



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 099**

Limited and controlled release of wheat and barley
genetically modified for altered grain composition or
nutrient utilisation efficiency

**Applicant: Commonwealth Scientific and Industrial
Research Organisation**

June 2010

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 099) from Commonwealth Scientific and Industrial Research Organisation (CSIRO). The licence authorises dealings involving the limited and controlled release of up to 11 lines¹ of genetically modified (GM) wheat and 3 lines of GM barley into the 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 or not to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public².

The application

CSIRO has applied for a licence for dealings involving the intentional release of up to 11 lines of GM wheat and 3 lines of GM barley on a limited scale and under controlled conditions. Four of the GM wheat lines have been genetically modified for altered grain composition. The remaining GM wheat lines and the 3 GM barley lines have been genetically modified for enhanced nutrient utilisation efficiency. The trial will take place at two sites, one in the shire of Narrabri (NSW) and the other in the shire of Corrigin (WA), on a maximum area of 2 ha per year, between June 2010 and June 2013.

Four of the GM wheat lines contain a gene fragment designed to decrease expression of a gene involved in determining grain qualities important for dough making and human nutrition. Decreased expression of the targeted gene results in alterations to the starch composition of the grain. Seven of the GM wheat lines and the three GM barley lines contain a gene from barley that encodes an enzyme involved in nitrogen utilisation. Expression of this gene is expected to result in an increase in plant biomass and yield. All of the GM wheat and barley lines contain a selectable marker gene.

The purpose of the trial is to assess the growth and yield characteristics of the GM plants when grown under field conditions. The applicant also intends to generate sufficient grain to assess any changes in grain composition for the GM plants relative to non-GM plants and how this may affect dough characteristics and end-product quality. The GM wheat and barley would not be used for human food or animal feed.

¹ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

² 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 (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

CSIRO proposed a number of controls to restrict the spread and persistence of the GM wheat and barley lines and the introduced genetic materials in the environment. These controls were considered during the evaluation of the application.

Confidential Commercial Information

Some details, including the name and sequence of the genes and some regulatory sequences, the identity of vectors, specific phenotypes, and some testing methods 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), previous approvals, relevant scientific/technical knowledge and advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology are postulated (risk scenarios), and these scenarios are evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight risk scenarios were postulated, including 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 wheat and barley; or produce unintended changes in the biochemistry of the GMO. The opportunity for gene flow to other organisms, and its effects if it were to occur, was also assessed.

A **risk** is only identified for further assessment when a risk scenario is considered to have some chance of causing harm. Pathways 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 risk scenarios in relation to both the seriousness and likelihood 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.

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

Risk management plan

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through the licence conditions.

As none of the eight risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed 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, conditions are imposed to restrict the spread and persistence of

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

The licence conditions require CSIRO to **limit** the release to a total area of 2 ha per year at two sites from the date of issue of the licence until June 2013. The **control** measures include containment provisions at the trial site, 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 the Regulator's transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concluded that this proposed limited and controlled release of up to 11 GM wheat lines and 3 GM barley lines on a maximum total area of 2 ha per year over three growing seasons in the shire of Narrabri (NSW) and the shire of Corrigin (WA), poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

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Abbreviations

the Act	<i>Gene Technology Act 2000</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
CCI	Confidential Commercial Information as declared under section 185 of the <i>Gene Technology Act 2000</i>
CaMV	Cauliflower mosaic virus
CSIRO	Commonwealth Scientific Industrial Research Organisation
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
HGT	Horizontal gene transfer
HPT	Hygromycin phosphotransferase
km	kilometre
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NPT	Neomycin phosphotransferase
NUE	Nitrogen Use Efficiency
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase Chain Reaction
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RNA	Ribonucleic Acid
TGA	Therapeutic Goods Administration

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Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of application (DIR 099) from Commonwealth Scientific and Industrial Research Organisation (CSIRO). The licence authorises dealings involving the limited and controlled release of up to 11 lines³ of genetically modified (GM) wheat and 3 lines of GM barley into the 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 or not to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public⁴.

The application

CSIRO has applied for a licence for dealings involving the intentional release of up to 11 lines of GM wheat and 3 lines of GM barley on a limited scale and under controlled conditions. Four of the GM wheat lines have been genetically modified for altered grain composition. The remaining GM wheat lines and the 3 GM barley lines have been genetically modified for enhanced nutrient utilisation efficiency. The trial will take place at two sites, one in the shire of Narrabri (NSW) and the other in the shire of Corrigin (WA), on a maximum area of 2 ha per year, between June 2010 and June 2013.

Four of the GM wheat lines contain an introduced synthetic gene designed to reduce or silence the expression of a specific wheat gene by a mechanism known as RNA interference (RNAi). The gene introduced is under the control of an endosperm-specific promoter and targets Starch Metabolic Enzyme B (*SMEB*) gene which, when silenced, alters the composition of grain starch.

Seven of the GM wheat lines and all of the GM barley lines contain the *Me1* gene which encodes a metabolic enzyme. This gene is naturally present in non-GM barley plants and its expression is expected to enhance the efficiency of nitrogen utilisation. Expression of the introduced *Me1* gene is under the control of a tissue specific promoter derived from rice. The *Me1* gene has previously been overexpressed in canola and rice and resulted in a phenotype of increased plant biomass and increased yield. A similar effect is expected for overexpression of *Me1* in wheat and barley.

All of the GM wheat lines contain one of two selectable marker genes derived from bacteria: either the *nptII* gene which confers resistance to aminoglycoside antibiotics related to

³ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

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 (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

kanamycin and neomycin, or the *hpt* gene which confers resistance to the antibiotic hygromycin. All of the GM barley lines contain the selectable marker gene *hpt*. These were used as selectable markers during early stages of development of the GM plants in the laboratory.

Other short regulatory sequences that control expression of the genes will also be present in the GM wheat and barley lines. These are derived from wheat, rice, Cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens* (a common soil bacterium). Although some of these sequences are derived from plant pathogens (*A. tumefaciens* and CaMV), the regulatory sequences comprise only a small part of the pathogen's total genome, and in themselves have no pathogenic properties.

The GM wheat lines were produced by transforming plants of the wheat cultivars Bobwhite 26 (nine lines) and Frame (two lines). The 3 GM barley lines were produced by transforming plants of the barley cultivar Golden Promise.

The purpose of the trial is to assess the growth and yield characteristics of the GM plants when grown under field conditions. The applicant also intends to generate sufficient grain to assess any changes in grain composition for the GM plants relative to non-GM plants and how this may affect dough characteristics and end-product quality.

The GM wheat and barley would not be used for human food or animal feed.

CSIRO proposed a number of controls to restrict the spread and persistence of the GM wheat and barley lines and the introduced genetic materials in the environment. These controls were considered during the evaluation of the application.

Confidential Commercial Information

Some details, including the name and sequence of the genes and some regulatory sequences, the identity of vectors, specific phenotypes, and some testing methods, 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), previous approvals, relevant scientific/technical knowledge and issues relating to risks to human health and safety and the environment raised in submissions received from consultation with a wide range of prescribed experts, agencies and authorities (included in Appendix A of the RARMP) as well as the public (included in Appendix B of the RARMP).

The reference documents, *The Biology of Triticum aestivum L. em Thell (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. The documents are available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology are postulated (risk scenarios), and these scenarios are evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight risk scenarios were postulated, including 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 wheat and barley; or produce unintended changes in the biochemistry of the GMO. The

opportunity for gene flow to other organisms, and its effects if it were to occur, was also assessed.

A **risk** is only identified for further assessment when a risk scenario is considered to have some chance of causing harm. Pathways 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 risk scenarios in relation to both the seriousness and likelihood 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 principal reasons for this include:

- limits on the size, locations and duration of the release proposed by CSIRO
- suitability of controls proposed by CSIRO to restrict the spread and persistence of the GM wheat and barley plants and their genetic material
- limited ability and opportunity for the GM wheat and barley to transfer the introduced genes to other wheat plants or other sexually compatible species
- none of the GM plant materials or products will be used for human food or animal feed
- widespread presence of the same or similar proteins encoded by the introduced genetic material in the environment and lack of known toxicity or evidence of harm from them.

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

Risk management plan

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through the licence conditions.

As none of the eight risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed 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, conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and to limit the release to the size, locations and duration requested by the applicant, as these were important considerations in establishing the context for assessing the risks.

Licence conditions

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

- limit the release to a total area of up to 2 ha per growing season at two sites, one in the LGA of Narrabri (NSW) and the other in the LGA of Corrigin (WA), from the date of issue of the licence until June 2013
- locate the trial sites at least 50 m away from natural waterways
- establish a 10 m zone around the trial sites in which any related species are prevented from flowering and which is maintained in a manner that does not attract or harbour rodents

- surround the GM wheat and barley with an inspection zone of up to 200 m in which growth of sexually compatible species is controlled
- ensure no other crops of wheat or barley are within 200 m of the trial sites
- enclose the trial site in the shire of Corrigin with a livestock-proof fence with lockable gates and ensure that livestock are excluded from trial site in the shire of Narrabri
- harvest the GM wheat and barley plant material separately from other crops
- clean the sites and equipment used on the sites following harvest
- apply measures to promote germination of any wheat and barley seeds that may be present in the soil after harvest, including irrigation
- monitor the site for at least 24 months after harvest and destroy any wheat and/or barley plants that may grow until no volunteers are detected for a continuous 6 month period
- destroy all GM plant material not required for further analysis or future trials
- transport material from the GMOs in accordance with the Regulator's guidelines
- not permit any GM wheat or barley plant material to be used in human food or animal feed, or in the production of therapeutic goods.

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 Standards 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)⁵.

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

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 these GM wheat and barley lines, or to justify a reduction in containment conditions. This would include:

- additional data on the potential allergenicity or toxicity of plant materials from the GM wheat and barley lines
- additional phenotypic characterisation of the GM wheat and barley lines, in particular pest disease susceptibility and characteristics indicative of weediness including measurement of altered reproductive capacity and competitiveness
- characterisation of the introduced genetic material in the plants, including copy number and genotypic stability.

⁵ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR).

Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

Suitability of the applicant

The Regulator determined, at the commencement of the assessment process for this application, that CSIRO was suitable to hold a DIR licence under the requirements of section 58 of the Act. The Regulator is satisfied that CSIRO remains suitable as no relevant convictions have been recorded, and no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment.

Conclusions of the RARMP

The risk assessment concluded that this proposed limited and controlled release of up to 11 GM wheat lines and 3 GM barley lines on a maximum total area of 2 ha per year over three growing seasons in the shire of Narrabri (NSW) and the shire of Corrigin (WA), poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, locations and duration proposed by the applicant, and to require controls in line with those proposed 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 potential risks to the health and safety of people or the environment posed by the proposed release are assessed (Figure 1).

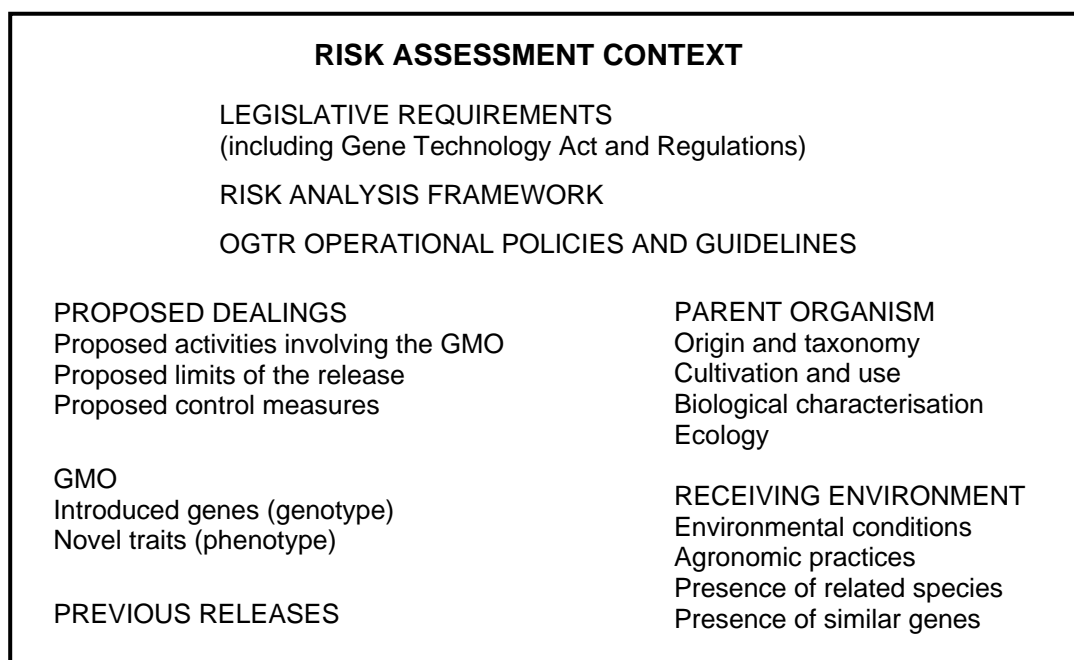


Figure 1. Parameters used to establish the risk assessment context

2. The risk assessment context is developed within the framework of the *Gene Technology Act 2000* and Gene Technology Regulations 2001 (Section 2), the *Risk Analysis Framework*, and operational policies and guidelines (see <http://www.ogtr.gov.au>).

3. In addition, establishing the risk assessment context for this application includes consideration of:

- the proposed dealings (Section 3)
- the parent organism (Section 4)
- the GMOs and the nature and effect of the genetic modification (Section 5)
- the receiving environment (Section 6)
- previous releases of these or other GMOs relevant to this application (Section 7).

Section 2 The legislative requirements

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

5. In accordance with section 50A of the Act, the Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the

applicant to conduct experiments. In addition, limits have been proposed on the size, locations and duration of the release and controls have been proposed by the applicant to restrict the spread and 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 is considered to be a limited and controlled release and the Regulator has prepared a RARMP for this application.

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

7. Section 52(2)(ab) 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). The decision is provided in Section 3 of Chapter 2.

Section 3 The proposed dealings

8. The Commonwealth Scientific and Industrial Research Organisation (CSIRO) proposes to release up to eleven lines⁶ of genetically modified (GM) wheat and three lines of GM barley into the environment under limited and controlled conditions. Four of the GM wheat lines have been modified for altered grain composition. The other seven GM wheat lines and three GM barley lines have been modified for enhanced nutrient utilisation efficiency.

9. The dealings involved in the proposed intentional release would include:

- propagating, growing, raising or culturing the GMOs
- breeding the GMOs
- using the GMOs in the course of manufacture of a thing that is not the GMOs
- conducting experiments with the GMOs
- transporting the GMOs
- disposing of the GMOs.
- possession, supply or use of the GMOs for the purposes of any of the above.

10. These dealings are detailed further throughout the remainder of the current Chapter.

11. Some details of the application including the name, sequence and function of the gene targeted by RNAi, the name and sequence of the introduced gene for enhanced nutrient utilisation efficiency, promoters, the identity of vectors, some of the specific phenotypes observed in these lines, and some testing methods have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was

⁶ The term ‘line’ is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

considered during the preparation of the RARMP and was made available to the prescribed expert groups and authorities that were consulted.

3.1 The proposed activities

12. The applicant has stated that the purpose of the trial is to:

- assess whether expression of the genes results in altered growth, biomass and yield characteristics in the GM plants, compared to non-GM wheat and barley plants, when grown under field conditions
- assess the impact of expression of the genes on grain characteristics and evaluate how such changes may affect performance of flour from the GM grain in foods.

13. The GM wheat and barley lines proposed for release would be grown between June 2010 and June 2013, over three growing seasons.

14. The applicant expects that approximately one-third of each site would be planted in each of the three growing seasons. The proposed area for planting would be dependant on the availability of seeds and the result of the trials from the previous year. The area sown in the first year will be determined by the availability of seeds from field trials currently being conducted on the same GMOs under licences DIR 092 and DIR 094. In the proposed trial, the yield from year 1 will determine the area sown in year 2. Similarly, the yield from year 2 will determine the area sown in year 3. In each location, the area sown in year 1 will not be used in year 2, and the area sown in year 2 will not be used in year 3. The applicant states that not planting in the same area each year will lower the chance of soil borne disease and facilitate monitoring and destruction of volunteers from the previous years' sowing. The applicant has requested to sow the area used in year 1 in year 3, if needed. The applicant proposes to provide full details of the areas planted at each location, in each growing season, to the OGTR prior to planting.

