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

Risk Assessment and Risk Management Plan for

DIR 112

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

Applicant: Commonwealth Scientific and Industrial Research Organisation

March 2012

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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 112) from the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The licence authorises dealings involving the limited and controlled release of genetically modified (GM) wheat and 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 public[1].

The application

CSIRO has applied for a licence for dealings involving the intentional release of up to 118 lines of GM wheat and 40 lines of GM barley on a limited scale and under controlled conditions. Of the wheat lines, 23 have been genetically modified for altered grain composition, while the remainder, and all the barley lines, have been genetically modified for enhanced nutrient (nitrogen) utilisation efficiency. The trial is authorised to take place at a site in the New Genes for New Environments (NGNE) facility, near Merredin, Western Australia (WA), between May 2012 and June 2015. This facility is operated by the Department of Agriculture and Food, Western Australia (DAFWA).

Some of the GM wheat lines contain part of a gene derived from wheat, which is expected to suppress the function of the corresponding endogenous gene in the GM plants, resulting in altered starch composition in grains. The remainder of the GM wheat lines, and all of the GM barley lines, contain a gene from barley that is expected to enhance nitrogen utilisation efficiency. In addition, most of the GM wheat and barley lines contain one of two selectable marker genes, derived from a common gut bacterium. These genes were used to select genetically modified plant cells and plants during initial development of the GM plants in the laboratory. Some of the GM wheat and barley lines contain no selectable marker gene.

The primary purpose of the three year field trial is to assess whether the respective genetic modifications result in increased biomass and yield of the GM plants with respect to unmodified plants. Further, some grain will be retained each year for replicated field trials in years two and three. Finally, the trial will provide material to assess the impact of the respective genetic modifications on grain protein composition, dough making properties and end product quality.

A number of the GM wheat and barley lines authorised for release have previously been approved by the Regulator for field trial under other licences. The risk assessments conducted for those applications included consideration of all the genes and partial gene sequences that are the subject of this licence.

[1] More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (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>.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals and current scientific/technical knowledge. Advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP was also considered. 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 those that warrant detailed characterisation are determined. This process is described as risk identification.

Six 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 GMOs. 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 six risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant and considering both the short and long term, did not identify any risks that could be greater than negligible. Therefore, they did not warrant further detailed assessment.

Risks to the health and safety of people, or the environment, from the proposed release of the GM wheat and barley lines into the environment are 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 licence conditions.

As none of the six 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 proposed release to the size, location and duration 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 1.0 ha per year at one site between May 2012 and June 2015, inclusive. 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 or other specific condition; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

Conclusions of the consultation RARMP

The risk assessment concluded that this limited and controlled release of up to 118 GM wheat lines and 40 GM barley lines on a maximum total area of 1 ha per year over three growing seasons in the shire Merredin (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, location 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

Act	The *Gene Technology Act 2000*
AlaAT	Alanine aminotransferase
APHIS	Animal and Plant Health Inspection Service (United States Department of Agriculture)
ATP	Adenosine triphosphate
CaMV	Cauliflower mosaic virus
CCI	Confidential Commercial Information as declared under section 185 of the *Gene Technology Act 2000*
CSIRO	Commonwealth Scientific Industrial Research Organisation
DAFWA	Department of Agriculture and Food, Western Australia
DIR	Dealings involving Intentional Release
EFSA	European Food Safety Authority
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GUS	β-glucuronidase
GWD	Glucan water dikinase
ha	Hectare
hpt	Hygromycin phosphotransferase
m	Metres
NGNE	New Genes for New Environments, a field trial facility established by DAFWA
nos	Nopaline synthase
nptII	Neomycin phosphotransferase type II gene
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment level 2
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RNAi	RNA interference
siRNA	Short interfering RNA
T-DNA	Transfer DNA
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*

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Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 112) from the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The licence authorises dealings involving the limited and controlled release of genetically modified (GM) wheat 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 public[2].

The application

CSIRO has applied for a licence for dealings involving the intentional release of up to 118 lines of GM wheat and 40 lines of GM barley on a limited scale and under controlled conditions. Of the wheat lines, 23 have been genetically modified for altered grain composition, while the remainder, and all the barley lines, have been genetically modified for enhanced nutrient (nitrogen) utilisation efficiency. The trial is authorised to take place at a site in the New Genes for New Environments (NGNE) facility, near Merredin, Western Australia (WA), between May 2012 and June 2015. This facility is operated by the Department of Agriculture and Food, Western Australia (DAFWA).

