends to use the physical barrier plants to gather data on the level of gene flow from the GM barley plants. If any non-GM plants are planted with the release they must be treated as GM plants.

198. The zones of cleared land and mown vegetation proposed by the applicant (refer to Figure 2 for details) will serve as a measure to control rodent damage/feeding at the release site that might result in dispersal of the GMOs (Event 3). Whilst there are differing reports regarding the average territory size of mice, the use of reduced vegetation and short grassland vegetation have been shown to help reduce rodent numbers in agricultural settings (referred to as habit modification in Event 3). In a previous RARMP (DIR 071/2006), a 10 m monitoring zone with reduced vegetation has been considered to be an effective measure to deter rodents from entering the GM wheat site. Combined with rodent baits placed around the perimeter of

the release site, these control measures will significantly limit the number of rodents entering the release site.

199. The applicant has proposed a number of conditions to minimise the persistence of any GM wheat and barley plants at the proposed release site after harvest of the proposed trial (Event 3) via the introduction of GM wheat and barley seeds into the seed bank. These conditions include hand harvesting of plants to minimise seed spillage, ploughing in residual straw left after harvest, followed by a single irrigation of the site a month after the final plants have been harvested and destruction of any plants which have germinated. After the harvest of the plants at the proposed release site, the applicant has proposed to monitor the proposed release site for 24 months or until the site has been clear of any volunteers for one growing season. All volunteers will be destroyed by hand pulling or by herbicide application.

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

201. It is considered that three irrigations, combined with tillage to the depth of the initial cultivation, and monitoring for and destruction of volunteers would more effectively reduce survival and persistence of viable wheat and barley seeds in the soil at the completion of the release than a single irrigation. The initial irrigation should take place at the time that residual straw is ploughed into the soil will encourage surface seed to germinate. The second irrigation at least 28 days after will further assist volunteer seed germination. The third irrigation to occur 10 to 18 months after the proposed trial is harvested is designed to encourage germination of any remaining viable seed that may have been placed at too greater a depth for germination to be encouraged by previous irrigation events. These measures will minimise the persistence of the GMOs in the environment (Event 2).

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

203. The applicant has stated that any plant material taken off-site for experimental analysis will be transported according to the OGTR *Guidelines for the transport of GMOs, July 2007* (<http://www.ogtr.gov.au/pubform/handbook.htm#guidelines>) and will be destroyed by autoclaving or steam sterilization immediately after analysis. These are standard protocols for the handling of GMOs to minimise exposure of the GMO to human and other organisms (Event 1), dispersal into the environment (Event 3), and gene flow/transfer (Event 4).

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

204. A number of licence conditions have been imposed to limit and control the release, which are described in detail in the licence. These include requirements to:

- ♦ conduct the release at one site of up to 400 m² in the local government area of Marion, South Australia, between June 2008 and June 2009
- ♦ establish a 10 m monitoring zone around the trial site that is free of any related species and maintained in a manner that does not attract or harbour rodents
- ♦ maintain an isolation zone of at least 200 m around each trial site free of any sexually compatible species
- ♦ enclose the trial site within a 1 m high fence with lockable gates and placing rodent baits within the fenced area
- ♦ locate the trial site at least 50 m away from natural waterways
- ♦ harvest the GM wheat and barley 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
- ♦ clean all equipment, including clothing, used on the site
- ♦ after harvest, apply measures to promote germination of any wheat and/or barley seeds that may be present in the soil
- ♦ monitor the site for at least 24 months and destroy any wheat and/or barley plants that may grow until no volunteers are detected for a continuous 6 month period.

4.1.3 Measures to control other activities associated with the trial

205. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, July 2007; 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.

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

4.2 Other risk management considerations

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

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

4.2.1 Applicant suitability

208. 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.

209. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers The University of Adelaide suitable to hold a licence.

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

211. The University of Adelaide must continue to have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Compliance and contingency plans

212. Prior to planting the GM wheat and barley lines, The University of Adelaide is required to submit a plan detailing how it intends to ensure compliance with the licence conditions and to document that compliance.

213. The University of Adelaide is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan would detail measures to be undertaken in the event of any unintended presence of the GM wheat and barley lines outside of the permitted areas.

214. The University of Adelaide 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

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

4.2.4 Reporting structures

216. 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.

217. 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.

218. 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

219. 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 site.

220. 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.

221. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. These include the provision for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to health and safety of people or the environment could result.

Section 5 Issues to be addressed for future releases

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

- ♦ characterisation of the introduced genetic material in the plants, including gene copy number and genotypic stability, and additional information on the 50 kb insert present in some of the GM barley lines
- ♦ additional data on the potential toxicity of plant materials from the GM wheat and barley lines
- ♦ additional data on the allergenicity of proteins encoded by the introduced genes for enhanced tolerance to abiotic stresses or increased beta glucan; and
- ♦ characteristics indicative of weediness including measurement of altered reproductive capacity; tolerance to environmental stresses; and disease susceptibility.