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

15. The release is proposed to take place at two sites located in the shires of Narrabri (NSW) and Corrigin (WA) on a maximum area of 2 ha per year over three years from May 2010 to June 2013.

16. Only trained and authorised staff would be permitted access to the locations.

3.3 The proposed controls to restrict the spread and persistence of the GMOs and their genetic material in the environment

17. The applicant has proposed a number of controls to restrict the spread and persistence of the GM wheat and barley lines and the introduced genetic material in the environment including:

- locating the trial sites at least 1 km away from natural waterways
- restricting animal access by using an isolated paddock as the trial site in the shire of Narrabri and surrounding the trial site in the shire of Corrigin with a fence and lockable gates, and by using mouse baiting and trapping around the perimeter of each site
- locating the trial sites at least 200 m from all other wheat and barley plantings
- surrounding the GM wheat and barley with a 10 m monitoring zone in which growth of related species is controlled
- maintaining a 200 m zone around the trial sites free of any sexually compatible species in the first growing season, then reducing this to 50 m (in addition to the 10 m monitoring

zone) provided no non-GM wheat and barley or sexually compatible species were identified during the first growing season⁷

- post harvest monitoring of the trial site on a monthly basis for 24 months and destroying any volunteer wheat and/or barley before flowering
- destroying all plant material from the trial not required for testing or future trials
- transporting and storing of the GMOs in accordance with the Regulator's guidelines
- not allowing the GM plant material or products to be used for human food or animal feed.

18. These controls, and the limits outlined in 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 4.2.1.

Section 4 The parent organism

19. The parent organisms are bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), both of which are exotic to Australia. Commercial wheat and barley cultivation occurs in the wheat belt from south eastern Queensland through New South Wales, Victoria, southern South Australia and southern Western Australia (OGTR 2008b). A small amount of barley is also grown in Tasmania (OGTR 2008a). Further detailed information about the parent organism is contained in the reference documents, *The Biology of Triticum aestivum L. em Thell (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/or barley plants (OGTR 2008a; OGTR 2008b). These documents are available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

20. The wheat cultivars used to generate the GM wheat lines are Bobwhite 26 and Frame. The Bobwhite cultivar is not favoured as a commercial bread wheat as it is considered to be of lower quality than most commercial cultivars (Bhalla et al. 2006) but is commonly used in genetic modification work because it is relatively easy to transform and has previously been used in conventional (non-GM) wheat breeding programs. The cultivar Frame is commercially cultivated in Australia.

21. The GM barley lines in the proposed release were derived from the barley cultivar Golden Promise. Golden Promise is a two-row, malting barley, which is not grown commercially in Australia but is commonly used in genetic modification work.

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

5.1 Introduction to the GMOs

22. Twenty three GM wheat and barley lines are proposed for release. These GM lines are currently approved for release under licences DIR 092 and DIR 094.

23. These GM wheat and barley lines were generated using two different gene constructs. The first construct was used to generate four GM wheat lines and contains a gene designed to decrease expression of a wheat gene which encodes an enzyme referred to as starch metabolic enzyme B (SMEB) that contributes to grain composition (Table 1). The specific identity of

⁷ CSIRO requested a reduction in inspection requirements after the release of the consultation RARMP.

the *SMEB* gene has been declared CCI. The decrease in *SMEB* expression is brought about by a mechanism known as gene silencing or RNA interference (RNAi). RNAi was recently discussed in the RARMP for DIR 092 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir092>). RNAi is a natural plant defence mechanism against infecting RNA viruses, and works by using short RNA molecules to recognise matching (homologous) foreign sequences, which are then destroyed by enzymes (reviewed by Baulcombe 2004). For an introduced gene to induce RNAi against an endogenous gene, the transcript from the introduced gene must mimic the structure of the double-stranded RNA viruses which naturally induce RNAi, using sequences from the gene to be silenced (the target gene) (reviewed by Waterhouse & Helliwell 2003). Because the transcript from the construct is destroyed in this process, no proteins are produced.

24. The second construct was used to generate seven GM wheat lines and three GM barley lines and contains a gene encoding a metabolic enzyme which is expected to enhance the efficiency of nitrogen utilisation. The introduced gene, referred to metabolic enzyme 1 gene (*Me1*), is derived from *H. vulgare*. Expression of the *Me1* gene is controlled by a tissue specific promoter derived from *Oryza sativa* (rice). The specific identities of the *Me1* gene and the promoter have been declared CCI.

25. The lines are grouped into five different categories of GMOs and include GM control lines:

- Wheat Category 1: Three lines contain an RNAi construct that suppresses the expression of the *SMEB* gene that encodes the SMEB enzyme. The fourth line is a negative segregant of one of these lines. While this GM line no longer contains the RNAi construct it may contain the *nptII* gene used as a selectable marker to select GM plants in the laboratory. All lines are in the cultivar Bobwhite 26
- Wheat Category 2: Three lines transformed with a plasmid vector containing *Me1* and two control lines which have been transformed but do not contain the *Me1* gene. All lines are in the cultivar Bobwhite 26.
- Wheat Category 3: Two lines transformed with a linearised fragment of DNA containing *Me1* and two control lines containing an empty plasmid vector. All lines are in the cultivar Bobwhite 26. A different co-bombardment plasmid was used in the transformation process than in Wheat Category 2.
- Wheat Category 4: Two lines transformed with a linearised fragment of DNA containing *Me1* and two control lines containing an empty vector. All lines are in the cultivar Frame. The same co-bombardment plasmid was used in Wheat Categories 3 and 4.
- Barley Category 1: Three lines containing *Me1* and three control lines containing an empty plasmid vector. All lines are in the cultivar Golden Promise.

26. The GM plants also contain one of two antibiotic resistance genes derived from *Escherichia coli* that were used as selective markers during the initial development of the GM wheat and barley lines in the laboratory (see Table 1). These genes, *nptII* and *hpt*, confer resistance to antibiotics such as G-418 and hygromycin, respectively.

27. In addition, the wheat and barley lines also contain bacterial selectable marker genes. All of the GM wheat lines contain the β -lactamase (*bla*) gene, a gene derived from *E. coli* which confers resistance to the antibiotic ampicillin. Wheat Category 2 also contains a kanamycin resistance gene. The bacterial selectable marker gene in the GM barley lines has been declared CCI. Expression of these selectable marker genes is controlled by sequences which only direct transcription of the genes in bacterial cells, and therefore they are not expressed in the GM wheat and barley lines.

28. Short regulatory sequences (promoters and transcription termination sequences) that control expression of the introduced genetic material in plant cells are also present in the GM wheat and barley lines. These are derived from wheat, maize and *Oryza sativa* (rice), the plant virus Cauliflower mosaic virus (CaMV), and the bacterium *Agrobacterium tumefaciens*.

Table 1. The introduced genes in the genetically modified wheat and barley

Gene	Accession No (Genbank)	Protein produced	Protein function	Source	Intended purpose
RNAi targeting <i>SMEB</i> sequence	CCI	None	Suppression of endogenous <i>SMEB</i> gene	<i>Triticum aestivum</i> (wheat)	Altered grain starch composition
Metabolic enzyme gene <i>Me1</i>	CCI	Metabolic enzyme	metabolism enhanced nutrient utilisation efficiency	<i>Hordeum vulgare</i> (barley)	Enhanced nitrogen use efficiency
<i>nptII</i>	AAF65403	neomycin phosphotransferase type II	Kanamycin and neomycin resistance	<i>Escherichia coli</i>	Selection of transformants
<i>hpt</i>	AAA92252	hygromycin phosphotransferase	hygromycin resistance	<i>Escherichia coli</i>	Selection of transformants
<i>bla</i>	AJ847363	β -lactamase (not produced in GM plants)	Ampicillin resistance	<i>Escherichia coli</i>	Bacterial selectable marker

5.2 The introduced genetic material and their associated effects

5.2.1 The *SMEB* gene and the effect of silencing

29. The DIR 099 application includes GM wheat lines carrying an RNAi construct designed to reduce expression of Starch Metabolic Enzyme B (*SMEB*, four lines), resulting in changes to grain starch composition. The specific identities of this gene and the phenotypes resulting from its silencing have been declared CCI and are not discussed further in this Section.

5.2.2 Toxicity/allergenicity of the effects associated with the introduced RNAi construct

30. Several types of allergic and immune reactions to wheat products have been recorded, with bakers asthma and coeliac disease being the best characterised. Bakers asthma is a respiratory allergy to inhaled flour and dust from grain processing, which is one of the most important occupational allergies in many countries (reviewed by Tatham & Shewry 2008). Coeliac disease is an inflammatory disorder of the small intestine, triggered by gluten consumption, and resulting in poor nutrient absorption, which affects approximately one in 200 people (reviewed by Sollid 2002). Other less well studied reactions to wheat include dietary allergy and pollen allergy. A variety of wheat proteins contribute to these adverse reactions, and identification of specific problem proteins is complicated by similar allergy/intolerance responses being induced by different proteins in different individuals.

31. The most important contributor to bakers asthma is a group of α -amylase inhibitors found in wheat grains which inhibit mammalian and insect α -amylase enzymes (reviewed by Tatham & Shewry 2008). A wide variety of other proteins have been shown to bind to immunoglobulin E (IgE) from bakers asthma patients, indicating an involvement in the allergy, including wheat germ agglutinin, peroxidase, thioredoxin, α -, β -, γ -, and ω -gliadins, α - and β -amylase, acyl CoA oxidase, glyceraldehyde-3-phosphate dehydrogenase,

triosephosphate isomerase and serpin (reviewed by Tatham & Shewry 2008). Experiments involving testing patient serum IgE for immunoreactivity to blotted wheat proteins indicated individuals react to unique sets of 10 to 50 proteins, and only the more commonly occurring of these proteins have been identified (reviewed by Tatham & Shewry 2008). Some of these proteins are also known to be involved in dietary allergy to wheat, with gluten proteins recognised as the most important contributors.

32. Coeliac disease has been characterised as an intolerance to various epitopes (portions of proteins to which antibodies react) derived from gluten proteins including α - and γ -gliadins, and low molecular weight glutenin subunits (reviewed by Sollid 2002). It is thought to be initiated through a T-cell response to specific gluten peptides, often α -gliadins, in the small intestine. This leads to expression of the enzyme transglutaminase which damages the intestinal mucosa and also deamidates further gluten proteins, leading to an enhanced T-cell response (reviewed by van Herpen et al. 2006). The specific range of epitopes against which a coeliac patient reacts varies between individuals, however α -gliadins are particularly common.

33. In the GM wheat lines modified for grain composition, the use of RNAi has the direct effect of reducing the expression of endogenous transcripts, without the expression of novel proteins. However, secondary effects of silencing target genes can alter expression of untargeted proteins.

34. The silencing of SMEB results in changes to the starch composition of the GM wheat grains. Humans are exposed to vast amounts of dietary starch from a range of sources. Because current starch intakes result in no allergenic or toxic effects, it is highly unlikely that the changes occurring in the silencing lines would result in altered toxicity or allergenicity of the GM wheat lines compared to parental cultivars.

35. No studies on the toxicity or allergenicity of the GM wheat lines and their products have been undertaken to date as the proposed trial is at an early stage. Such studies would have to be conducted if approval was sought for the GMOs or their products to be used for human consumption in Australia.

5.2.3 The introduced gene for enhanced nutrient utilisation efficiency, and its encoded protein

36. Nitrogen use efficiency (NUE) is an important factor in crop plant productivity. Nitrogen based fertilizers are used extensively in modern agriculture, including for wheat and barley. Further details of NUE are given in the RARMP for DIR 094 (available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir094>>).

37. The introduced gene for enhanced nutrient utilisation efficiency, *Mel*, encodes an enzyme involved in nitrogen utilisation. The specific identities of *Mel* and the promoter used to drive its expression have been declared CCI and are not discussed further in this Section.

5.2.4 The end products/effects associated with the introduced gene for enhanced nutrient utilisation efficiency

38. The aim of the genetic modification is to increase the ability of the GM plants to utilise nutrients, specifically nitrogen, from the soil. This is achieved by tissue specific expression of the *Mel* gene. The same gene has been expressed in *Brassica napus* (canola) and rice and the resulting GM plants showed higher biomass and higher yields than control plants.

5.2.5 Toxicity/allergenicity of the protein/end products encoded by the introduced gene for enhanced nutrient utilisation efficiency

39. The *Mel* gene introduced into the GM plants was isolated from barley. Although barley contains a number of anti-nutritional factors and allergens that, in extreme cases, may have a

toxic effect (OGTR 2008a), the protein encoded by the *Me1* gene is not expected to have any toxic or allergenic effects. Homologues of the encoded metabolic enzyme occur naturally in a wide range of organisms, including animals, bacteria, yeast and plants consumed by people and animals (see discussion in Section 6.5). On this basis, people and other organisms have a long history of exposure to the enzyme encoded by *Me1*.

40. It is possible that the GM wheat and barley plants will produce altered levels of some metabolites in both below and above ground tissues. These metabolites are ubiquitous in nature and consumed widely by humans.

41. No studies on the toxicity or allergenicity of the GM wheat or barley lines have been undertaken to date as the proposed trial is still at an early stage. Such studies would have to be conducted if approval was sought for the GMOs, or products derived from the GMOs, to be used for human consumption in Australia.

42. A comprehensive search of the scientific literature yielded no information to suggest that *Me1* is toxic or allergenic to people, or toxic to other organisms.

5.2.6 The antibiotic resistance marker gene *nptII* and the encoded protein

43. The GM wheat from Categories 1 and 2 (ie nine of the GM wheat lines, including three control lines, Section 5.1) contain the antibiotic resistance selectable marker gene, neomycin phosphotransferase type II (*nptII*). This gene, encoding the enzyme neomycin phosphotransferase (NPT), was derived from *E. coli* and confers resistance to antibiotics such as kanamycin or G-418 on the GM plant. The *nptII* gene was used as a selective marker to identify transformed plant tissue during initial development of the GM plant lines in the laboratory. To prevent expression of *nptII* in prokaryotes, the gene has been interrupted by insertion of the 198 bp intron 2 of the potato *stls2* gene into the N-terminal part of the *nptII* coding region.

44. A kanamycin resistance marker gene is also present in Wheat Category 2. This gene is under the control of its own bacterial promoter and terminator and therefore is not expressed in the GM plants. The gene was used in the laboratory prior to the production of the GM lines.

45. The *nptII* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in previous RARMPs, and in more detail in the RARMPs for DIR 070/2006 and DIR 074/2007 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir070-2006>) and <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir074-2007>) or by contacting the OGTR), regulatory agencies in Australia and in other countries have assessed the use of the *nptII* gene in GMOs as not posing a risk to human or animal health or to the environment. The most recent detailed international evaluation of *nptII* in terms of human safety was by the European Food Safety Authority, which concluded that the use of the *nptII* gene as a selectable marker in GM plants (and derived food or feed) does not pose a risk to human or animal health or to the environment (EFSA 2009).

5.2.7 The antibiotic resistance marker gene *hpt* and the encoded protein

46. The GM wheat lines from Categories 3 and 4 and all of the GM barley lines contain the *hpt* gene from *E. coli*, which confers resistance to the antibiotic hygromycin B.

47. The *hpt* gene encodes the hygromycin phosphotransferase (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.

48. The *hpt* gene has been used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in the RARMP for DIR 073/2007 and more recently DIR 077/2007 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir073-2007>) and <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir077-2007>), the use of *hpt*, or other HPT encoding genes, as marker genes in GM plants has been assessed as not posing a risk to human health and safety or the environment. The HPT protein is easily digested by simulated gastric juices and the amino acid sequence contains no similarities to known allergens (Lu et al. 2007). 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).

5.2.8 The antibiotic resistance marker gene *bla* and the encoded protein

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

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

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

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

5.3 The regulatory sequences

53. The plasmid vectors used to generate the GM wheat and barley lines are shown in Table 2, together with the regulatory sequences used to control expression of the introduced genetic material.

5.3.1 Regulatory sequences for expression of the wheat RNAi construct

54. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. The regulatory sequences included in the RNAi construct are listed in Table 2. The Bx17 promoter, which controls expression of the RNAi construct in the GM wheat lines (Category 1), from the wheat high molecular weight glutenin subunit gene, is endosperm specific, and naturally controls the expression of a seed storage protein in wheat (information provided by applicant).

55. Introns are DNA sequences within a gene that may help regulate the timing and extent of gene expression, but are not translated into protein. Separation of the inverted-repeat arms of the RNAi construct with an intron has been shown to increase the effectiveness of silencing (Smith et al. 2002). The introns used to separate the arms of the RNAi construct in the GM wheat lines are derived from rice intron sequences (Table 2).

56. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA termination region for the RNAi construct in the GM wheat lines is derived from the *Agrobacterium tumefaciens* nopaline synthase (*nos*) gene

(Bevan 1984). Although *A. tumefaciens* is a plant pathogen, the regulatory sequence comprises only a small part of its total genome, and is not in itself capable of causing disease.

5.3.2 Regulatory sequences for expression of the introduced gene for enhanced nutrient utilisation efficiency

57. The *Me1* gene sequence present in the GM wheat and barley is under the control of a tissue specific promoter derived from rice.

58. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA termination region for the *Me1* gene in the GM wheat and barley is derived from the *nos* gene from *A. tumefaciens*.

5.3.3 Regulatory sequences for the expression of the selectable marker genes

59. Expression of the *nptII* and *hpt* genes in GM wheat plants in Category 1 and 2 is controlled by the *35S* gene promoter from Cauliflower mosaic virus (CaMV) (Odell et al. 1985) and the *35S* mRNA termination region from CaMV. Although CaMV is a plant pathogen, the regulatory sequence comprises only a small part of its total genome and is not in itself capable of causing disease. The *nptII* gene also contains an intron derived from the potato *stls2* gene.

60. Expression of the *hpt* gene in GM wheat plants in Categories 3 and 4 is controlled by the *Ubiquitin1 (Ubi1)* promoter from *Zea mays* (maize) and the *nos* gene mRNA termination region from *A. tumefaciens* (Bevan 1984).

61. The *35S* and *Ubi1* are constitutive promoters and direct the marker genes to be expressed in most plant tissues and throughout the plant lifecycle.

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

Construct	Promoter	Gene	Terminator	Categories of GMOs	Transformation method
vector 1	pBx17	<u>Inverted repeat target SMEB sequence</u> : 581 nt <i>SMEB</i>	<i>Nos</i>	Wheat Category 1 (3 experimental lines, 1 control line)	Biolistic
pCMneoSTLS2	<i>35S</i>	<i>nptII</i>	<i>35S</i>		
vector 2	CCI	Metabolic enzyme <i>Me1</i>	<i>Nos</i>	Wheat Category 2 (3 experimental lines, 2 control lines)	Biolistic
pCMneoSTLS2	<i>35S</i>	<i>nptII</i>	<i>35S</i>		
vector 3, linearised fragment	CCI	Metabolic enzyme <i>Me1</i>	<i>Nos</i>	Wheat Categories 3 and 4 (4 experimental lines, 4 control lines)	Biolistic
pIC1600	<i>Ubi1</i>	<i>Hpt</i>	<i>nos</i>		
vector 3	CCI	Metabolic enzyme <i>Me1</i>	<i>Nos</i>	Barley Category 1, (3 experimental lines, 3 control lines)	<i>Agrobacterium</i> -mediated
	<i>35S</i>	<i>Hpt</i>	<i>35S</i>		

5.4 Method of genetic modification

62. Two different methods were used to generate the GM wheat and barley lines in the proposed release (see Table 2) – biolistic transformation (wheat) or *A. tumefaciens*-mediated transformation (barley). Biolistic transformation (Pellegrineschi et al. 2002) involved coating very small gold particles with two transformation constructs, one containing a plant selectable marker and a second containing the gene of interest. The particles were then ‘shot’ into

embryos from *T. aestivum* cultivar Bobwhite 26 (Categories 1, 2 and 3) or Frame (Category 4). Genetically modified plant tissues were recovered by survival on tissue culture media containing the selective agent G-418 (Categories 1 and 2) or hygromycin (Categories 3 and 4).

63. The constructs used to generate the GM wheat lines from Category 1 were vector 1, containing the RNAi construct, and a second vector, pCMneoSTLS2, containing the intron interrupted *nptII* gene (Table 2). The constructs used to generate GM wheat lines from Category 2 were vector 2 containing the *Me1* gene and pCMneoSTLS2, containing the intron interrupted *nptII* gene (Table 2). GM wheat lines from Categories 3 and 4 were generated by co-transforming a linearised fragment of DNA containing the *Me1* gene with pIC1600, containing the *hpt* gene (Table 2).

64. *A. tumefaciens*-mediated transformation was used to generate the GM barley lines. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants (Van Larebeke et al. 1974), through transfer of DNA (transfer-DNA or T-DNA, located between specific border sequences on a resident plasmid) from *A. tumefaciens*.

65. Disarmed *Agrobacterium* strains have been constructed specifically to facilitate genetic modification of plants with desired genes without causing disease. The disarmed strains used for genetic modification do not contain the genes responsible for the overproduction of auxin and cytokinin (*iaaM*, *iaaH* and *ipt*), which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). *Agrobacterium* plasmid vectors used to transfer T-DNAs contain well characterised DNA segments required for their replication and selection in bacteria, and for transfer of T-DNA from *Agrobacterium* and its integration into the plant cell genome (Bevan 1984; Wang et al. 1984).

66. To generate the GM barley lines in the current application, a vector containing both the *Me1* gene and the *hpt* selectable marker gene (see Table 2) was introduced by electroporation into the disarmed *Agrobacterium* strain AGL1. The *Agrobacterium* was inoculated onto the scutellum of immature embryos of the *H. vulgare* cultivar Golden Promise. Callus tissue was then cultured from transformed embryo cells, which was regenerated into plantlets in tissue culture in the presence of the selective agent hygromycin.

67. Each GM line was generated in a single transformation.

68. Both biolistic and *Agrobacterium*-mediated transformation have been widely used in Australia and overseas for introducing new genes into plants and are not known to cause any adverse effects on human health and safety or the environment.

5.5 Characterisation of the GMOs

5.5.1 Stability and molecular characterisation

69. All constructs used to generate the GM wheat and barley lines were sequenced prior to transformation.

70. Molecular characterisation of the GM wheat and barley lines has not been carried out, as the project is in early stages. The genomic locations of the introduced DNA has not been characterised, and the number of copies of the transgenes present in each line is currently unknown.

71. The parent wheat and barley lines used for transformation have stable genotypes. The applicant states that the transgenes are stably inherited (as monitored by PCR assays) over five generations for Wheat Category 1 GM lines, and over an unspecified number of generations for Wheat Categories 2, 3 and 4 and for Barley Category 1.

5.5.2 Characterisation of the phenotype of the GM wheat and barley

72. Analysis of wheat endosperm proteins in T3⁸ *SMEB* RNAi lines (Wheat Category 1) by western blotting showed that the *SMEB* protein was reduced to undetectable levels. Analysis of the starch from these lines showed that suppression of *SMEB* resulted in a decrease in one of the metabolites measured. Except for this decrease, no structural or quantitative modifications of starch composition have been found by the applicant. Amylose content and chain length distribution of debranched amylopectin were unchanged for two of the lines and decreased in the third line, although this decrease was not statistically significant. These results indicate that no other enzyme involved in starch composition is affected by the silencing of *SMEB*.

73. The levels of enzymes involved in the breakdown, or catabolism, of starch were assayed in the mature endosperm from T4 seeds to determine the effect of silencing *SMEB* on these enzymes. β -amylase activity was significantly increased in one of the GM wheat lines but not the other two GM wheat lines. α -glucosidase activity was significantly increased in one GM wheat line, significantly decreased in another and unchanged in the third GM wheat line. Further details of analysis of grain composition in the *SMEB* RNAi lines have been declared CCI and are not discussed further in this Section.

74. Altered growth characteristics were observed in the *SMEB* RNAi lines during growth of the plants in the glasshouse. Growth analysis was conducted on the GM wheat lines at the T2 stage in pots, and at the T4 stage in garden beds at a density that simulated sowing rates in the field. The altered growth characteristics were seen in both the pot and simulated plot trials. The relative starch content of the seed did not change during these trials, which suggests that the altered growth characteristics do not affect composition of the seeds. The changes in growth are not outside what is normal for wheat lines produced through traditional breeding.

75. The applicant has not conducted a comprehensive phenotypic analysis of the GM wheat and barley lines that express the introduced *Me1* gene as these GM lines are at an early stage of development. Monocots and dicots genetically modified to express the *Me1* gene have a similar phenotype. Based on this information, the applicant expects a similar phenotype in the GM wheat and barley lines that contain the introduced *Me1* gene.

76. Preliminary characterisation of the GM wheat lines from Wheat Category 2 has not revealed a significant difference in seed number, average seed weight or total yield between plants that contain the *Me1* gene and plants that do not contain the introduced gene. Similarly, preliminary results from the GM wheat lines from Categories 3 and 4 show that there is no difference in seed number, total yield and shoot dry weight between the GM wheat lines and the control lines.

77. Preliminary characterisation of the GM barley lines (Barley Category 1) has shown that two of the three GM lines have altered growth characteristics compared to the controls. These results are within the normal range for non-GM barley and have been considered in Chapter 2. The details of this characterisation have been declared CCI and are not discussed further in this Section.

78. While there may be changes in the levels of products produced as a result of the activity of the encoded protein, no new products should be produced by expression of the introduced

⁸ The generation of a GM plant is identified by the letter T (transgenic) followed by a numeral indicating the number of generations for which it has been maintained, beginning at T0 for the original transformed plant.

gene for enhanced nutrient utilisation efficiency. However, there may be unintended effects due to random insertion of the introduced genetic material (see Chapter 2, Risk scenario 7).

Section 6 The receiving environment

79. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the geographic regions where the release would occur and any relevant biotic/abiotic properties of these locations; the intended agronomic practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2009).

80. The size, locations and duration of the proposed release are outlined in Section 3.2. The proposed dealings involve planting at two sites. One site is located in the shire of Narrabri (NSW) on established farmland run by a government agricultural research organisation, approximately 2 km from the Australian Cotton Research Institute (ACRI). The other proposed site is located on private property in the shire of Corrigin (WA), in productive agricultural land used for commercial purposes.

81. The closest population centres to the proposed Narrabri shire site are Wee Waa (population 2000) and Narrabri (population 7500).

82. The closest population centre to the proposed Corrigin shire site is the town of Corrigin (population 1200), which is around 30 km from the proposed site.

6.1 Relevant abiotic factors

83. The abiotic factors relevant to the growth and distribution of commercial wheat and barley can be found in *The Biology of Triticum aestivum L. (bread wheat)* (OGTR 2008b) and *The Biology of Hordeum vulgare L. (barley)* (OGTR 2008a).

84. The release is proposed to take place in the shires of Narrabri (NSW) and Corrigin (WA). The shire of Narrabri is a cotton growing region and has a typical climate of summer cotton growing regions in Australia, with warm summers and higher summer than winter rainfall (see Table 3).

Table 3. Monthly temperature and rainfall statistics for proposed release sites*

Site	Nearest weather station	Mean max temp (°C) Summer	Mean min temp (°C) Summer	Mean max temp (°C) Winter	Mean min temp (°C) Winter	Mean monthly rainfall (mm) Summer	Mean monthly rainfall (mm) Winter
Narrabri	Narrabri Post Office	33.3	18.7	18.8	4.5	74	46
Corrigin	Corrigin	31.4	15.2	16.0	5.2	15	57

*data taken from the Australian Bureau of Meteorology website (<http://www.bom.gov.au/climate/averages/>). Temperature and rainfall data are an average of 41 to 120 years of records for Narrabri, and 63 to 101 years of records for Corrigin.

Summer entries are averages of monthly data from December to February, and winter entries are averages of monthly data from June to August.

85. The shire of Corrigin is located 235 km southeast of Perth, in the central wheatbelt of WA. The region has a dry climate, with hot dry summers and cool wet winters (see Table 3). Rainfall and soil types influence land use, which is based on dryland agricultural production using annual, winter growing pastures and crops (Source: www.agric.wa.gov.au/PC_93283.html).

86. The Namoi River is about 2 km to the south of the proposed Narrabri site, and a temporary floodway is about 2 km to the north (information supplied by the applicant).

87. The nearest waterway is approximately 1 km away from the proposed Corrigin site (information supplied by the applicant).

6.2 Relevant biotic factors

88. The biotic factors relating to the growth and distribution of commercial wheat and barley in Australia are discussed in the reference documents, *The Biology of Triticum aestivum L. em Thell. (bread wheat)* (OGTR 2008b) and *The Biology of Hordeum vulgare L. (barley)* (OGTR 2008a). In addition, the following points are of particular relevance to this release:

- Both sites are located within established agricultural areas. It is therefore possible that wheat and barley will be grown near the proposed trial sites. Cotton (*Gossypium hirsutum*, *Gossypium barbadense*), sorghum (*Sorghum bicolor*) and/or millet (*Pennisetum glaucum*, *Panicum miliaceum*, *Echinochloa spp.*) are proposed to be grown within 200 m of the Narrabri site. Lupins, canola or field peas are proposed to be grown within 200 m of the Corrigin site.
- Wheat was grown at the Narrabri site in 2008 and at the Corrigin site in 2009. No barley has been grown at either the Narrabri or Corrigin site.
- No wheat or barley breeding lines will be grown within 10 km of the Narrabri trial site, or within 2 km of the Corrigin site.
- GM cotton has been previously grown at the proposed trial site in the shire of Narrabri. GM canola is currently growing at the trial site in the shire of Corrigin (under licence DIR 020/2002). GM wheat has also previously been grown a number of years ago in the shire of Corrigin under licence DIR 053/2004.

89. Invertebrates, vertebrates and microorganisms could be exposed to the introduced genetic material, their encoded proteins and/or end products. In particular, rodents and native birds may visit the proposed release sites.

6.3 Relevant agricultural practices

90. It is anticipated that the agronomic practices used by the applicant for the cultivation of the GM wheat and barley will not differ significantly from conventional practices, with the exception that the applicant proposes to harvest using a small mechanical single row harvester at the Narrabri site. A plot harvester is proposed to be used to harvest at the Corrigin site. Conventional cultivation practices for wheat and barley are discussed in more detail in *The Biology of Triticum aestivum L. em Thell. (bread wheat)* (OGTR 2008b) and *The Biology of Hordeum vulgare L. (barley)* (OGTR 2008a).

91. There are a number of pests and diseases of wheat and barley, which may require management (eg application of pesticides such as herbicides or insecticides) during the growing season (for further details, see OGTR 2008a; OGTR 2008b). Weed control using specific classes of herbicides may involve a pre- or post-emergence application.

92. The parental wheat and barley cultivars are spring cultivars. In Australia, spring wheat and barley varieties are commonly grown as a winter crop and are usually planted in late autumn or early winter, depending on variety and location. Harvest of the mature grain generally occurs in early summer.

93. The trial is proposed to take place over three growing seasons. The applicant states that specific planting arrangements for this trial at each site over the three seasons will be determined by yields from each season. For each planting, the applicant aims to avoid re-use of plots within the site for the purpose of managing soil disease. For example, the area sown

in year 1 will not be used in year 2, nor will the area used in year 2 be used in year 3. However, first season areas may be replanted in the third season.

94. Multiple plots will be sown for each GM line and non-GM control lines so that the yield potential under different nutrition regimes can be assessed for each line. At the Narrabri site, the applicant anticipates planting in plots that are approximately 2 m x 5 m. The plots will be approximately 1.3 m x 5 m at the Corrigin site.

95. Waste material collected from harvesting will be destroyed by autoclaving or incineration. Non-propagative plant material remaining at the field location after harvest (for example, residual stem stubble) would be ploughed into the ground after the trial. Excess seed not required for experimental analysis, or future trials, would be removed from the site and destroyed.

96. The applicant does not propose to irrigate the sites following harvest.

97. The applicant proposes to grow break crops in areas within the trial sites which are not being used for growing the GM wheat and barley. The break crop at the proposed Narrabri site is likely to be cotton, millet or sorghum. The break crop at the proposed Corrigin site is likely to be canola, lupins or field peas. Any break crops grown will be treated with a selective herbicide to ensure that any wheat plants growing within these crops do not flower.