Section 6 Conclusions of the RARMP

223. The risk assessment concludes that this proposed limited and controlled release of up to 30 GM wheat and barley lines on a maximum total area of 400 m² over one growing season in the South Australian local government area of Marion poses **negligible** risks to the health and safety of people and the environment as a result of gene technology.

224. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. If a licence were to be issued, conditions are proposed to restrict the

proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risk

References

- ABB Grain (2006). Flagship malting barley. AAB Grain.
- Anderson, R.L., Soper, G. (2003). Review of volunteer wheat (*Triticum aestivum*) seedling emergence and seed longevity in soil. *Weed Technology* **17**: 620-626.
- Arts, J., Mommers, C., de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. *Critical review in Toxicology* **36**: 219-251.
- Bhalla, P.L., Ottenhof, H.H., Singh, M.B. (2006). Wheat transformation - an update of recent progress. *Euphytica* **149**: 353-366.
- Blattner, F.R., Plunkett, G., Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B., Shao, Y. (1997). The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453-1462.
- Bradford, K.J., van Deynze, A., Gutterson, N., Parrot, W., Strauss, S.H. (2005). Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. *Nature Biotechnology* **23**[4], 439-444.
- Brennan, C.S., Cleary, L.M. (2005). The potential use of cereal (1->3),(1->4)- β -D-glucans as functional food ingredients. *Journal of Cereal Science* **42**: 1-13.
- Brennan, R.F., Adcock, K.G. (2004). Incidence of boron toxicity in spring barley in southwestern Australia. *Journal of Plant Nutrition* **27**: 411-425.
- Brown, P.R., Davies, M.J., Singleton, G.R., Croft, J.D. (2004). Can farm-management practices reduce the impact of house mouse populations on crops in an irrigated farming system? *Wildlife Research* **31**: 597-604.
- Bruening, G. (1998). Plant gene silencing regularized. *Proceeding of the National Academic of Science, USA* **95**: 13349-13351.
- Buckeridge, M.S., Rayon, C., Urbanowicz, B., Tine, M.A., Carpita, N.C. (2004). Mixed Linkage (1'3),(1'4)-beta-d-Glucans of Grasses. *Cereal Chemistry* **81**: 115-127.
- Buckeridge, M.S., Vergara, C.E., Carpita, N.C. (1999). The mechanism of synthesis of a mixed-linkage (1->3),(1->4) β -D-glucan in maize. Evidence for multiple sites of glucosyl transfer in the synthase complex. *Plant Physiology* **120**: 1105-1116.
- Burton, R.A., Wilson, S.M., Hrmova, M., Harvey, A.J., Shirley, N.J., Medhurst, A., Stone, B.A., Newbigin, E.J., Bacic, A., Fincher, G.B. (2006). Cellulose synthase-like *CsIF* genes mediate the synthesis of cell wall (1,3;1,4)- β -D-glucans. *Science* **311**: 1940-1942.
- Burton, R.A., Jobling, S.A., Harvey, A.J., Shirley, N.J., Mather, D.E., Bacic, A., Fincher, G.B. (2008). The genetics and transcriptional profiles of the cellulose synthase-like *HvCsIF* gene family in barley (*Hordeum vulgare* L.). *Plant Physiology* **107**.

- Caimi, P.G., McCole, L.M., Klein, T.M., Hershey, H.P. (1997). Cytosolic expression of the *Bacillus amyloliquefaciens* *SacB* protein inhibits tissue development in transgenic tobacco and potato. *New Phytol* **136**: 19-28.
- Caimi, P.G., McCole, L.M., Klein, T.M., Kerr, P.S. (1996). Fructan accumulation and sucrose metabolism in transgenic maize endosperm expressing a *Bacillus amyloliquefaciens* *SacB* gene. *Plant Physiol* **110**: 355-363.
- Canadian Food Inspection Agency (2006). Addendum II: Terms and conditions for confined research field trials of wheat (*Triticum aestivum* L.); Terms and conditions 2006. <http://www.inspection.gc.ca/english/plaveg/bio/dt/term/2006/triturge.shtml>.
- Cartwright, B., Zarcinas, B., Mayfield, A. (1984). Toxic concentrations of boron in a red-brown earth at Gladstone, South Australia. *Australian Journal of Soil Research* **22**: 261-272.
- Cartwright, B., Zarcinas, B.A., Spouncer, L.R. (1986). Born toxicity in South Australian barley crops. *Australian Journal of Agricultural Research* **37**: 351-359.
- Central Science Laboratory (2001). Final project report: Control of rat populations without the use of pesticides. Report No. VCO321, Government of the United Kingdom,
- Chandra, G.S., Proudlove, M.O., Baxter, E.D. (1999). The structure of barley endosperm - An important determinant of malt modification. *Journal of the Science of Food and Agriculture* **79**: 37-46.
- Code of Federal Regulations (2006). *Federal Seed Act Regulations* - (Title 7 Agriculture, Subtitle B Regulations of the Department of Agriculture, Chapter 1 Agricultural Marketing Service (Standards, Inspections, Marketing Practices), Part 201). 7(1)§201, 76-78 available at http://www.access.gpo.gov/nara/cfr/waisidx_06/7cfr201_06.html.
- Condon, K. (2004). Understanding frost risk by monitoring on-farm temperature. Ground Cover [49]. GRDC, <http://www.grdc.com.au/growers/gc/gc49/frost.htm>.
- DAFWA (2006). Effect of boron, soil acidity and aluminium toxicity on barley. http://www.agric.wa.gov.au/servlet/page?_pageid=449&_dad=portal30&_schema=PORTAL30&p_start_url=/pls/portal30/docs/FOLDER/IKMP/FCP/CER/BAR/CP/BARLEY_ESTAB_PADD.HTM.
- De Vries, J., Meier, P., Wackernagel, W. (2001). The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. *FEMS Microbiology Letters* **195**: 211-215.
- Directorate General for the Environment and European Commission (2004a). Notification report - *Field trials with marker gene in barley, followed by a 6 year research program (2003-2008) - Iceland*. Report No. B/IS/04/01, Joint Research Centre of the European Commission, available at http://gmoinfo.jrc.it/gmp_report.aspx?CurNot=B/IS/04/01.
- Directorate General for the Environment and European Commission (2004b). Notification report - *Fungal resistant wheat Germany 2004*. Report No. B/DE/03/151, Joint Research Centre of the European Commission, available at http://gmoinfo.jrc.it/gmp_report.aspx?CurNot=B/DE/03/151.