6.4 Presence of related plants in the receiving environment

98. The GM wheat and barley lines proposed for release will be grown together at the field trial sites. Barley and wheat are not known to hybridise with each other under natural conditions (OGTR 2008a; OGTR 2008b).

99. The applicant has indicated that both of the proposed trial sites are located in established agricultural areas. Therefore, it is possible that wheat and barley will be grown near the proposed trial sites. However, the applicant proposes to maintain a 200 m zone in which there is no cultivation of wheat or barley plants around the site of the trial for the full duration of the trial.

100. Apart from commercially cultivated bread and durum wheat, other *Triticum* species are not known to be present in Australia. Other species belonging to the genera *Elytrigia*, *Elymus*, *Hordeum*, and *Secale* are known to occur in Australia. Wild barley, *H. vulgare* ssp. *spontaneum*, is not known to be present in Australia either.

101. Wheat is sexually compatible with many species within the genus *Triticum*. Hybridisation with *Hordeum vulgare* has not been reported. Wheat can hybridise with *Hordeum marinum* but only with substantial human intervention (Pershina et al. 1998; Islam & Colmer 2008) and the resultant hybrids are usually infertile (Islam et al. 2007). Of the species that may perhaps hybridise with bread wheat under natural conditions, few are known to be present in Australia. *Aegilops* spp are recognised as quarantine weeds in Australia and are not known to be present naturally. The interspecific crossing potential of wheat is discussed in more detail in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008b).

102. *Hordeum vulgare* ssp. *spontaneum* (wild barley) is the only species that can cross with cultivated barley under natural conditions (Nevo 1992; OGTR 2008a). As mentioned above, wild barley is not found in Australia (OGTR 2008a). There are no reports of barley forming hybrids with cultivated wheat under natural conditions.

6.5 Presence of the introduced genetic material or similar genetic material and encoded proteins and/or end products in the environment

103. All of the introduced sequences were isolated from naturally occurring organisms that are already widespread and prevalent in the environment.

104. The RNAi construct consists of a wheat high molecular weight glutenin subunit promoter (from the *Bx17* gene), controlling expression of an inverted repeat of a wheat sequence targeted for silencing the wheat grain composition gene (see Table 2). The inverted repeats are separated by intron sequences isolated from the rice *SBE1* gene (introns 4 and 9). Wheat and rice are widespread and prevalent in the environment and have been safely consumed by humans and animals for centuries.

105. The introduced gene for enhanced nutrient utilisation efficiency was isolated from barley, which is already widespread and prevalent in the environment and consumed by humans and animals. In addition, homologues of the *Me1* gene occur naturally in animals (including humans), plants, yeast and bacteria.

106. The promoter driving expression of the *Me1* gene was obtained from rice. The *Ubi1* promoter for the *hpt* gene used in wheat Categories 2 and 3 was derived from maize. The *nptII* gene contains an intron derived from the potato *stls2* gene. Rice, maize and potato have all been safely consumed by humans and animals for centuries.

107. The *nptII*, *hpt* and *bla* genes are derived from the common gut bacteria *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 humans routinely encounter the encoded proteins through contact with plants and food. In some of the GM wheat lines, the *nptII* gene is interrupted by an intron sequence from the potato *stls2* gene. Potato is widespread and prevalent in the environment and has been safely consumed by humans for centuries.

108. The 35S promoter for the *nptII* gene and the *hpt* gene used in the wheat and barley GM lines was derived from CaMV. Expression termination sequences were originally isolated from CaMV and the soil bacterium *A. tumefaciens*. Although CaMV and *A. tumefaciens* are plant pathogens, the regulatory sequences comprise only a small part of their total genomes and are not capable in themselves of causing disease. CaMV is a virus which infects many human food crops and would be commonly consumed in food. *A. tumefaciens* is also widespread in the environment. No proteins are encoded by the introduced regulatory elements.

Section 7 Australian and international approvals

7.1 Australian approvals of GM wheat and barley

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

109. The GM wheat and barley lines proposed for release are currently approved for limited and controlled release under licences DIR 092 and DIR 094. DIR 092 was issued to CSIRO for GM wheat with altered grain composition on 1 ha in May 2009. DIR 094 was issued to CSIRO for GM wheat and barley with enhanced nutrient utilisation efficiency on 1 ha in July 2009.

110. The Regulator has issued six licences for the limited and controlled release of other GM wheat and/or barley lines: 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 0.412 ha in the Australian Capital Territory; DIR 071/2006 was issued to Department of Primary Industries – Victoria for GM drought

tolerant wheat on 0.315 ha in Victoria; DIR 077/2007 was issued to the University of Adelaide for GM wheat and barley with enhanced tolerance to abiotic stresses or increased beta glucan on 0.04 ha in South Australia; DIR 080/2007 was issued to Department of Primary Industries – Victoria for GM drought tolerant wheat on 0.225 ha in Victoria; DIR 093 was issued to CSIRO for GM wheat and barley with altered grain starch composition on 1 ha in June 2009.

111. Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been five field trials of different types of GM wheat ranging in size from 325–1500 plants: PR65 (1996), PR66 (1996), PR102 (1998), PR102X (2000), and PR107 (1999). Five field trials of different types of GM barley also occurred under GMAC. They ranged in size from 400–2940 plants: PR88 (1998), PR92 (1998), PR106 (1998), PR88X (1999) and PR139 (2000).

112. 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

113. 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), Food Standards Australia New Zealand (FSANZ), and the Australian Pesticides and Veterinary Medicines Authority (APVMA). This is discussed further in Chapter 3.

114. The applicant does not intend any material from the GM wheat or barley lines proposed for release to be used in animal feed or human food. All genetically modified foods intended for sale in Australia must undergo a safety evaluation by FSANZ. Accordingly, the applicant has not applied to FSANZ to evaluate the GM wheat or barley lines. FSANZ approval would be required before materials or products derived from the GM wheat or barley lines could be sold for human consumption.

7.2 International approvals of GM wheat and barley

115. There have been no releases of these GM wheat and barley lines internationally. However, there have been releases of other GM wheat and barley plants. The traits which have been modified include; novel protein production, disease resistance, altered grain properties and herbicide tolerance⁹.

⁹<<http://www.aphis.usda.gov/brs/status/relday.html>>, <<http://gmoinfo.jrc.ec.europa.eu>> accessed 6 March 2009.

Chapter 2 Risk assessment

Section 1 Introduction

116. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

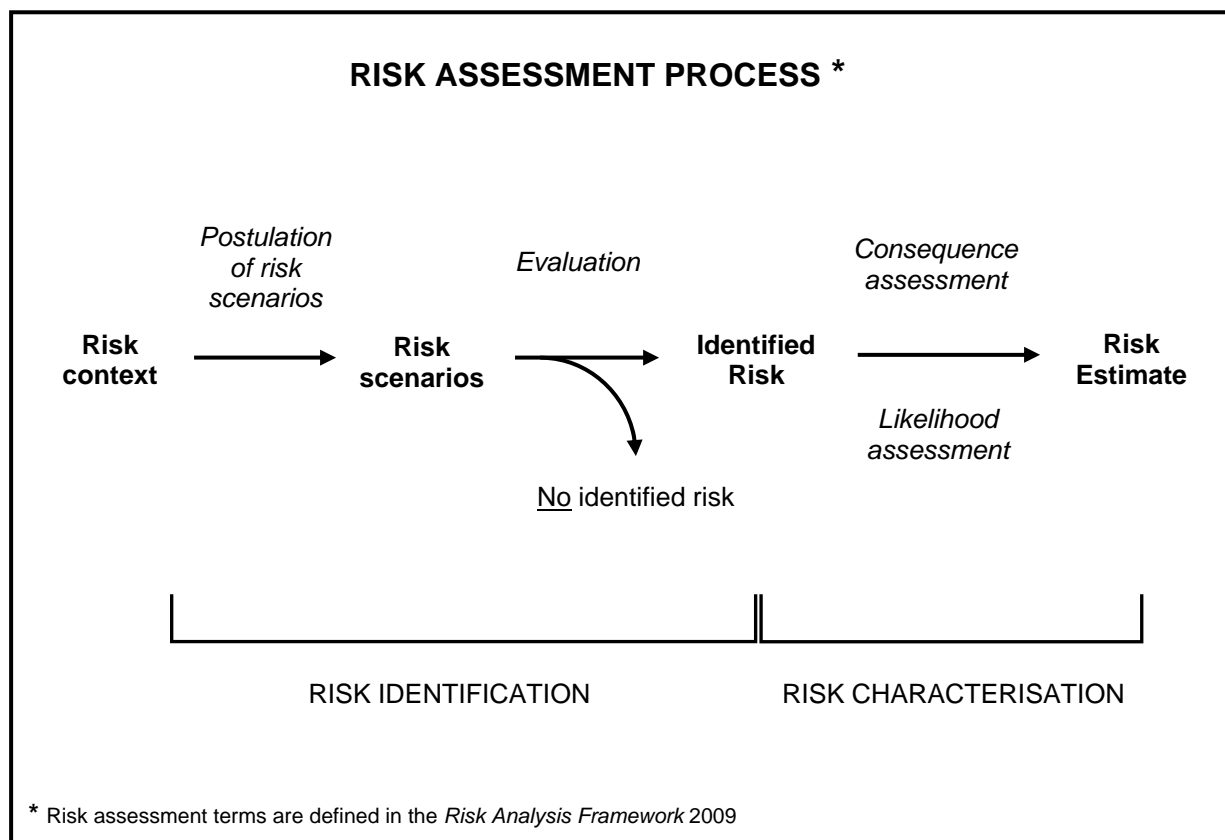


Figure 2. The risk assessment process

117. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios).

118. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

119. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2009). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

120. Identified risks are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments.

Section 2 Risk identification

121. The following factors are taken into account when postulating relevant risk scenarios:

- 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 parent organism(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the biotic and abiotic environment at the site(s) of release
- agronomic management practices for the GMOs.

122. Eight risk scenarios were identified and evaluated. These are summarised in Table 4, where circumstances that share a number of common features are grouped together in broader risk categories. None of the risk scenarios were considered to lead to an identified risk that required further assessment. More detail of the evaluation of these scenarios is provided later in this Section.

123. As discussed in Chapter 1, Sections 5.2.6 and 5.2.7, the GM wheat and barley lines contain one of two selectable marker genes; the *hpt* gene encoding the HPT protein which confers tolerance to the antibiotic hygromycin; and the *nptII* gene encoding the NPT protein which confers tolerance to the antibiotics such as kanamycin and G-418.

124. The prevalence of the *hpt* and *nptII* genes in the environment and the lack of evidence for toxicity or allergenicity of the HPT and NPT proteins to humans and animals have been discussed previously (see Chapter 1, Sections 5.2.6 and 5.2.7). The use of *hpt* or *nptII* has been assessed as not posing a risk to human health or the environment (EFSA 2004; EFSA 2007). Therefore, the potential effects of the *hpt* and *nptII* genes will not be further assessed for this application.

125. As discussed in Chapter 1, Section 5.2.8, the GM wheat lines also contain the antibiotic resistance selectable marker gene, *bla*. The *bla* gene, encoding β -lactamase, is not expressed in the GM wheat lines as it is linked to a bacterial promoter that does not function in plants, and it is widespread in the environment, and therefore it will not be assessed further.

Table 4. Summary of risk scenarios from dealings with GM wheat and barley.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Exposure to GM plant material containing the introduced genetic material, encoded products, end products, or other characteristics associated with the genetic modification	Increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The GM wheat lines containing the introduced RNAi construct do not express novel proteins. The encoded protein for enhanced nutrient utilisation efficiency and its end product 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 minimise exposure of people and other organisms to the GM plant material.
Section 2.2 Spread and persistence of the GM wheat and/or barley plants in the environment	2. Expression of the introduced genetic material improving the survival of the GM wheat and/or barley plants	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Many abiotic and biotic factors are expected to limit the spread and persistence of wheat and barley in the areas proposed for release, for example low intrinsic competitive ability, nutrient availability, pests and diseases. The limits and controls proposed for the release would minimise spread and 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"> Dispersal would be minimised by the proposed limits and controls which include locating the site away from natural waterways, measures to control rodent numbers, measures to exclude livestock and transporting materials according to the Regulator's guidelines.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced genetic material other wheat and/or barley plants	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Pollen-mediated gene transfer in wheat and barley occurs at low rates, and generally over short distances. A 200 m separation between the GM lines proposed for release and other non-GM wheat and barley will restrict pollen-mediated gene flow. The other proposed limits and controls would also minimise gene flow. The toxicity, allergenicity and weediness potential of the GM wheat and barley lines were assessed in Risk scenarios 1-3 and no risks were identified.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
	5. Expression of the introduced genetic material, or regulatory sequences 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"> • Pollen-mediated gene transfer in wheat and barley occurs at low rates, and generally over short distances. • The other proposed limits and controls (eg the 10 m and 200 m zones surrounding the location) would also minimise gene flow. • The toxicity, allergenicity and weediness potential of the GM wheat and barley lines were assessed in Risk scenarios 1-3 and no risks were identified.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	6. Expression of the introduced genetic material in other 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 sequences composing the introduced RNAi construct and/or the introduced gene sequences and the introduced regulatory sequences or components thereof are already present in the environment and are available for transfer via demonstrated natural mechanisms • Risk scenarios 1-5 associated with expression of the introduced genes did not constitute identified risks for people or the environment
Section 2.5 Unintended changes in biochemistry, physiology or ecology	7. Changes to biochemistry, physiology or ecology of the GM wheat and/or barley plants resulting from expression, or random insertion, of the introduced genetic material	Weediness; increased allergic reactions in people or toxicity in people and other organisms, increased pest pressure	No	<ul style="list-style-type: none"> • Unintended, adverse effects, if any, would be minimised by the proposed limits and controls. • Obvious unexpected alterations are likely to have been detected and eliminated during the production of the GM wheat and barley lines.
Section 2.6 Unauthorised activities	8. Use of the GMOs outside the proposed licence conditions	Potential adverse outcomes mentioned in Sections 2.1 to 2.6	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

126. 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).

127. 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).

128. A range of organisms may be exposed directly or indirectly to the introduced RNAi construct for modified grain composition and the protein (and end products) encoded by the introduced gene for enhanced nutrient utilisation efficiency, and their associated effects. Workers cultivating the GM wheat and barley would be exposed to all plant parts. Organisms may be exposed directly to the end products of the introduced sequences and/or protein through biotic interactions with GM wheat and barley plants (vertebrates, invertebrates, 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, invertebrates, fungi and/or bacteria).

Risk scenario 1. Exposure to GM plant material containing the introduced genetic material

129. Expression of the introduced RNAi construct to alter grain composition and the introduced gene for enhanced nutrient utilisation efficiency could potentially result in the production of novel toxic or allergenic compounds in the GM wheat and barley lines, or alter the expression of endogenous wheat and barley proteins. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these humans or other organisms.

130. Non-GM wheat and barley are not known to be toxic to humans or other organisms. However, non-GM wheat and barley flour can produce allergic and autoimmune 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). These properties are not expected to be altered in the GM wheat and barley lines proposed for release.

131. Although no toxicity studies have been performed on the GM wheat plant material, the RNAi construct is composed of partial gene sequences isolated from naturally occurring organisms that are already widespread and prevalent in the environment. It is not expected that any novel products would be produced as a result of the expression of the introduced RNAi construct as it is likely to be degraded upon initiating RNAi, before transcription can occur. The components of the introduced RNAi construct were isolated from non-GM wheat and rice (see Chapter 1, Section 5.2.2). Similarly, the *Mel* gene was isolated from barley, which is also widespread and prevalent in the environment and consumed by humans and animals. It is not expected that any novel products would be produced as a result of the expression of the introduced gene, as the gene was isolated from barley, and an expressed sequence tag with 96% identity to the barley *Mel* gene has been identified in wheat. This level of identity indicates that wheat already contains a protein nearly identical to that encoded by the introduced gene.

132. No information was found to suggest that the changes brought about by the protein encoded by the introduced *Mel* gene or its end products are toxic or allergenic to people or toxic to other organisms. However, changes brought about by the introduced RNAi construct could potentially affect the production of endogenous wheat toxins and allergens (Chapter 1, Section 5.2.2).