Directorate General for the Environment and European Commission (2004c). Notification report - *Study of the stability of the transgene and his hereditability of genetically modified wheat under of open field conditions - Italy*. Report No. B/IT/04/02, Joint Research Centre of the European Commission, available at http://gmoinfo.jrc.it/gmp_report.aspx?CurNot=B/IT/04/02.

Directorate General for the Environment and European Commission (2006). Notification report - *Increase of grain protein content in winter wheat - Germany*. Report No. B/DE/06/178, Joint Research Centre of the European Commission, available at http://gmoinfo.jrc.it/gmp_report.aspx?CurNot=B/DE/06/178.

EFSA (2004). Opinion of the scientific panel on genetically modified organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. *The EFSA Journal* **48**: 1-18.

Felsot, A.S. (2000). Insecticidal genes. Part 2: Human health hoopla. *Agrichemical & Environmental News* **168**: 1-7.

Forster, B.P. (2001). Mutation genetics of salt tolerance in barley: An assessment of Golden Promise and other semi-dwarf mutants. *Euphytica* **120**: 317-328.

Garlinge, J. (2005). 2005 crop variety sowing guide for Western Australia. Report No. 4655, State of Western Australia,

Gay, P., Le Coq, D., Steinmetz, M., Ferrari, E., Hoch, J.A. (1983). Cloning structural gene sacB, which codes for exoenzyme levansucrase of *Bacillus subtilis*: expression of the gene in *Escherichia coli*. *The Journal of Bacteriology* **153**: 1424-1431.

Gebhard, F., Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* **64**: 1550-1554.

Glover, J. (2002). Gene flow study: Implications for the release of genetically modified crops in Australia. Bureau of Rural Sciences, Australian Government Department of Agriculture, Fisheries and Forestry, Canberra.

Gomez-Macpherson, H. (2001). *Hordeum vulgare*. http://ecoport.org/ep?Plant=1232&entityType=PL****&entityDisplayCategory=full.

Goodman, R.E., Vieths, S., Sampson, H.A., Hill, D., Ebisawa, M., Taylor, S.L., van Ree, R. (2008). Allergenicity assessment of genetically modified crops - what makes sense? *Nature Biotechnology* **26**: 73-81.

Guadagnuolo, R., Savova-Bianchi, D., Keller-Senften, J., Febler, F. (2001). Search for evidence of introgression of wheat (*Triticum aestivum* L.) traits into sea barley (*Hordeum marinum* s.str. Huds) and bearded wheatgrass (*Elymus caninus* L.) in central and northern Europe, using isozymes, RAPD and microsatellite markers. *Theor Appl Genet* **103**: 191-196.

Hang, A., Obert, D., Gironella, A.I.N., Burton, C.S. (2007). Barley amylose and β -glucan: Their relationships to protein, agronomic traits, and environmental factors. *Crop Science* **47**: 1754-1760.