133. The proposed limits and controls for 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 trial site in the shire of Narrabri will not have a fence, however access by livestock is prevented as the site is isolated and there is a fence around the farm where it is located

(information supplied by the applicant). The proposed trial site in the shire of Corrigin will be surrounded by a fence with lockable gates. Mouse baiting and trapping will be carried out around the perimeter of each trial site. Only approved staff with appropriate training will have access to each site. These measures will minimise exposure of the public and animals to the GM plant material. Livestock and other animals would not be intentionally exposed as the GM plant material will not be used as feed.

134. Contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff. There is little potential for exposure of the public to GM plant material via ingestion, skin contact or inhalation as no GM plant material will be used as human food, animal feed or plant products. The short duration (2010-2013) and small size (2 ha) of the proposed trial would also limit the potential for exposure to the GM plant material.

135. **Conclusion:** The potential for allergic reactions in people, or toxicity in people and other organisms as a result of exposure to GM plant materials with altered grain composition as a result of the introduced RNAi construct, and/or the protein encoded by the introduced gene for enhanced nutrient utilisation efficiency, or its end products, is **not an identified risk** and will not be assessed further.

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

136. 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 given in *The biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008b) 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 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 as long as growing conditions permit, high seed output, high seed dispersal and long-distance seed dispersal (Keeler 1989). In addition, wheat and barley have been bred to avoid seed shattering, and white wheats and modern barley cultivars have little seed dormancy (OGTR 2008a; OGTR 2008b).

137. Scenarios that could lead to increased spread and persistence of the GM wheat and barley lines include expression of the introduced RNAi construct for altered grain composition and/or the introduced gene for enhanced nutrient utilisation efficiency conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These risk scenarios could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins.

Risk scenario 2. Expression of the introduced genetic material improving the survival of the GM wheat and/or barley plants

138. If the GM wheat or barley plants were to establish or persist in the environment they could increase the exposure of humans and other organisms to the GM plant material. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials has been considered in Risk scenario 1 and was not considered an identified risk.

139. If the expression of the introduced RNAi construct for altered grain composition and/or the introduced gene for enhanced nutrient utilisation were to provide the GM wheat and barley plants with a significant selective advantage over non-GM wheat and barley plants and if they were able to establish and persist in favourable non-agricultural environments, this

may give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. Similarly, the GM wheat and barley plants could adversely affect agricultural environments if they exhibited a greater ability to establish and persist than non-GM wheat and barley.

140. The impact of the genetic modification on survival of the GM wheat lines containing the introduced RNAi construct is uncharacterised. The applicant has provided data demonstrating that the introduced RNAi construct results in minor compositional changes in grain from the GM wheat lines grown in glasshouse conditions (see Section 5.5.2 for details). Other data suggesting altered growth characteristics in the GM wheat lines has also been provided by the applicant; however these results are within the range of phenotypes observed in conventionally bred wheat (see Section 5.5.2 for details). The RNAi construct is composed of wheat and rice partial gene sequences, and does not result in the expression of novel proteins in the GM wheat lines.

141. The alteration of grain composition in the GM wheat lines containing the introduced RNAi construct could possibly have secondary effects on seed germination or seedling vigour. Carbohydrates and proteins are the major storage compounds present in cereal grains, accumulating in the endosperm. These energy reserves support germinating plants until they become photosynthetically active. Mobilisation of storage reserves can begin before germination is completed, with major mobilisation occurring after the completion of germination (reviewed by Nonogaki 2008). The composition of storage compounds is changed in the GM wheat lines containing the RNAi construct, leading to the possibility of changed availability of energy to germinating seeds. Processes occurring before the point of germination, such as seed dormancy, which do not rely on energy reserves from the endosperm, are unlikely to be altered by the changes in grain composition in the GM wheat lines.

142. Starch reserves are mobilised by enzymes catalysing hydrolysis of the linkages between glucosyl residues, with α -amylases breaking down α -(1-4) linkages, and debranching enzymes breaking down α -(1-6) linkages (reviewed by Nonogaki 2008). The rate at which starch is acted upon by hydrolytic enzymes, or the total amount of energy available, could be changed as a result of changes to starch composition in the GM wheat lines. Effects on processes utilising the energy from storage starch, including germination and early seedling growth, could follow from these changes.

143. The impact of the genetic modifications on the survival of the GM wheat and barley lines containing the introduced gene for enhanced nutrient utilisation efficiency is also uncharacterised. However, a number of predictions can be made based on knowledge of gene function and on the observed phenotypes of other GM plants expressing the same gene (see Section 5.2.3 and 5.2.4 for detail).

144. Seven of the GM wheat lines and three of the GM barley lines contain the *Me1* gene for enhanced nutrient utilisation efficiency, which if successful would confer improved growth in soil with low nitrogen levels. In an environment in which nitrogen availability was the main factor limiting the spread and persistence of wheat and barley, expression of the gene for enhanced nutrient utilisation could result in weediness of the GM wheat and barley lines. Further, the lines could deplete nitrogen from soil and ‘starve’ surrounding vegetation.

145. It is possible that the GM wheat and barley lines containing the *Me1* gene may show an increase in total nitrogen content. Nitrogen content in wheat seeds can affect dormancy

(Morris & Paulsen 1985). A high level of nitrogen fertilisation has been shown to increase pre-harvest sprouting, indicating reduced dormancy. However, this relationship varied among varieties and depended on environmental conditions being conducive to pre-harvest sprouting (Morris & Paulsen 1985). If the GM wheat and barley plants showed an increase in nitrogen content in the seed, it is possible this could lead to reduced dormancy.

146. In other GM plants, expression of the *Me1* gene has resulted in increased biomass and seed yield. It is possible that the GM wheat and barley lines may also show these phenotypic changes, which could impact on the spread and persistence of the GM wheat and barley plants. Preliminary data have indicated that expression of the *Me1* gene in the GM wheat lines do not show altered growth characteristics. Preliminary characterisation of the GM barley lines has shown that two of the three GM lines have altered growth characteristics compared to the controls. However, these changes were not outside the normal range for non-GM barley.

147. Modern wheat and barley cultivars, some of which are bred for high vigour, are not recognised as significant weeds in Australia, and there have been no reports of bread wheat or barley becoming an invasive pest in Australia or overseas. Additionally, the spread and persistence of the GM wheat and barley plants would still be limited by lack of seed shattering and low intrinsic competitive ability, as well as temperature, nutrient availability (other than nitrogen), 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). However, if there were any developmental advantages conferred to the GM wheat lines as a result of altered grain composition or enhanced nutrient utilisation efficiency, their persistence at the release site would be limited by the controls proposed by the applicant.

148. The proposed limits and controls for the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM wheat and barley lines proposed for release. The release would be of limited size and short duration and the applicant proposes a number of control measures, including destruction of all plant materials not required for further analysis, and post harvest monitoring of the proposed site.

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

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

150. If the GM wheat and barley plants were to be dispersed from the release sites there could be increased exposure of humans and other organisms to the GM plant material and/or the GM plants may establish and persist in the environment. The effects of contact, inhalation or ingestion of the GM wheat and barley plants have been assessed in Risk scenario 1 and were not an identified risk. The potential for the introduced genetic material to result in improved survival of the GM wheat and barley plants in the environment was considered in Risk scenario 2 and was not an identified risk.

151. Dispersal of reproductive GM plant materials, for example viable grain, could occur in a variety of ways including: endozoochory (dispersal through ingestion by animals); the activity of animals such as rodents and herbivores; or through extremes of weather such as flooding or high winds. Seed yield may be increased in the GM wheat and barley lines. Other seed production and dispersal characteristics, such as grain number per spike, may also be altered compared to non-GM parental cultivars.

152. Seed dispersal for wheat or barley through endozoochory has not been reported, however it is possible that wheat or barley seeds could germinate after passage through the digestive system of some mammals. For example, viable wheat and barley seeds have been detected in cattle dung (Kaiser 1999). Seeds which survive chewing and digestion by animals are typically small and dormant (Malo & Suárez 1995). The GM wheat lines proposed for release are in white wheat parental backgrounds, which have large seeds with low dormancy and thin seed coats (Hansen 1994; DPI Vic 2005), and are therefore likely to be easily broken down in the digestive system of mammals. Barley also produces large seeds and the parental cultivar, Golden Promise, is a malting barley, which typically have low levels of dormancy (Briggs 1978).

153. Kangaroos, rabbits and mice are known pests of wheat and barley crops, and cattle or sheep may graze at the farm in the shire of Corrigin. The applicant has proposed to surround the trial site in the shire of Corrigin with a fence which will prevent livestock from accessing the trial site. The proposed field site in the shire of Narrabri (NSW) will not be surrounded by a fence. However, access by livestock is prevented as the proposed site is isolated and there is an external fence around the farm where the site is located.

154. Rabbits favour soft, green, lush grass (Myers & Poole 1963) and select the most succulent and nutritious plants first (Croft et al. 2002). Although viable seeds from a variety of plant species have been found in rabbit dung, viable wheat seeds were not among them (Malo & Suárez 1995). Other studies have shown that generally very few viable seed are obtained from rabbit dung (Wicklow & Zak 1983; Welch 1985).

155. Habitat modifications such as reduced plant cover have been reported to be a deterrent to the movement of mice (White et al. 1998; Central Science Laboratory 2001; AGRI-FACTS 2002; Brown et al. 2004) and therefore the proposed 10 m wide zone of reduced plant cover around the trial site is expected to discourage dispersal by mice. The applicant proposes to place mouse baits and traps around the perimeter of each site, which will further limit seed dispersal by mice.

156. 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 it elsewhere for storage or consumption. However, there are no reports on the ability of birds such as cockatoos and galahs, which are known to consume loose seeds found on the soil surface following harvest, to disperse viable seeds. Reports of seed dispersal via birds are generally confined to fruit-eating birds which consume succulent fruits and berries and then disseminate the undigested seeds at considerable distances (McAtee 1947; Barnea et al. 1991; VanDer Wall et al. 2005), rather than grain-eating birds which intentionally consume seed grains including wheat and barley (Diaz 1990; Thompson et al. 1991). It has been stated that seeds of wheat will germinate after passage through an emu's digestive system, although no experimental evidence was provided (Davies 1978). The thin seed coats of the wheat cultivars used in this trial will promote digestion of the seeds during passage through bird and animal intestines and therefore dispersal of viable GM wheat seed is likely to be low. An extensive search of the literature did not identify any reports of other birds transporting and dispersing wheat or barley seed. The possibility of dispersal of GM wheat seed by birds was considered in detail in the RARMP for DIR 071/2006 which is available from the OGTR, and is discussed in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008b). Barley seed dispersal by birds has been considered in the *The Biology of Hordeum vulgare L. (Barley)* (OGTR 2008a).

157. Wheat lacks seed dispersal characteristics such as stickiness, burrs, and hooks, which can contribute to seed dispersal via animal fur (Howe & Smallwood 1982). Barley seeds,

however, have special bristles on the spikelet structures and seeds could potentially adhere to animals and the clothing of people, thus facilitating dispersal (OGTR 2008a). The proposed release site in the Corrigin shire (WA) will be surrounded by a fence with a locked gate, while the farm where the Narrabri site will be located is surrounded by a fence, reducing the possibility of seed dispersal by livestock or by unauthorised people accessing the site. Dispersal by authorised people entering the proposed trial sites would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing. All GM plant material will be transported in accordance with the Regulator's transport guidelines which will minimise the opportunity to disperse the GM material.

158. Extremes of weather may cause dispersal of plant parts. However, control measures have been proposed by the applicant to minimise dispersal outside the trial site (Chapter 1, Section 3.3). These include locating the proposed release away from natural waterways to prevent dispersal in the event of flooding.

159. **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

160. 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 cross-pollination between the crop and neighbouring crops, related weeds or native plants (Glover 2002).

161. Baseline information on vertical gene transfer associated with non-GM wheat and barley plants can be found in the *The Biology of Triticum aestivum L. em Thell (Bread Wheat)* (OGTR 2008a) and *The Biology of Hordeum vulgare L. (Barley)* (OGTR 2008a; OGTR 2008b). Plant genotypes and environmental context and conditions, such as wind direction and humidity, can influence gene flow. In summary, wheat and barley plants are predominantly self-pollinating and the chances of natural hybridisation occurring with commercial crops or other sexually compatible plants are low.

Risk scenario 4. Expression of the introduced genetic material in other wheat and/or barley plants

162. Transfer and expression of the RNAi construct and/or the introduced gene for enhanced nutrient utilisation efficiency to other wheat and barley plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

163. 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 to, and no greater than, those from endogenous regulatory elements.

164. As discussed in Risk scenario 1, 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 gene for enhanced nutrient utilisation efficiency. However, changes brought about by the introduced RNAi construct could potentially affect the production of endogenous

wheat toxins and allergens. This will be the same if the introduced genetic material is transferred to other wheat or barley plants.

165. Both wheat and barley are predominantly self-pollinating (94-99%) and any outcrossing occurs through wind pollination (reviewed in OGTR 2008a; OGTR 2008b). Intraspecific gene flow generally occurs over much shorter distances for small scale experimental releases compared to commercial scale, although gene flow levels are highly variable. The majority of gene flow from small scale fields of wheat occurs up to ten metres from the pollen source, and only low levels of gene flow have been detected as far as 300 m away (Matus-Cadiz et al. 2004). Gene flow in barley rapidly decreases at distances beyond a few metres (Gatford et al. 2006). However, cross fertilisation with very low frequencies has been observed at distances of up to 60 m (Wagner & Allard 1991).

166. Studies under Australian field conditions (South Australia and the ACT), indicate that gene flow occurs at extremely low frequencies and over very short distances. Wheat gene flow occurred at less than 12 m; 0.012% and 0.0037% in the ACT and South Australia, respectively (Gatford et al. 2006). Pollen flow from GM barley was found to be 0.005% over a distance of less than 10 m at a site in South Australia that was part of the same small scale study (Gatford et al. 2006).

167. 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 (see Risk scenario 2). Expression of the introduced RNAi construct or introduced gene for enhanced nutrient utilisation efficiency in other wheat and barley plants would result in plants also limited by these factors.

168. The applicant proposes to prevent cultivation of non-GM wheat and barley, other than as part of the trial within the site, within 200 m of the site. These measures are further discussed in Chapter 3, Section 4.1.1. Isolation from other wheat and barley cultivation will greatly restrict the potential for pollen flow and gene transfer.

169. Cross pollination between GM wheat lines proposed for release at each site must be considered in relation to combining ('stacking') of GM traits possibly contributing to weediness of the resultant GM wheat lines. If the GM wheat lines containing the RNAi construct for altered grain composition cross-pollinated with the GM wheat lines containing the gene for enhanced nutrient utilisation efficiency, these characteristics may contribute to the spread and persistence of the GM wheat lines.

170. The applicant proposes to save seeds from each harvest for replanting in subsequent years. This gives rise to the possibility of low-level cross pollination between GM wheat lines during the first year of the trial, and subsequent amplification of a cross pollination event through propagation in later years. Low levels of gene flow between the trials may lead to stacking of GM traits, which may give rise to GM wheat and barley plants with altered weediness compared to any individual GM wheat or barley line in the proposed release.

171. The combination of these traits is likely to contribute only incrementally to the potential weediness of the GM wheat plants, the spread and persistence of which would be limited by factors such as lack of seed shattering, low intrinsic competitive ability, temperature, pests and diseases and other environmental factors that normally restrict the spread and persistence of wheat plants in Australia. The persistence of such lines would also be controlled by measures proposed by the applicant to restrict the persistence of the GM wheat and barley lines at the release site.

172. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for pollen flow and gene transfer to non-GM wheat and barley plants. In

particular, the applicant proposes to isolate the trial site from other plantings of wheat and barley, and the majority of the pollen is expected to fall within the trial site or the 10 m area directly surrounding the trial site. The applicant also proposes to perform post harvest monitoring of the site for twenty four months and to destroy any volunteer plants found at the site. The latter measure would ensure any remaining GM wheat or barley, potentially the product of gene flow, that germinate in these areas are destroyed.

173. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the genetic material, and regulatory sequences in other wheat and barley plants as a result of gene transfer is **not an identified risk** and will not be assessed further.

Risk scenario 5. Expression of the introduced genetic material and regulatory sequences in other sexually compatible plants

174. Transfer and expression of the introduced RNAi construct for altered grain composition and the introduced gene for enhanced nutrient utilisation efficiency to other sexually compatible plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

175. As discussed in Risk scenario 1, 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 RNAi construct or the introduced gene for enhanced nutrient utilisation efficiency. Similarly, if the introduced RNAi construct and introduced gene for enhanced nutrient utilisation efficiency are expressed in other sexually compatible species, allergenicity and toxicity are also not expected to be altered.

176. Expression of the introduced RNAi construct and introduced gene for enhanced nutrient utilisation efficiency in other sexually compatible plants is unlikely to give these plants a significant selective advantage. The conditions that restrict the spread and persistence of hybrids between non-GM wheat or barley and other sexually compatible plants would be expected to restrict the spread and persistence of any hybrids between the GM wheat or barley and other sexually compatible species.

177. *Hordeum vulgare* ssp. *spontaneum* (wild barley) is the only species that can cross with cultivated barley under natural conditions (Nevo 1992; OGTR 2008a). Wild barley is not found in Australia (OGTR 2008a).

178. As discussed in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008b), there are few species outside the *Triticum* genus that are sexually compatible with wheat and known to form hybrids under natural conditions. Examples include: *Aegilops cylindrica*, *Ae. ovata*, *Ae. biuncialis* and possibly *Secale cereale*. The hybrids obtained are generally male sterile and often have reduced female fertility. Hybridisation between wheat and other species in the *Elymus* and *Hordeum* genera have been recorded, and typically result in sterile hybrids. Artificial hybrids between wheat and *Secale cereale* have been reported, but no natural hybrids between these species have been observed in Europe or the USA (Eastham & Sweet 2002). However, some non-peer reviewed reports exist of naturally formed hybrids from Canada (Hegde & Waines 2004). Hybrids obtained between wheat and *S. cereale* are completely male sterile but female fertile (Hegde & Waines 2004). Furthermore, any hybridisation would require synchronicity of flowering between the GM wheat lines and compatible species to enable cross-pollination and gene flow to occur.

179. Information in addition to that discussed in the *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008b) has been identified in relation to the possibility of hybrids forming between *Triticum* and *Aegilops*. In Europe, hybrids between *Triticum* and

Aegilops species have been reported. However these were obtained from seed resulting from cross hybridising plants at close proximity from established mixed populations, hand crosses and crosses conducted under controlled conditions. The fertility of these hybrids varied greatly depending on the *Aegilops* species and wheat cultivars used in the experiments. Although some fertile hybrids were obtained, most showed compromised fertility and were generally male sterile (Schoenenberger et al. 2005; Schoenenberger et al. 2006; Loureiro et al. 2006; Loureiro et al. 2009).

180. Of the species that might hybridise with bread wheat under natural conditions, few are known to be present in Australia. Apart from commercially cultivated bread and durum wheat, other *Triticum* species are not known to be present in Australia. Durum wheat (*Triticum turgidum* subsp. Durum) can cross with wheat, although there are no reports of gene flow beyond 40 m (Matus-Cadiz et al. 2004). Other species belonging to the genera *Elytrigia*, *Elymus*, *Hordeum*, and *Secale* are known to occur in Australia. *Aegilops* spp are recognised as a quarantine weed species but are not known to be present naturally (see Chapter 1, Section 6.4).

181. The applicant has indicated that both sites are located within established agricultural areas. It is therefore possible that wheat and barley will be grown near the proposed trial sites. In addition, 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 (OGTR 2008a; OGTR 2008b).

182. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for pollen flow and gene transfer to sexually compatible plants. In particular, the applicant proposes to isolate the trial sites from other sexually compatible species, and the majority of the pollen is expected to fall within the trial site or the 10 m area directly surrounding the trial site. The applicant proposes to manage the area immediately surrounding the trial site to prevent sexually compatible species from flowering at the same time as the GM wheat and barley. This area will be inspected before flowering of the GM wheat and barley, and any plants found will be destroyed. The applicant proposes to inspect the experimental site for volunteer plants at monthly intervals following harvest. This will prevent the persistence of residual seed carrying over in the form of volunteer plants.

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

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

184. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but could be part of a scenario potentially leading to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or by altering the expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

185. Risks that might arise from horizontal gene transfer have been considered in previous RARMPs (eg DIR 057/2004 and DIR 085/2008), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. From the current scientific evidence,

HGT from GM plants to other organisms presents negligible risks to human health and safety or the environment due to the rarity of such events, relative to those HGT events that occur in nature, and the limited chance of providing a selective advantage to the recipient organism.

186. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. All of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

Risk scenario 6. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer

187. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of potential recipient organisms and the nature of the introduced genetic material. Risks that might arise from HGT from a GMO to another organism have been recently reviewed (Keese 2008) and considered in detail in a previous RARMP (DIR 085/2008) which is available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office.

188. HGT could result in the presence of sequences from the introduced RNAi construct for modified grain composition, and/or the introduced gene for enhanced nutrient utilisation efficiency, in bacteria, plants, animals or other eukaryotes. The sequences comprising the RNAi construct were isolated from wheat and rice, which are widespread in the environment (Chapter 1, Sections 6.5) and already available for transfer via demonstrated natural mechanisms. Importantly, the RNAi construct includes only a part of the coding sequence rather than the entire sequence of the gene. If HGT were to occur, it could result in the expression of a short protein fragment, which may or may not include the functional domain of the protein encoded. Similarly, the *Me1* gene was isolated from barley and homologues of the encoded protein occur naturally in animals, plants, yeast and bacteria. The regulatory sequences are also widespread in the environment (see Chapter 1, Section 6.5). Therefore, the RNAi sequence, *Me1* gene and regulatory sequences are already available for transfer via demonstrated natural mechanisms.

189. The nature of the RNAi construct is considered unlikely to change the potential frequency of HGT, which is known to occur at an extremely low frequency from plants to other eukaryotes or prokaryotes (Keese 2008). The nature of the RNAi construct is also considered unlikely to result in harmful consequences, should HGT occur. The RNAi construct differs from most other transgenes previously evaluated for intentional release in the arrangement of DNA sequences into an inverted repeat. Sequencing of the *Arabidopsis* genome has indicated that a significant proportion of plant genes are arranged in arrays of tandem repeats, and within this group there are many examples of local inversions (The Arabidopsis Genome Initiative 2000). During genome evolution, genome rearrangement, including the generation of inverted duplications, is a frequent occurrence (reviewed by Shapiro 2005). Transcribed inverted repeats of protein coding sequences appear to be the evolutionary origin of microRNAs, a conserved endogenous silencing mechanism which is involved in the regulation of hundreds, perhaps thousands, of plant genes (Axtell & Bowman 2008). This evidence shows that inverted repeat sequences, including those with functional promoters, are commonly available for HGT from plants.

190. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced genes rather than HGT *per se* (Thomson 2000). If the introduced RNAi construct, introduced gene for enhanced nutrient utilisation efficiency or their end products are not associated with any risk then even in the unlikely event of HGT occurring, they should not pose any risk to humans, animals or the environment. Conclusions reached for Risk scenarios 1 - 5 associated with the expression of

the introduced genetic material did not represent an identified risk. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

191. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. Most of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

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

2.5 Unintended changes in biochemistry, physiology or ecology

193. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy¹⁰ (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 biochemical pathways incorporating the protein encoded by the introduced gene.

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

Risk scenario 7. Changes to biochemistry, physiology or ecology of the GM wheat and/or barley plants resulting from expression or random insertion of the introduced genes

195. The applicant indicates that the GM wheat and barley lines have undergone very limited phenotypic characterisation as the project is in early stages. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced genetic material, have already been discussed in Risk scenarios 1 to 3, and were not considered identified risks.

196. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered responses to environmental stress, production of novel substances, and changes to levels of endogenous substances. Unintended changes that occur as a result of gene insertions are rarely

¹⁰ Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

advantageous to the plant (Kurland et al. 2003). Obvious unexpected alterations are likely to have been detected and eliminated during development of the GM wheat and barley lines.

197. Various biochemical pathways of the GM wheat and barley plants could be changed by the expression of the introduced RNAi construct and introduced gene for enhanced nutrient utilisation efficiency, resulting in the production of novel or higher levels of endogenous toxins, allergens or anti-nutritional compounds. Non-GM wheat can be toxic to animals if consumed in large quantities (due to nitrate poisoning), and flour from both wheat and barley is allergenic to some people and may also trigger coeliac disease. For further discussion regarding the toxicity and allergenicity of non-GM wheat and barley see *The Biology of Triticum aestivum L. em Thell. (bread wheat)* (OGTR 2008a) and *The Biology of Hordeum vulgare L. (barley)* (OGTR 2008a; OGTR 2008b).

198. In plants, RNAi constructs can give rise to off-target silencing effects, where short sequences from the RNAi construct closely match non-target sequences expressed in the same cells. Homology of as little as 20 nucleotides can give rise to off-target silencing (reviewed by Small 2007). The strength of silencing of the non-target gene generally increases with greater lengths of homology and the strongest effects are expected to occur between highly homologous gene family members (Miki et al. 2005). This is expected to occur only in tissues in which the RNAi construct is expressed, which is expected to be only in the endosperm in the GM wheat lines (information supplied by applicant). Potential off-target silencing may be able to be predicted if the sequence of the host genome is known, however this is not the case for wheat. Similar to the effect of random insertions discussed above, any strong off-target silencing effect is likely to be detrimental to the plant, so likely to be detected during production of the GM wheat lines.

199. Unintended secondary effects occurring as a result of altered grain composition could include changes in seed germination and seedling vigour (as discussed in Risk scenario 2), pest preference for grain, seed dormancy, timing of flowering and seed set, outcrossing tendency or disease susceptibility. While the GM wheat lines have not undergone thorough phenotypic analysis, it is expected that substantial changes in these parameters would have been detected in the time these lines have been under development.

200. Unintended secondary effects occurring as a result of enhanced nutrient utilisation efficiency and any potential increase in nitrogen content of the plants could change pest and disease susceptibility. For example, aphids are known to prefer plants with higher levels of nitrogen compared to plants with lower levels of nitrogen, which could result in an increase in aphid-transmitted plant viruses (Thompson et al. 1993; Duffield et al. 1997; Ponder et al. 2000). Infection by powdery mildew may also increase (Thompson et al. 1993). While the GM wheat and barley lines have not undergone thorough analysis, any adverse effects would be minimised by the proposed small scale and short duration of the trial. Pests and diseases would also be managed during the trial by normal agricultural practice.

201. The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the scale and duration of the trial would minimise the potential for adverse effects. Access to the proposed trial sites would be by private road, which limits exposure of the public to the GM plant material. The public and livestock would not be intentionally exposed as the GM plant material will not be used as food or animal feed.

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

2.6 Unauthorised activities

Risk scenario 8. Use of GMOs outside the proposed licence conditions (non-compliance)

203. If a licence were to be issued, non-compliance with the conditions of the licence could lead to spread and persistence of the GM wheat and barley plants outside of the proposed release areas and/or increased exposure of people and other organisms to GM material. The adverse outcomes that this risk scenario could cause are the same as those 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 to 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.

204. **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

205. The risk assessment begins with postulation of potential pathways that might lead to harm to the health and safety of people or the environment during the proposed release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and within the context of the receiving environment.

206. Eight risk scenarios were postulated 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.

207. A **risk** is only identified when a risk scenario is considered to have some chance of causing harm. Pathways 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.

208. The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood 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 principal reasons for this include:

- limits on the size, locations and duration of the release proposed by CSIRO
- suitability of controls proposed by CSIRO to restrict the spread and persistence of the GM wheat and barley plants and their genetic material
- limited ability and opportunity for the GM wheat and barley plants to transfer the introduced genetic material to commercial wheat and barley crops or other sexually related species
- none of the GM plant materials or products will be used human food or animal feed
- widespread presence of the same genes or sequences in the environment and lack of known toxicity or evidence of harm from them.

209. 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 plants into the environment are considered

to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment¹¹.

Section 4 Uncertainty

210. 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 (consequence and likelihood) are always uncertain to some degree.

211. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹². 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 to treat an identified risk.

212. For DIR 099, which involves early stage research, uncertainty is noted particularly in relation to the characterisation of:

- Risk scenario 1, regarding potential increases in allergenicity or toxicity through ingestion or contact with plant material containing the introduced RNAi construct and/or the protein encoded by the introduced gene for enhanced nutrient utilisation efficiency
- Risk scenario 2, associated with a potential for increased survival of the GMOs and the persistence of seeds at the trial site following harvest
- Risk scenario 7, due to incomplete characterisation of the GMOs.

213. 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 these GM wheat and barley lines if they are selected for further development.

214. Chapter 3, Section 5 discusses information that may be required for future release.

¹¹ As none of the proposed dealings are 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 has allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

¹² A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2009) available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

Chapter 3 Risk management plan

215. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through proposed licence conditions. The risk management plan informs the Regulator's decision-making process. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

216. 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.

217. All licences are required to be subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions contemplate the Regulator maintaining oversight of licensed dealings: 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.

218. It is further provided 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. 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

219. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National 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 agencies¹³.

220. 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.

221. FSANZ is responsible for human food safety assessment, including GM food. As the trial involves early stage research, the applicant does not intend any material from the GM wheat and barley plants to be used for human food. Accordingly, the applicant has not applied

¹³ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

to FSANZ to evaluate the GM wheat and barley plants. However, in the event of a commercial release, FSANZ approval would need to be obtained before materials from the GM wheat and barley plants could be sold for human consumption.

222. No other approvals are required.

Section 3 Risk treatment measures for identified risks

223. The risk assessment of the risk scenarios 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 2009), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

224. These risk scenarios were considered in the context of the scale of the proposed release (a maximum area of 2 ha per year on two sites, one in the shire of Narrabri (NSW) and the other in the shire of Corrigin (WA), between May 2010 and June 2013), the proposed containment measures (Chapter 1, Section 3), and the receiving environment (Chapter 1, Section 6).

Section 4 General risk management

225. Licence conditions are imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and limit the release to the size, locations 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 Section 4.1.2.

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by CSIRO

226. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls proposed by CSIRO in their application, and these are discussed in the eight risk scenarios characterised for the release in Chapter 2. The appropriateness of these controls is considered further below.

227. The proposed release would be confined to a maximum of 2 ha per year on two sites, one located in the shire of Narrabri (NSW) and the other in the shire of Corrigin (WA), and the duration of the proposed release will be limited to three growing seasons. The applicant does not intend to use any of the GM plant material as human food or animal feed. Only staff with appropriate training would be allowed access to the trial sites. These measures will minimise the potential exposure of humans, vertebrates and other organisms to the GMOs (Risk scenario 1) and the potential for the GM wheat and barley lines to disperse and establish outside the proposed release site (Risk scenario 3). To further minimise the exposure of humans to the products of the GM wheat and barley, a licence condition has been imposed which prohibits the use of the GM products in the production of therapeutic goods as defined in the *Therapeutic Goods Act 1989*.

228. The applicant's proposal to restrict gene flow from the GM wheat and barley (Risk scenario 4 and Risk scenario 5) includes surrounding the proposed release site with a 10 m wide zone in which vegetation is controlled and related species are prevented from flowering, and a 200 m zone in which no other wheat or barley plants are to be grown (including breeding lines).

229. As discussed in Chapter 1, Section 6.4 and Risk scenario 5, there are few species with which wheat can naturally form hybrids, and the fertility of hybrids formed is typically low.

Hordeum vulgare ssp. *spontaneum* (wild barley) is the only species that can cross with cultivated barley under natural conditions (Nevo 1992; OGTR 2008a). Wild barley is not found in Australia (OGTR 2008a). There are no reports of barley forming hybrids with cultivated wheat under natural conditions.

230. Differences in pollen flow have been observed in different pollen flow studies in both wheat and barley. A number of variables, particularly pollen source size, climatic conditions and the difficulty of detecting rare events, could influence the accuracy and reproducibility of these measurements. In Northern America, levels of gene flow in wheat have been shown to be dependent on the size of the field (reviewed by OGTR 2008b). For an experimental scale wheat field, extremely low rates of gene flow (0.002 – 0.003%) were detected at distances up to 100 m. For commercial scale fields, outcrossing rates of 0.25% were detected at 61 m and in one instance gene flow was recorded up to 2.75 km from a commercial wheat field (Matus-Cadiz et al. 2007).