- Haslberger, A.G. (2003). Codex guidelines for GM foods include the analysis of unintended effects. *Nature Biotechnology* **21**: 739-741.
- Hayes, J.E., Reid, R.J. (2004). Boron tolerance in barley is mediated by efflux of boron from the roots. *Plant Physiology* **136**: 3376-3382.
- Jefferies, S.P., Barr, A.R., Karakousis, A., Kretschmer, J.M., Manning, S., Chalmers, K.J., Nelson, J.C., Islam, A.K.M.R., Langridge, P. (1999). Mapping of chromosome regions conferring boron toxicity tolerance in barley (*Hordeum vulgare* L.). *Theor Appl Genet* **98**: 1293-1303.
- Kahl, G. (2001). *The dictionary of gene technology: genomics, transcriptomics, proteomics*. Wiley-VCH, Weinheim, Germany. pp 1-941.
- Kokic, P., Davidson, A., and Boero-Rodriguez, V. (2006). Australia's grains industry: factors influencing productivity growth. Report No. ABARE research report 06.22,
- Lazaridou, A., Biliaderis, C.G. (2007). Molecular aspects of cereal β -glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science* **46**: 101-118.
- Lopato, S., Bazanova, N., Morran, S., Milligan, A.S., Shirley, N., Langridge, P. (2006). Isolation of plant transcription factors using a modified yeast one-hybrid system. *Plant Methods* **2**:
- Lu, Y., Xu, W., Kang, A., Luo, Y., Guo, F., Yang, R., Zhang, J., Huang, K. (2007). Prokaryotic Expression and Allergenicity Assessment of Hygromycin B Phosphotransferase Protein Derived from Genetically Modified Plants. *Journal of Food Science* **72**: M228-M232.
- Mantri, N.L., Ford, R., Coram, T.E., Pang, E.C.K. (2007). Transcriptional profiling of chickpea genes differentially regulated in response to high-salinity, cold and drought. *BMC Genomics* **8**: -<http://www.biomedcentral.com/1471-2164/8/303>.
- Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A., Flint, H.J. (1999). Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* **65**: 6-10.
- Miki, B., McHugh, S. (2004). Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *Journal of Biotechnology* **107**: 193-232.
- Miwa, K., Takano, J., Omori, H., Seki, M., Shinozaki, K., Fujiwara, T. (2007). Plants tolerant of high boron levels. *Science* **318**: 1417.
- Newsome, A.E. (1969). A Population Study of House-Mice Permanently Inhabiting a Reed-Bed in South Australia. *The Journal of Animal Ecology* **38**: 361-377.
- Nielsen, K.M. (1998). Barriers to horizontal gene transfer by natural transformation in soil bacteria. *Acta pathologica, microbiologica, et immunologica Scandinavica* **106**: 77-84.
- Nielsen, K.M., van Elsas, J.D., Smalla, K. (2000). Transformation of *Acinetobacter* sp strain BD413(pFG4 Delta nptII) with transgenic plant DNA in soil microcosms and effects of

kanamycin on selection of transformants. *Applied and Environmental Microbiology* **66**: 1237-1242.

OECD (2008). OECD Seed Schemes 2008. Organisation for Economic Co-operation and Development,

Ogg, A.G. and Parker, R. (2000). Control of volunteer crop plants. Report No. EB 1523, Washington State University Cooperative Extension,

OGTR (2005a). *Risk Analysis Framework*. Australian Government, Canberra, ACT.

OGTR (2005b). The Biology and Ecology of Bread Wheat (*Triticum aestivum* L. em Thell.) in Australia. Document prepared by the Office of the Gene Technology Regulator, Canberra, Australia, available online at <http://www.ogtr.gov.au/>,

OGTR (2007). Risk Analysis Framework. Report No. Version 2.2, Document produced by the Australian Government Office of the Gene Technology Regulator, available online from <http://www.ogtr.gov.au/>,

OGTR (2008a). The biology of *Hordeum vulgare* L. (barley). Document prepared by the Office of the Gene Technology Regulator, Canberra, Australia, available online at <http://www.ogtr.gov.au/>,

OGTR (2008b). The biology of *Triticum aestivum* L. em Thell. (Bread Wheat). Document prepared by the Office of the Gene Technology Regulator, Canberra, Australia, available online at <http://www.ogtr.gov.au/>,

Okamuro, J.K., Caster, B., Villarroel, R., Van Montagu, M., Jofuku, K.D. (1997). The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in *Arabidopsis*. *Proceedings of the National Academy of Sciences* **94**: 7076-7081.

Pickett, A.A. (1989). A review of seed dormancy in self-sown wheat and barley. *Plant Varieties and Seeds* **2**: 131-146.

Pierce, J.C., Sauer, B., Sternberg, N. (1992). A Positive Selection Vector for Cloning High Molecular Weight DNA by the Bacteriophage P1 System: Improved Cloning Efficacy. *Proceedings of the National Academy of Sciences* **89**: 2056-2060.

Pretty, J. (2001). The rapid emergence of genetic modification in world agriculture: contested risks and benefits. *Environmental Conservation* **28**: 248-262.

Queensland Department of Primary Industries and Fisheries (2007). Barley - Planting, nutrition and harvesting. http://www.dpi.qld.gov.au/cps/rde/xchg/dpi/hs.xsl/26_3514_ENA_HTML.htm#Nutrition.