231. In barley, outcrossing rates are generally lower than those found for wheat and gene flow is mostly detected between adjacent plants (reviewed in OGTR 2008a), with very low levels of outcrossing (0.1%) occurring at up to 60 m (Wagner & Allard 1991). In an Australian study, gene flow from a small planting of GM wheat occurred at a frequency of 0.012% and 0.0037% over 8 m and for barley gene flow occurred at 0.005% over a maximum distance of 10 m (Gatford et al. 2006). However, gene flow was not measured beyond 10 m and therefore the true rate may be higher.

232. Isolation distances for GM field trials vary greatly amongst different countries. For example, 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 the European Union, various GM wheat field trials have been approved, requiring border plantings of non-GM wheat or other plants ranging up to 30 m wide and isolation distances from other wheat ranging from 2 to 50 m (European Commission Directorate General for the Environment 2009). A field trial release of GM barley in Iceland requires a separation distance of at least 300 m from other barley fields (European Commission Directorate General for the Environment 2009).

233. In Australia, requirements for basic and certified seed production for both wheat and barley are aligned with Organisation for Economic Cooperation and Development (OECD) rules (Australian Seeds Authority Ltd. 2006). OECD rules (OECD 2008) stipulate a maximum acceptable level of off-types or other cultivars of the same species of 0.1% for basic wheat or barley seed and 0.3% for certified wheat or barley seed (1st generation). Seed crops of self-fertilising cereals (eg wheat and barley) are required to be separated from other cereal crops by a barrier or space sufficient to prevent seed mixture during harvest. Similarly, the United States *Federal Seed Act Regulations* does not specify an isolation distance for either wheat or barley used for seed production. However, for hybrid seed production (where the phenotype may be variable and determination of contamination levels is difficult) a distance of 300 feet (approximately 100 m) is required for the US and 25–100 m for the OECD (Code of Federal Regulations 2006; OECD 2008).

234. The applicant has proposed to maintain a 200 m separation between the GM wheat and barley lines and other wheat and barley cultivation. If gene flow to a breeding line were to occur, and the breeding line was to eventually become commercially successful, this could lead to increased propagation and dispersal of the GMOs. However, on the basis of the scientific literature on gene flow, international containment measures for GM wheat and barley trials, and the rules for producing basic and certified seed, a 200 m isolation zone clear of sexually compatible species is considered adequate to restrict gene flow from the GM

wheat and barley plants to any other wheat and barley (including breeding lines) or other sexually related species outside the release site (Risk scenario 4 and Risk scenario 5) and is therefore imposed as a licence condition. This consideration is reflected in a licence condition in recent DIR wheat licences (eg DIRs 092, 093 and 094), where an isolation zone of 200 m is imposed and must be inspected for sexually compatible species, which if found must be destroyed before flowering.

235. While the applicant proposes to maintain the 200 m separation from any other non-GM wheat and barley plantings, they propose that inspections of this 200 m zone are carried out for the first growing season, and then reduced to the first 50 m of this zone in the following growing seasons¹⁴. This reduction in inspection requirements is based on data indicating that gene flow for wheat or barley is low over distances of 60 m for wheat (see discussion above) and even lower for barley. In particular, the Australian trial by Gatford et al (2006) and the study by Wagner and Allard (1991) suggests that, at 60 m separation, cross-fertilisation between barley plants would occur at less than 1 in every 10,000 gametes. In effect the applicant's proposal is for the GMOs to be surrounded by a 10 m monitoring zone which, coupled with the first 50 m of the inspection zone would give 60 m in total requiring inspection and control/removal of sexually compatible species by herbicide treatment, ploughing back into the soil, or uprooting before they flower.

236. The likelihood of non-GM wheat or barley or any sexually compatible species occurring within the 200 m zone will depend on the occurrence of sexually compatible species as well as the environmental/climatic context, previous cropping history and management practices for the site. The applicant has indicated that the proposed trial sites are in productive agricultural areas, so it is likely that wheat and/or barley plants will be grown in the wider area (ie beyond 200 m) during the trial. As a result, there is the possibility that non-GM volunteers may germinate in the 200 m isolation zone during the proposed trial. However, the likelihood of this occurring differs between the two sites. No barley has been grown at either the Narrabri or Corrigan site, including the proposed isolation zones. However, wheat was grown at the Narrabri site in 2008 and at the Corrigan site in 2009.

237. As discussed above, a 200 m isolation zone clear of sexually compatible species is considered adequate to minimise gene flow from the GM wheat and barley plants to any other wheat and barley (including breeding lines). Considering the cropping history of the areas and environmental/climatic context, it is possible non-GM wheat plants may occur within the 200 m isolation zone in the first year or two of the trial. Therefore, a licence condition has been imposed that initially requires inspection for, and destruction of, related species in the total area of the 200 m isolation zone. It allows CSIRO to request a reduction of inspection requirements within the isolation zone to 50 m, two years after any non-GM wheat was grown and if no non-GM wheat or sexually compatible species have been found during monitoring of the site in the first year. A requirement to maintain a minimum separation distance of 200 m from other wheat and barley crops will not be affected by any reduction in inspection area.

238. As discussed in Risk scenario 5, natural hybrids between *T. aestivum* and *Secale* or *T. aestivum* and *Elytrigia* (*Elymus*) have not been recorded. There is no evidence to indicate that viable hybrids could be generated between *H. vulgare* and *E. scaber*, if present. Other species with which *T. aestivum* could potentially form hybrids, albeit with highly compromised fertility, such as a few selected *Aegilops* species. *Aegilops* spp. are recognised as quarantine weeds in Australia and are not known to be present naturally. There are no

¹⁴ CSIRO requested a reduction in inspection requirements after the release of the consultation RARMP.

reports of barley forming hybrids with cultivated wheat under natural conditions. In the context of the proposed release site(s) and on the basis of the scientific literature on interspecific and intergeneric gene flow sexually compatible species which must be inspected for and controlled will be restricted to the *Triticum* genus and *Hordeum vulgare*.

239. In determining post-harvest monitoring requirements, it is important to consider the potential dispersal of grain during sowing and harvesting (mechanical dispersal). This is most likely to result in dispersal of grain into the area immediately around the trial. As this area would be subject to inspections, GM plants arising from this seed would be detected and controlled.

240. The applicant proposes to surround the trial site in the shire of Corrigin with a fence, with lockable gates, which will prevent access by livestock to the GM wheat and barley lines at this site. A requirement to surround the Corrigin trial site with a fence is imposed as a licence condition. The trial site in the shire of Narrabri will not be surrounded by a fence, however access by livestock will be prevented as the site is isolated and there is a fence around the farm where the site is located. This will minimise the potential exposure of livestock to the GMOs (Risk scenario 1) and the potential dispersal of the GMOs (Risk scenario 3).

241. The proposed 10 m monitoring zone, with little or no vegetation, will serve as a deterrent to rodent activity at the proposed release site (Risk scenario 3). The applicant also proposes to conduct mouse baiting and trapping around the perimeter of each site, which will aid in reducing the size of the mouse population which may have access to the GM wheat and barley lines. Whilst there are differing reports regarding the average territory size of mice, the use of reduced vegetation has been shown to help reduce rodent numbers in agricultural settings. As viable seed may remain on the soil surface after harvest, a licence condition is imposed requiring rodent reduction measures to continue after harvest and until remaining seeds have been incorporated into the soil through post harvest tillage. This will minimise the potential exposure of vertebrates to the GMOs (Risk scenario 1) and the potential dispersal of the GMOs (Risk scenario 3).

242. The applicant has stated that the trial sites will be located at least 1000 m from the nearest waterways. A standard DIR licence condition is imposed requiring the trial site to be located at least 50 m from a natural waterway which will limit the dispersal of viable GM plant material in the event of flooding (Risk scenario 3).

243. The applicant has proposed a number of measures to restrict the persistence of any GM wheat or barley plants and seeds in the seed bank at the proposed release site after harvest of the trial (Risk scenario 2). These measures include monthly monitoring of the trial site for volunteer wheat and barley plants following harvest, and destroying volunteer plants that emerge after harvest by either spraying with herbicide, ploughing back into the soil, or pulling out volunteer plants before they flower. Plant material remaining at the site after harvest will be ploughed in at an unspecified time after harvest. The applicant has also proposed to monitor the proposed release site for 24 months after harvest. All volunteers will be destroyed before flowering.

244. The loss of wheat seed at harvest is estimated to range between 0.8 and 6%, depending upon factors including the harvest machinery used, the genetic tendency of a variety to shed seed and weather conditions (reviewed by Anderson & Soper 2003). The applicant has proposed to harvest the GM wheat and barley plants at the Narrabri site with a small mechanical single row harvester. Harvesting at the Corrigin site is proposed to be done with a plot harvester. Wicks et al. (2000) reported that small plot headers are less efficient than commercial harvesters, so self-sown wheat and barley may be a greater problem under experimental conditions.

245. Cereal grains require an after-ripening period before germination can occur, which takes up to nine months depending upon genetic background and environmental conditions (Pickett 1989; Anderson & Soper 2003). The process of after-ripening is favoured by hot dry conditions, which can be facilitated in the field by retaining any seed remaining after harvest on the soil surface prior to irrigation (Pickett 1993). Although the time required for after-ripening of the GM wheat and barley lines under the expected field conditions is unknown, retention of dropped seed on the soil surface for at least 28 days is imposed as a licence condition.

246. The persistence of seed depends on several factors which contribute to seed dormancy: cultivar genetics, environmental conditions during seed formation, crop nutrition, environmental conditions after shedding, and field treatment (reviewed by Anderson & Soper 2003). Viable seeds persist in the soil for longer periods in dry than in moist conditions (Anderson & Soper 2003), and wheat seeds present as un-threshed ears have longer dormancy than loose seeds (Komatsuzaki & Endo 1996). Shallow tillage after harvest, combined with irrigation, will germinate much of the grain dropped at harvest (Ogg & Parker 2000), while deep tillage encourages burial-induced dormancy (reviewed by Anderson & Soper 2003). Shallow tillage concurrent with irrigation would also serve to enable degradation of the plant material remaining at the site after harvest.

247. There is high variability in volunteer wheat and barley emergence, and various field studies report volunteer emergence up to two years following harvest (reviewed by Anderson & Soper 2003). In a study in Germany, a small proportion of barley seeds were recovered after 15 months (Rauber 1988). In a Scottish survey, volunteer winter barley was reported to persist for up to five seasons, and volunteer spring barley persisted for two seasons, in some rotations (Davies & Wilson 1993). A Canadian field study of spring wheat persistence reported low levels of volunteer germination three years after wheat seeds were dropped in test plots (Harker et al. 2005). Dormancy of cereals is reduced in warmer temperatures (reviewed by Pickett 1989), and so dormancy is expected to be reduced in Australian field conditions compared to western Canada, Germany and Scotland.

248. The applicant proposes not to irrigate the trial sites following harvest. Instead, each site will be monitored for volunteer plants at monthly intervals for 24 months after harvest, and any volunteer plants will be destroyed. However, without sufficient irrigation (or sufficient rainfall) of a post-harvest site, seed could persist for a longer period of time. Therefore, it is considered that three irrigations (or sufficient rainfall), combined with an appropriate tillage regime, and monitoring for and destruction of volunteers for at least 24 months, would effectively reduce survival and persistence of viable wheat and barley seeds in the soil. The imposed licence conditions require an initial irrigation to take place within 60 days of harvest to encourage surface seed to germinate. Two further irrigations will be required at intervals of at least 28 days, with the last irrigation occurring during the final six months of the monitoring period. These treatments will promote germination by ensuring any remaining seeds are exposed to sufficient moisture and placed at an appropriate depth for germination and will also encourage the microbial decomposition of any residual seed. Post harvest monitoring of the release site for at least 24 months after harvest, with no volunteers observed in the most recent six months, needs to be completed before an application that inspection conditions no longer apply can be made to the Regulator. These measures will minimise the persistence of the GMOs in the environment (Risk scenario 2).

249. During the growing season, the applicant proposes to plant break crops in the areas of each trial site not being used for growing the GM wheat and barley. These crops would help to remediate the soil and prevent the build up of disease. The crops proposed at the Narrabri site are cotton, millet or sorghum. These crops will be grown to maturity and then harvested. The crops proposed at the Corrigin site are canola, lupins or field peas. These crops will be

ploughed prior to flowering. During growth of the break crops, the site would be managed so as to not interfere with the detection and destruction of volunteer wheat and barley plants, or treated with selective herbicides, thereby destroying any volunteer wheat or barley plants present. It is further specified in the licence conditions that the selective herbicide be used such that any volunteers are destroyed before flowering.

250. Although, as indicated above, volunteer wheat and barley plants should be destroyed before flowering, their presence may go undetected in a break crop. This would be taken into account by the Regulator in considering any application for site sign off if a break crop was grown in the six month period prior to the application for sign off.

251. Non-GM wheat will be planted amongst the GM wheat and barley plants at the trial sites (refer to Chapter 1, Section 5.1 for details). As there is a possibility of viable GM material being present in the non-GM plants, a licence condition has been imposed requiring any non-GM plants at the trial site to be treated as the GMOs and therefore subject to all the same licence conditions. This will restrict the dispersal of viable GM plant material (Risk scenario 3).

252. The applicant has stated that any plant material taken off-site for experimental analysis will be transported according to the Regulator's *Guidelines for the transport of GMOs*, <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/transport-guide-1>>. These are standard protocols for the handling of GMOs to minimise exposure of the GMOs to human and other organisms (Risk scenario 1) and dispersal of GMOs or GM material into the environment (Risk scenario 3, 4 and 5).

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

253. A number of licence conditions have been imposed to limit and control the release, based on the above considerations. These include requirements to:

- limit the release to a total area of up to 2 ha per growing season at two sites, one in the LGA of Narrabri (NSW) and the other in the LGA of Corrigin (WA), from the date of issue of the licence until June 2013
- locate the trial sites at least 50 m away from natural waterways
- establish a 10 m zone around the trial sites in which any related species are prevented from flowering and which is maintained in a manner that does not attract or harbour rodents
- surround the GM wheat and barley with an inspection zone of up to 200 m in which growth of sexually compatible species is controlled
- ensure no other crops of wheat or barley are within 200 m of the trial sites
- enclose the trial site in the shire of Corrigin with a livestock-proof fence with lockable gates and ensure that livestock are excluded from trial site in the shire of Narrabri
- harvest the GM wheat and barley plant material separately from other crops
- clean the sites and equipment used on the sites following harvest
- apply measures to promote germination of any wheat and barley seeds that may be present in the soil after harvest, including irrigation
- monitor the site for at least 24 months after harvest and destroy any wheat and/or barley plants that may grow until no volunteers are detected for a continuous 6 month period
- destroy all GM plant material not required for further analysis or future trials
- transport material from the GMOs in accordance with the Regulator's guidelines

- not permit any GM wheat or barley plant material to be used in human food or animal feed, or in the production of therapeutic goods.

4.1.3 Measures to control other activities associated with the trial

254. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs; Policy on transport and supply of GMOs*). Licence conditions based on these guidelines and policies have been 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.

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

4.2 Other risk management considerations

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

- applicant suitability
- contingency 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

257. 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.

258. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers CSIRO suitable to hold a licence.

259. 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.

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

4.2.2 Contingency plan

261. CSIRO 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.

262. CSIRO 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 would be 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

263. The persons covered by the licence would be 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

264. 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.

265. 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.

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

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

4.2.5 Monitoring for Compliance

267. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

268. 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.

269. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides 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

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

- additional data on the potential allergenicity or toxicity of plant materials from the GM wheat and barley lines
- additional phenotypic characterisation of the GM wheat and barley lines, in particular pest disease susceptibility and characteristics indicative of weediness including measurement of altered reproductive capacity and competitiveness
- characterisation of the introduced genetic material in the plants, including copy number and genotypic stability.