Reiting, R., Broll, H., Waiblinger, H.-U., Grohmann, L. (2007). Collaborative study of a T-nos real-time PCR method for screening of genetically modified organisms in food products. *Journal of Consumer Protection and Food Safety* **2**: 116-121.

Roessner, U., Patterson, J.H., Forbes, M.G., Fincher, G.B., Langridge, P., Bacic, A. (2006). An investigation of boron toxicity in barley using metabolomics. *Plant Physiology* **142**: 1087-1101.

Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., Oono, Y., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., Satou, M., Akiyama, K., Taji, T., Yamaguchi-Shinozaki, K., Carninci, P., Kawai, J., Hayashizaki, Y., Shinozaki, K. (2002). Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *Plant Journal* **31**: 279-292.

Shinozaki, K., Yamaguchi-Shinozaki, K., Seki, M. (2003). Regulatory network of gene expression in the drought and cold stress responses. *Current Opinion in Plant Biology* **6**: 410-417.

Slee, D. (2003). Wheat yields: 3 times better than you thought. Ground Cover [44]. GRDC, http://www.grdc.com.au/growers/gc/gc44/wheat_yields.htm.

Smith, P. and Baxter, L. (2002). South Australian Seed Certification Scheme - Procedures and Standards Manual. Seed Services, Primary Industries & Resources South Australia, Plant Research Centre, Hartley Grove, Urrbrae, SA 5064, available online at http://www.ruralsolutions.sa.gov.au/_data/assets/pdf_file/0005/43349/seeds_manual.pdf.

Sorensen, I., Pettolino, F.A., Wilson, S.M., Doblin, M.S., Johansen, B., Bacic, A., Willats, W.G.T. (2000). Mixed linkage (1->3),(1->4)-beta-D-glucan is not unique to the Poales and is an abundant component of Equisetum arvense cell walls. *The Plant Journal* **Postprint**:

Staudte, R.G., Woodward, J.R., Fincher, G.B., Stone, B.A. (1983). Water-soluble (1->3),(1->4)-β-D-Glucan from barley (*Hordeum vulgare*) endosperm. III. Distribution of cellotriosyl and cellotetraosyl residues. *Carbohydrate Polymers* **3**: 299-312.

Sutton, T., Baumann, U., Hayes, J., Collins, N.C., Shi, B.-J., Schnurbusch, T., Hay, A., Mayo, G., Pallotta, M., Tester, M., Langridge, P. (2007). Boron-toxicity tolerance in barley arising from efflux transporter amplification. *Science* **318**: 1446-1449.

Thomson, J.A. (2000). Horizontal transfer of DNA from GM crops to bacteria and to mammalian cells. *Journal of Food Science* **66**: 188-193.

Tsuchiya, K., Urahara, T., Konishi, T., Kotake, T., Tohno-oka, T., Komae, K., Kawada, N., Tsumuraya, Y. (2005). Bioynthesis of (1->3),(1->4)-β-glucan in developing endosperms of barley (*Hordeum vulgare*). *Physiologia Plantarum* **125**: 181-191.

US FDA (2006). Agency response letter GRAS notice no. GRN 000207. United States Food and Drug Administration,

USDA-APHIS (1994). Environmental Assessment and finding of No Significant Impact - application (APHIS Number 94-221-01) - field test with genetically engineered (transgenic) wheat (*Triticum aestivum*) plants. Report No. 94-221-01 available at <http://www.isb.vt.edu/biomon/releapdf/9422101r.ea.pdf>.

van Gool, D. and Vernon, L. (2006). Potential impacts of climate change on agricultural land use suitability: barley. Report No. 302, Department of Agriculture, Western Australia.

Vij, S., Tyagi, A.K. (2007). Emerging trends in the functional genomics of the abiotic stress response in crop plants. *Plant Biotechnology Journal* **5**: 361-380.

- Waines, J.G., Hedge, S.G. (2003). Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. *Crop Science* **43**: 451-463.
- Wang, W., Vinocur, B., Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* **218**: 1-14.
- White, J., Horskins, K., Wilson, J. (1998). The control of rodent damage in Australian macadamia orchards by manipulation of adjacent non-crop habitats. *Crop Protection* **17**: 353-357.
- Wood, P.J. (2007). Cereal β -glucans in diet and health. *Journal of Cereal Science* **46**: 230-238.
- Yamaguchi-Shinozaki, K., Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annual Review of Plant Biology* **57**: 781-803.
- Yau, S.K. (2002). Interactions of boron-toxicity, drought, and genotypes on barley root growth, yield, and other agronomic characters. *Australian Journal of Agricultural Research* **53**: 347-354.

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 authorities²⁰ on the consultation RARMP for DIR 077/2007

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

Summary or issues raised	Comment
Suggested that the isolation zone be increased from 200 m to 1,000 m due to concern over potential pollen flow to non-GM plants.	The scientific literature referenced in the submission was considered in the preparation of the RARMP Events 4 & 5. Considering the available scientific literature the proposed isolation zone is considered to be adequate to limit and control the trial and minimise gene flow to non-GM plants (Ch 3, Section 4.1).
The GMOs expressing the gene introduced for soil boron tolerance also contain a large non coding region of introduced DNA. Superfluous or uncharacterised DNA sequences pose a degree of hazard to the environment. These sequences could facilitate unintended genomic rearrangements or promote horizontal gene transfer.	The large non-coding region of introduced DNA is <i>Hordeum vulgare</i> genomic DNA and is not expected to act differently from DNA transferred through conventional breeding. It contains putative repetitive elements that are common to non-coding regions of the barley genome (Event 6). Characterisation of the 50 kb insert has been identified as information that may be required for any possible future releases of these GM lines.
The <i>SacB</i> gene is not required to produce the desired trait. The large non-coding region of DNA inserted may contain sequences that are able to drive the expression of the <i>SacB</i> gene.	The <i>SacB</i> gene is not expected to be expressed in the GM plants. If the <i>SacB</i> gene was to be expressed in the GM plants, the GM plants would be expected to have reduced fitness as tissue damage to plants expressing the <i>SacB</i> gene has been reported.

²⁰ GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment, Heritage & the Arts and the Local council where the release may occur.

Summary or issues raised	Comment
<p>Suggests several areas of further research before future releases of the lines are considered:</p> <ul style="list-style-type: none"> ♦ stability of the genotypes; ♦ details of the survival of the GMOs in the environment under the relevant abiotic stresses; ♦ metabolic and proteomic alterations due to the presence of the introduced gene. 	<p>Noted. The RARMP identified additional information that may be required by the OGTR to assess an application for a large scale or commercial release of these GM wheat or barley lines. The additional information identified in the RARMP is similar to the issues raised in this submission.</p>
<p>Notes that if any future applications were made that involve produce being used in food or feed the OGTR would need to examine the potential for altered allergenicity or toxicity, or for altered metabolism of pesticides applied to the GM plants. If altered metabolism resulted in changes in the nature/quality of chemical residues a revised Acceptable Daily Intake may have to be established, and the definition of the herbicide residue in and on products may have to be reset, based on relevant toxicology and metabolism data.</p>	<p>Noted. Toxicity and allergenicity of the GMOs has been identified as a future research requirement.</p> <p>APVMA is responsible for pesticide use and chemical residues.</p>
<p>Notes that the creation or breeding of plants able to survive in bushland or wilderness areas without intervention, due drought resistance or seed viability changes, is a major environment concern. This could result in weeds that are harder to control or resistant herbicides or other control mechanisms currently used.</p>	<p>Wheat and barley are not problem weeds in Australia and lack most characteristics common to weeds. These characteristics are not expected to be altered by the introduced genes (Events 2, 3). Control measures have been imposed to minimise survival and persistence of the GM lines.</p>
<p>Notes that placement of GM plants with the environment requires consideration to prevent cross pollination, spread of weeds and “genetic pollution” of natural plants.</p>	<p>Noted. The risk of weediness and an adverse outcome as a result of gene transfer are considered negligible for the current limited and controlled field trial (Events 2,3,4).</p>

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 077/2007

The Regulator received five submissions from the public on the consultation RARMP. These submissions, summarised in the table below, 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 raised: **A:** administration; **AG:** agricultural production; **AR:** antibiotic resistance; **B:** benefits of gene technology; **CCI:** confidential commercial information; **DR:** data requirements; **EA:** expert advice; **EN:** environmental risks; **GT:** gene transfer; **H:** human health and safety; **HGT:** horizontal gene transfer; **L:** labeling; **M:** marketing concerns; **Mor:** moratorium; **RA:** risk assessment process; **RM:** risk management; **S:** segregation; **UE:** unintended effects

Other abbreviations: **CCD:** colony collapse disorder; **FSANZ:** Food Standards Australia New Zealand; **GM:** Genetically Modified; **GMO:** genetically modified organism; **GTR:** Gene Technology Regulator; **GTTAC:** the Gene Technology Technical Advisory Committee; **OSA:** outside scope of assessment

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

Sub. No:	Type	Issue	Summary of issues raised	Comment
1	I	AG	The use of GM crops will lead to increased farming of dryland ecosystems, exacerbating the results of the green revolution.	OSA. Decisions on where and when to grow crops are primarily economic and planning issues outside the scope of the Act.
		H	Human health and safety is put at risk by GM which attempts to alter the genetic heritage of natural selection.	Risk to human health and safety as a result of this limited and controlled release has been assessed as negligible.
2	IG	None	Proposed release site is not within the Local Government Area therefore no comment is provided.	Noted.
3	I	Administrative processes		
		A	Concerned about administrative processes in the OGTR and the public service in general.	Noted.
		CCI	Feels that secrecy about introduced genes stifles debate and "Commercial in Confidence" claims must be justified in areas of public debate.	The GTR considers each application for CCI in accordance with the Act. CCI in relation to this application was granted by the Regulator on 6 December 2007.
		EA	Disclosure of interests of experts and advisors should be made public.	All committee members are subject to strict disclosure of interest provisions which are contained in the Gene Technology Regulations 2001. OGTR staff are subject to the same disclosure of interest provisions as all Australian public service employees.