Section 6 Conclusions of the RARMP

271. The risk assessment concluded that this proposed limited and controlled release of up to 11 GM wheat lines and 3 GM barley lines on a maximum total area of 2 ha per year over three growing seasons in the shire of Narrabri (NSW) and the shire of Corrigin (WA), poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

272. The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, locations and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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Appendix A Summary of issues raised in submissions received from prescribed experts, agencies and authorities¹⁵ on the consultation RARMP for DIR 099

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. A number of submissions received raised issues relating to risks to the health and safety of people and the environment as summarised below.

Summary of issues raised	Comments
Grains produced in the trial should be specifically excluded from use as inactive ingredients in 'therapeutic goods'. Consequently, wherever the risk management refers to exclusion of grains from 'human food or animal feed', reference should also be made to the exclusion of grains from 'therapeutic goods'.	Included in Chapter 3, Section 4 of the RARMP and the Licence.
Concerned with unintended effects of GM crops, noting that use of transgenic modification and RNA silencing both have the potential for effects that go beyond the original modifications intended. Not confident that screening GM wheat or barley lines for agronomic traits is sufficient to ensure safety for human health and the environment.	While the GM wheat and barley lines have not undergone thorough phenotypic analysis, the likelihood of any pleiotropic or other unintended effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the limited scale and duration of the trial and the prohibition of use of material from the GM wheat and barley in food, feed and therapeutic goods would reduce the potential for adverse effects.
Requests that assessment of grain composition be expanded to use profiling techniques (eg proteomics) as a non-targeted, unbiased approach to detect unintended effects of gene expression that might be relevant to human health and the environment.	Although profiling represents a powerful array of techniques to detect molecular differences between organisms, their current status precludes a reliance on these techniques within the present regulatory framework. Further research is required to validate different profiling methodologies and databases established for ranges typical of conventional varieties at different developmental stages and under a range of growth environments and stresses. Furthermore, changes identified by these methodologies require assessment with respect to identifying any associated harm to people or the environment. The Regulator adopts a weight of evidence approach, which includes extensive genetic, biochemical, physiological, morphological, ecological and agronomic data that encompass changes directly attributable to the genetic modification as well as secondary and unintended effects. The emphasis of the risk assessment is on those characteristics that provide evidence of potential harm to people or the environment. Metabolic differences, whether few or many, do not necessarily indicate the causation of harm. More useful are other indicators of harm, including toxicity and allergenicity, increased pathogenicity, increased

¹⁵ GTTAC, State and Territory Governments, Australian Government agencies and the Minister for Environment Protection, Heritage & the Arts.

Summary of issues raised	Comments
	ability to spread and persist in the environment, potential enhancement of harmful properties of sexually compatible relatives etc.
<p>Draft RARMP does not include discussion of the potential interaction or stacking of the GM traits in this trial with those traits present in other current wheat and/or barley trials. Early consideration of possible interactions of the expanding number of traits may lead to enhanced risk assessment outcomes. Recommends inclusion of a discussion of this nature.</p>	<p>Stacking of the GM traits in this release with other limited & controlled releases of GM wheat and barley was not discussed because it is extremely unlikely that stacking between GM plants in different trials will occur since the different trials are geographically remote and licence conditions imposed for each release effectively restrict gene flow. Therefore, a discussion on this issue is not warranted at this stage. A consideration of stacking and any potential risks that might arise as a consequence would be undertaken for a release where controls on gene flow were minimal or absent, such as for an application to commercially release GM wheat or barley.</p>
<p>GM lines modified for nitrogen use efficiency are likely to contain elevated levels of nitrogen compared to their unmodified counterparts. This is not discussed in RARMP.</p> <p>Changed nitrogen content may affect patterns of predation by aphids, which are known to preferentially consume plants containing elevated nitrogen levels over those containing a lesser nitrogen content.</p> <p>Changed pest populations could create secondary effects (eg changing the rate and spread of aphid-communicated plant viruses) which may require further risk analysis, particularly if the GM lines proceed to commercialisation.</p> <p>Suggest monitoring aphid populations in the trial toward increasing understanding of this issue should the trait proceed to commercial release.</p>	<p>Risk scenario 7 in the final RARMP has been modified to include a discussion on disease susceptibility of the GM wheat and barley lines. Additionally, the future research requirements in Chapter 3, Section 5 have been modified to include this issue.</p>

Appendix B Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 099

The Regulator received six submissions from the public on the consultation RARMP. These submissions, summarised in the table below, raised issues relating to human health and safety and the environment. These were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

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

Issues raised: **AP:** Assessment process; **C:** Controls; **E:** Economic issues; **EN:** Environmental issues; **F:** Food; **GT:** Gene technology; **H:** Human health and safety; **HGT:** Horizontal Gene Transfer; **L:** Legal issues; **LC:** Licence condition; **OSA:** Outside scope of assessment; **P:** Persistence; **R:** research; **S:** Segregation; **UE:** Unintended effects.

Other abbreviations: **Ch:** Chapter; **GE:** genetically engineered; **GM:** Genetically Modified; **GMO:** Genetically Modified Organism; **RARMP:** Risk Assessment and Risk Management Plan.

Type: **I:** individual; **G:** Group.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	n	C	For the DIR 053 GM wheat trial that took place north of Corrigin, birds dug up GM seed shortly after sowing. Bird netting was specified in the licence for harvest, but was subsequently erected after sowing in response to this bird damage. The OGTR was informed of this incident.	Noted. 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 it elsewhere for storage or consumption (Risk scenario 3). The report to the OGTR regarding this incident supports this statement. Parrots removed a small number of seedlings from the soil, and ate the seeds. The birds fed on already germinated seed, and as a result destroyed the viability of the GM plant material. Consequently, the overall assessment of the bird damage was that no viable GM wheat was removed from the site.
2	I	x	C, EN	Concerned that GM wheat and barley cannot be contained to trial sites, especially during storms, thereby contaminating non-GM wheat and barley.	Dispersal of GM material was considered in Risk scenario 3 and the RARMP concluded that there were negligible risks to people or the environment from the GM wheat and barley. The Regulator has imposed a range of measures to limit the trial to the proposed size, locations and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
3	I	x	GT	<p>Introduction of GM wheat and barley should be unnecessary in an ecologically balanced system. Their introduction is indicative of a failure to balance and restrict human needs within ecological imperatives.</p> <p>The government is failing to examine the causes of ecological collapse by supporting genetic engineering, and is promoting an unwanted and untested remedy.</p> <p>The Regulator is implicit in this process by claiming to protect human health and safety within too narrow a frame of reference.</p>	<p>The appropriateness of using gene technology is outside the scope of issues to which the Regulator must have regard when deciding whether or not to issue a licence.</p> <p>The frame of reference for risk assessment is defined by the <i>Gene Technology Act 2000</i>, which requires the Regulator to consider risks to human health and safety and the environment posed by or as a result of gene technology and to manage any identified risks.</p>
4	I	x	GT	GM technology is very different to breeding using plant reproductive processes.	Noted.
			L, AP	[The OGTR] does not have the legal or technical means to effectively and independently assess long term effects of genetic modification.	<p>The <i>Gene Technology Act 2000</i> (the Act) requires the Regulator to consider risks to human health and safety and the environment posed by or as a result of gene technology and to manage any identified risks.</p> <p>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 on whether or not to issue a licence to deal with a GMO. The decision is based upon a RARMP prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the <i>Risk Analysis Framework</i> and are finalised following consultation with a wide range of experts, agencies and authorities, and the public.</p> <p>The Regulator considers both short term and long term impacts, taking into account current scientific and technical knowledge, when assessing an application to release a GMO.</p>

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			EN, UE	Prior effective risk management strategies do not cover the possibility of unpredictable future downstream broad species destruction.	<p>DIR 099 is a limited and controlled release for the purpose of conducting experiments, including field characterisation of the GM wheat and barley lines. The RARMP concluded that there were negligible risks to people and the environment from this limited and controlled release of GM wheat and barley. The Regulator has imposed a range of measures to limit the trial to the proposed size, locations and duration and requires controls in line with those proposed by the applicant.</p> <p>Furthermore, the RARMP outlines future research requirements should the applicant seek a licence for a larger scale or commercial release. These include further information on the potential allergenicity or toxicity of the GM plants, additional phenotypic characterisation (including characteristics indicative of weediness), and characterisation of the genetic material in the plants. Such information would help inform the Regulator about any possible unintended effects for future applications.</p>
			E, EN, H	Conducting the GM trial in areas where non-GM wheat and barley are grown will lead to contamination of non-GM crops. This contamination will have negative economic impacts and unintended malevolent consequences.	<p>The trial is of small size and limited duration, and licence conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment. These include a requirement to maintain a 200 m isolation zone around the trial site in which no sexually compatible species, including cultivated wheat or barley, can be grown. The RARMP concluded that there were negligible risks to people and the environment from this limited and controlled GM wheat and barley trial.</p> <p>Economic issues are outside the scope of assessments required by the <i>Gene Technology Act 2000</i>.</p>
			R	No independent review or control trial of nutrient utilisation or stress tolerance gene modification has shown enhanced yield over subsequent generations. Largeesse funding, invested interests and multinational companies create an environment of conflict of interest and biased research.	<p>Potential benefits of the technology are outside the scope of assessments required by the <i>Gene Technology Act 2000</i>. The Regulator uses a variety of information sources to assess applications for the release of GMOs, including peer reviewed literature, biological principles, experts and technical reports, data generated by applicants, and broad consultation.</p>

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			UE	No eye witness testimony or research from results, studies and observations across species within many countries has ever convinced submitter to adopt GM. Abstinence and border protection are the only defence against unintended consequences of GM.	The RARMP concluded that this limited and controlled release of GM wheat and barley poses negligible risks to people and the environment.
5	G	x	UE	<p>It is not correct that insertion of a single gene into an organism will produce a single desired characteristic. Some genes are known to encode many protein variants by alternative splicing. Further, insertion of genetic material into the genome causes damage such as deletions, repetitions and scrambling. The behaviour of the genetic code can lead to unexpected complexities and results.</p> <p>Therefore, the insertion of genetic material into crops could result in changes in characteristics and proteins in seeds (eg allergens). CSIRO can not eliminate harmful genetic characteristics that could develop in several generations.</p>	<p>Unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (as discussed in Chapter 2, Section 2.5). While the GM wheat and barley lines have not undergone thorough phenotypic analysis, it is expected that substantial unintended changes would have been detected in the time these lines have been under development.</p> <p>Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced genetic material, have been discussed in Risk scenarios 1 to 3, and were not considered identified risks.</p> <p>The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the scale and duration of the trial would limit the potential for adverse effects. The public and livestock would not be intentionally exposed as the GM plant material will not be used as human food, animal feed or in therapeutic goods.</p>
			C, E, S	GM crops may contaminate non-GM crops, including outcrossing of introduced genes to related species of food plants and weeds. This is a threat to the livelihood of non-GM and organic farmers, and to the genetic diversity of food crops.	<p>This trial is of small size and limited duration, and licence conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment. These include a requirement to maintain a 200 m isolation zone around the trial site in which no sexually compatible species, including cultivated wheat or barley, can be grown.</p> <p>Economic and marketability issues are outside the scope of assessments required by the <i>Gene Technology Act 2000</i>.</p>
			H	Concerned that there is no independent testing of the safety of GM crops, and that Monsanto funds CSIRO.	The Regulator uses a variety of information sources to assess applications for the release of GMOs, including peer reviewed literature, biological principles, experts and technical reports, data generated by applicants, and broad consultation.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			AP	Concerned that the OGTR always issues a licence for GM crops upon receipt of an application.	<p>The <i>Gene Technology Act 2000</i> (the Act) requires the Regulator to consider risks to human health and safety and the environment posed by or as a result of gene technology and to manage any identified risks.</p> <p>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 on whether or not to issue a licence to deal with a GMO. The decision is based upon a RARMP prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the <i>Risk Analysis Framework</i> and are finalised following consultation with a wide range of experts, agencies and authorities, and the public.</p>
			EN	Concerned that GM crops are harmful to soil organisms, soil health, bees and other pollinating insects, and develop susceptibility to plant pests and diseases.	<p>The RARMP for this limited and controlled release concludes that risks to human health and the environment are negligible. Licence conditions have been imposed to restrict the release to the size, locations and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.</p> <p>The RARMP also outlines future research requirements should the applicant seek a licence for a larger scale or commercial release. The research requirements include further information on the potential allergenicity or toxicity of the GM plants, additional phenotypic characterisation (including characteristics indicative of weediness and plant disease susceptibility), and characterisation of the genetic material in the plants.</p>

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			HGT	Concerned about HGT to gut bacteria in humans, animals, bees and insects that have contact with or consume GM crops.	<p>Risks to human health and safety and environment arising as a result of HGT were assessed in the RARMP (risk scenario 6). Important considerations were that:</p> <ul style="list-style-type: none"> the sequences are already widely present in the environment, and so are naturally available for HGT from these other sources assessment of the potential toxicity or allergenicity of the expressed proteins did not identify a risk for human health and safety or the environment. <p>Given the rarity of HGT occurring and that adverse consequences are unlikely, it was concluded that the potential for an adverse outcome was not an identified risk.</p>
			EN, H	Concerned about the safety record of GM crops. Concerned that continued release may lead to large-scale health problems for people and animals.	<p>DIR 099 is a limited and controlled release for the purpose of conducting experiments, including field characterisation of the GM wheat and barley lines. The RARMP concluded that this limited and controlled release of GM wheat and barley poses negligible risks to people and the environment.</p> <p>The Regulator has imposed a range of measures to minimise exposure to the GMOs and their genetic material including preventing use in food and feed and to limit the trial to the proposed size, locations and duration.</p>
			E	Concerned that the continued release of GM crops may lead to loss of production and loss of markets.	<p>Economic issues are outside the scope of assessment required by the <i>Gene Technology Act 2000</i>.</p>

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6	I	x	H	Irresponsible and risky to conduct open air plantings of GM wheat and barley as there have been no studies on the toxicity and allergenicity of the GM wheat and barley proposed for release.	<p>DIR 099 is a limited and controlled release for the purpose of conducting experiments, including field characterisation of the GM wheat and barley lines.</p> <p>The Regulator has imposed a range of measures to minimise exposure to the GMOs and their genetic material including preventing use in food and feed and to limit the trial to the proposed size, locations and duration.</p> <p>The RARMP concluded that this limited and controlled release poses negligible risks to people or the environment.</p> <p>Furthermore, the RARMP outlines future research requirements should the applicant seek a licence for a larger scale or commercial release. The research requirements include further information on the potential allergenicity or toxicity of the GM plants.</p>
			C	The trial poses a degree of risk as the control measures listed in the RARMP are not able to completely contain the GM trial. The example of Canadian flax being contaminated by a de-registered GMO variety indicates the ability of [GM] plants to persist in the environment.	<p>DIR 099 is a limited and controlled release for the purpose of conducting experiments.</p> <p>The Regulator has imposed a range of measures to limit the trial to the proposed size, locations and duration and require controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, including post harvest inspections of the trial sites to ensure there is no persistence of the GMOs..</p> <p>The RARMP concluded that this limited and controlled release poses negligible risks to people or the environment.</p>

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			UE	<p>GM technology routinely creates unintended proteins or alters existing proteins. There are likely to be unintended effects (short- and long-term) which may not be detected or controlled.</p> <p>RARMP does not explain how minimised pleiotropic adverse effects which are impossible to predict constitute no risk, especially when there is no knowledge of what these risks may be.</p>	<p>As discussed in Section 2.5 of Chapter 2, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant. While the GM wheat and barley lines have not undergone thorough phenotypic analysis, it is expected that substantial unintended changes would have been detected in the time these lines have been under development.</p> <p>The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the GM plant material will not be used as human food, animal feed or in therapeutic goods and the scale and duration of the trial would limit the potential for adverse effects.</p> <p>The Act requires that the licence holder informs the Regulator of any unintended effects of the dealings authorised by the licence.</p> <p>The RARMP concluded that this limited and controlled release of GM wheat and barley poses negligible risks to people and the environment.</p>
			F	<p>Concerned about the safety of GMO foods, given the lack of independent research using longitudinal studies.</p>	<p>This is a limited and controlled release of GM wheat and barley and the licence stipulates that products from the trial cannot be used in human food, animal feed or in therapeutic goods. The regulation of GM foods is the responsibility of FSANZ.</p>