Sub. No:	Type	Issue	Summary of issues raised	Comment
			The GTR is being tested by applicants and assistance from the OGTR and other experts is inadequate. GTR must seek advice from unbiased, competent experts and ensure the OGTR is staffed by competent persons who keep up to date with the literature and submissions.	Members of GTTAC are experts that are appointed on the basis of their skills and experience in a range of subject areas relevant to gene technology. Scientific staff within the OGTR have postgraduate qualifications and research experience in a number of subject areas relevant to gene technology. The Office has ongoing programs for professional development including ensuring that staff have access to the latest scientific information in relevant areas of gene technology.
		Horizontal Gene Transfer (HGT)		
		HGT	<p>Concerned about the conclusion of the risk assessment of HGT in the current DIR 077/2007 and 080/2007 RARMPs (and their reference to DIR 057/2004).</p> <p>Considers that the OGTR and its experts, advisors and committees have not dealt appropriately with HGT and the risk assessment is biased against HGT.</p> <p>Notes that a current literature review on HGT has not been prepared by either the OGTR or the applicant.</p>	<p>The risk of HGT from the GMOs to microorganisms in the soil has been considered in the context of this limited and controlled release (Event 5).</p> <p>A scientist on the OGTR staff has recently had a review of the risk of HGT from GMOs accepted for publication in an international, peer reviewed journal. This paper is a comprehensive review of the current scientific, peer reviewed literature on HGT and cites over a hundred independent papers, including a number of papers published in 2008.</p>
		GT H UE	<p>Reasons provided for concern about HGT include;</p> <ul style="list-style-type: none"> Genes introduced by genetic modification are not as stable as natural genes in genomes and are therefore more likely to be transferred. A multitude of microenvironments would exist at the trial site including conditions that may be suitable for transformation. Sufficient sequence homology may exist for homologous recombination to occur between the DNA introduced into the GMOs and microorganisms and for the sequences to be expressed. Calculates that 10^9 new drought tolerant species of microorganisms could be produced as a result of the proposed release for DIR 077/2007. Natural environmental conditions could provide selective pressure for microorganisms to retain abiotic stress tolerance genes as a result of HGT. HGT of introduced abiotic tolerance genes from GM plants to microorganisms could lead to microorganisms more tolerant to abiotic stresses and could result in new and tougher species of pathogens with greater virulence, the consequences of which may not be immediately obvious but could have a significant impact on human health and safety. 	<p>The risk arising from such transfer from GMOs to microorganisms was assessed using the available evidence and was considered to be negligible (Event 5). Reasons include:</p> <ul style="list-style-type: none"> There is no evidence that the introduced genes are unstable or more likely to transfer to other organisms. None the less further evidence would be required if a larger scale release application was made. Transfer of plant DNA to microorganisms is extremely rare; although examples have been identified the genome sequencing of bacteria shows a lack of plant genes. There are a large number of events that must successfully occur before an adverse outcome could arise as a result of HGT, should it occur. Many of these events are considered unlikely. The introduced genes are from wheat and barley. Therefore, these genes have been in the agricultural environment and available for transfer for a significant amount of time. Even so, microorganisms have preferentially evolved other mechanisms to deal with drought and other adverse conditions. For example fungi develop spores which allow them to survive dry conditions. The small size and short duration of the proposed trial greatly reduce the chance of any adverse effect occurring as a result of HGT, should it occur.

Sub. No:	Type	Issue	Summary of issues raised	Comment
		Dispersal of GM Plant Material		
			Bird dispersal of GM seed was not considered in the RARMP. Use of bird scarers or nets should be considered.	<p>Dispersal of GM plant material has been considered in detail in previous RARMPs and reference documents prepared by the OGTR. A detailed consideration was undertaken for DIR 071/2006 (Event 8), and risks were considered negligible.</p> <p>A recent literature search was unable to find reports of wheat or other grain seeds being dispersed by birds in Australia.</p> <p>Bird damage has been reported for wheat crops. However, birds appear to prefer softer plant parts and are more likely to eat the GM wheat or grain on site rather than carry it elsewhere for storage or consumption.</p> <p>Dispersal of any GM seed is not expected to result in the establishment of volunteer plants as they would need appropriate environmental conditions for germination, survival and persistence. The spread and persistence of the GMOs outside of the trial site would be limited by multiple factors including temperature, low intrinsic competitive ability, nutrient availability, and pests and diseases (Event 2).</p>
		Data Requirements		
		DR	A number of lines are proposed for release but exact information (including interpretable Southern and Northern blots) on gene copy number, insertion sites, stability; and about phenotypic features, which should have been identified in laboratory and greenhouse trials, are not provided for each of them.	Some information on gene copy number was provided as was information on relevant phenotypic features. This is a 'proof of concept' trial and issues to be addressed by the applicant for future releases have been identified in the RARMP, including further characterisation of the introduced genetic material in the plants.
			<p>Suggested experimental tests that the OGTR should have required the applicant to undertake to test for HGT to soil microorganisms.</p> <p>Further data, such as transcription/expression in <i>E.coli</i> (and possibly yeast) and colony lifts of microorganisms in soil cultured with the construct DNA, should be required by the OGTR.</p>	Risks to the health and safety of people or the environment that might arise as a result of HGT from the GMOs to microorganisms in the soil has been considered in the context of this limited and controlled release (Event 5). Information suggested was not required for this release.
		Other concerns		
		B	Considers that greater corporate control, larger profits and royalties provide some financial benefit to others but no real benefit to the public or consumers. On the other hand there is a significant risk of establishing new and unpredictable pathogenic microorganisms.	Risks to the health and safety of people or the environment that might arise as a result of HGT from the GMOs to microorganisms in the soil has been considered in the context of this limited and controlled release (Event 5). The consideration of financial benefits of gene technology are outside the scope of issues the GTR must have regard to when deciding whether or not to issue a licence.

Sub. No:	Type	Issue	Summary of issues raised	Comment
4	A	M S	States that the Australian animal industries have a potential market advantage over international competitors through the ability to avoid GM feed stocks. Feels that, while beyond the scope of the current RARMP, more research should be conducted into improving on-farm safety and tracking systems for segregation of GM and non-GM feed stocks.	Noted. Agricultural production, segregation and marketing concerns are outside the scope of assessments required by the act.
5	IG	S	States US farmers have suffered a variety of problems with GM seed including; cross-pollination, seed drift, herbicide resistance and seed supply mix ups.	Noted. Licence conditions have been imposed to restrict the release in time and space. 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.
		S	Expresses concern about GM wheat and barley seed from the proposed trial being unintentionally mixed with non-GM seed.	Noted. Licence conditions have been imposed to restrict seed dispersal including harvesting by hand and separately from other crops, and transporting according to OGTR Guidelines.
		EN	States that GM pollen drift has been implicated in bee colony collapse disorder and the demise of bees would have adverse economic effects.	Risks to a range of organisms including insects (as a result of the proposed trial) were considered to be negligible. Speculation that there is a link between GM crops and CCD is not supported by scientific evidence. Current evidence suggests that CCD is likely to be linked to a combination of factors contributing to the stress of honey bees including; viral, bacterial and fungal diseases, parasitic mites, cultural practices (e.g. movement of hives, in-hive chemical use) and nutritional status of the adult bees. GM crops are not considered a likely cause of CCD.
		H	Raises several human health concerns. States that GM food crops have yet to be proven safe for human consumption and that independent scientists and professional bodies warn of a range of risks from allergies to cancer. Also states that overseas and Australian studies have resulted in adverse health effects in rats or mice. States that there is a risk that pollen from the GM plants may be inhaled by a larger section of the population than usual as the proposed trial site is in close proximity to urban housing.	None of the GM plant material will be used in human food or animal feed. The pollen from the GM plants is not expected to be any more allergenic than pollen from non-GM wheat or barley. All introduced genes, with the exception of the marker genes, were originally isolated from wheat or barley. Both selectable marker genes the used in construction of the GM wheat and barley lines are widespread in the environment and are not known allergens.
		L	In the absence of labelling the impact of GM consumption on human health cannot be monitored or identified.	OSA. Labelling of food is the responsibility of FSANZ.

Sub. No:	Type	Issue	Summary of issues raised	Comment
		AR	States that there is awareness that antibiotic resistance markers may cause increased pools of antibiotic resistant bacteria. There have been calls to avoid their use from certain bodies including the British Medical Association.	Risks to the health and safety of people and environment associated with the selectable marker genes were assessed as negligible. Both selectable marker genes the used in construction of the GM wheat and barley lines are widespread in the environment. The <i>hpt</i> gene which confers hygromycin resistance was included in a study by the European Food Safety Authority who concluded that inclusion of this gene in GM plants would not significantly affect the health of humans or animals. The <i>SacB</i> gene was originally isolated from a soil bacterium and is not an antibiotic resistance marker gene.
		RM	Concerned that the proposed licence condition that the site be enclosed by a 1 m high fence will not stop domestic pets (cats or dogs), rabbits which inhabit the site and foxes from spreading plant material, seeds or pollen in their fur and, therefore, the Risk Management Plan appears to be inadequate.	Risks associated with the dispersal of plant material by animals were considered negligible (Event 3). The larger area on which the proposed trial site is to be located is itself surrounded by a 2 m fence which should limit entry by domestic pets and foxes. Licence conditions have been imposed to limit gene flow and seed dispersal.