The GM wheat lines with altered grain composition contain a genetic modification for the down-regulation of the expression of the glucan water dikinase gene. Such a change in the expression of this gene is expected to alter the starch composition of the grain. Those GM wheat and barley plants genetically modified for enhanced nitrogen utilisation efficiency contain an alanine aminotransferase gene. The derived protein product of this gene is involved in the shuttling of carbon and nitrogen in plants. Most of the GM plants contain selectable marker genes.

The primary purpose of the three year field trial is to assess whether the respective genetic modifications result in increased biomass and yield of the GM plants with respect to unmodified plants. Further, some grain will be retained each year for replicated field trials in years two and three. Finally, the trial will provide material to assess the impact of the respective genetic modifications on grain protein composition, dough making properties and end product quality.

A number of the GM wheat and barley lines authorised for release have previously been approved by the Regulator for field trial under other licences. The risk assessments conducted for those applications included consideration of all the genes and partial gene sequences that are the subject of this licence.

[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>.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals and current scientific/technical knowledge. Advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP was also considered. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

Two 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 those that warrant detailed characterisation are determined. This process is described as risk identification.

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.

Six 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 lines; 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.

The characterisation of the six risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant and considering both the short and long term, did not identify any risks that could be greater than negligible. Therefore, they did not warrant further detailed assessment. The principal reasons for this include:

- limits on the size, location 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
- the genetic modifications are unlikely to give rise to adverse affects on human health and safety or the environment
- widespread presence of the same and similar genes and gene sequences in the environment and lack of evidence of harm from them
- limited ability and opportunity for the GM wheat and barley plants to transfer the introduced genes to commercial wheat and barley crops or other sexually related species
- the potential of the GM wheat and barley to spread and persistence would be restricted by a range of environmental factors that restrict non-GM wheat and barley
- none of the GM plant materials or products will enter human food or animal feed supply chains.

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.

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 proposed licence conditions.

As none of the six 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 proposed 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, location and duration 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 1.0 ha per year at one site between May 2012 and June 2015, inclusive. 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 or other specific condition; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

Licence conditions

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

- limit the release to a total area of up to 1 ha per growing season at one site in the fenced NGNE facility between May 2012 and June 2015
- surround the site by a 10 m monitoring zone in which sexually compatible plants must be destroyed before flowering or prevented from flowering
- surround the monitoring zone with a 190 m isolation zone in which no other crops of wheat and barley may be grown, and where sexually compatible species plants must be destroyed before flowering or prevented from flowering
- the monitoring zone must be maintained in a manner that does not attract or harbour rodents, and if rodent activity is detected in the site, measures must be implemented to control the rodents
- harvest the GM wheat and barley plant material separately from other crops
- clean the areas and equipment after use
- apply measures to promote germination of any wheat and barley seeds that may be present in the soil after harvest, including irrigation and tillage
- monitor 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
- all material from plants, whether GM or non-GM, grown within the site of the trial must be treated as if is GM
- destroy all GM plant material not required for further analysis or future trials
- if other sexually compatible GMOs are later approved and grown in the site, seed derived from concurrent trials must not be used for later commercial development
- not allow GM plant material to be used for human food or animal feed
- transport material from the GMOs in accordance with the Regulator's guidelines.

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. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated

with the use of gene technology. However, dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority, Therapeutic Goods Administration, National Industrial Chemicals Notification and Assessment Scheme and Australian Quarantine Inspection Service[3].

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

In addition, dealings authorised by the Regulator may be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

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 includes:

- additional data on the potential toxicity and allergenicity of plant materials from the GM wheat and barley lines
- additional phenotypic characterisation of the GM wheat and barley lines, particularly with respect to traits that may contribute to weediness, including tolerance to environmental stresses and disease susceptibility
- additional molecular and biochemical characterisation of the GM wheat and barley lines.

Suitability of the applicant

The Regulator has assessed the suitability of CSIRO to hold a DIR licence as required by the Act. CSIRO is considered suitable as the Regulator is satisfied that no relevant convictions have been recorded, no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment, and the organisation has the capacity to meet the conditions of the licence.

Conclusions of the consultation RARMP

The risk assessment concluded that this proposed limited and controlled release of up to 118 GM wheat lines and 40 GM barley lines on a maximum total area of 1 ha per year over three growing seasons in the shire Merredin (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, location 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.

[3] 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>.

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).

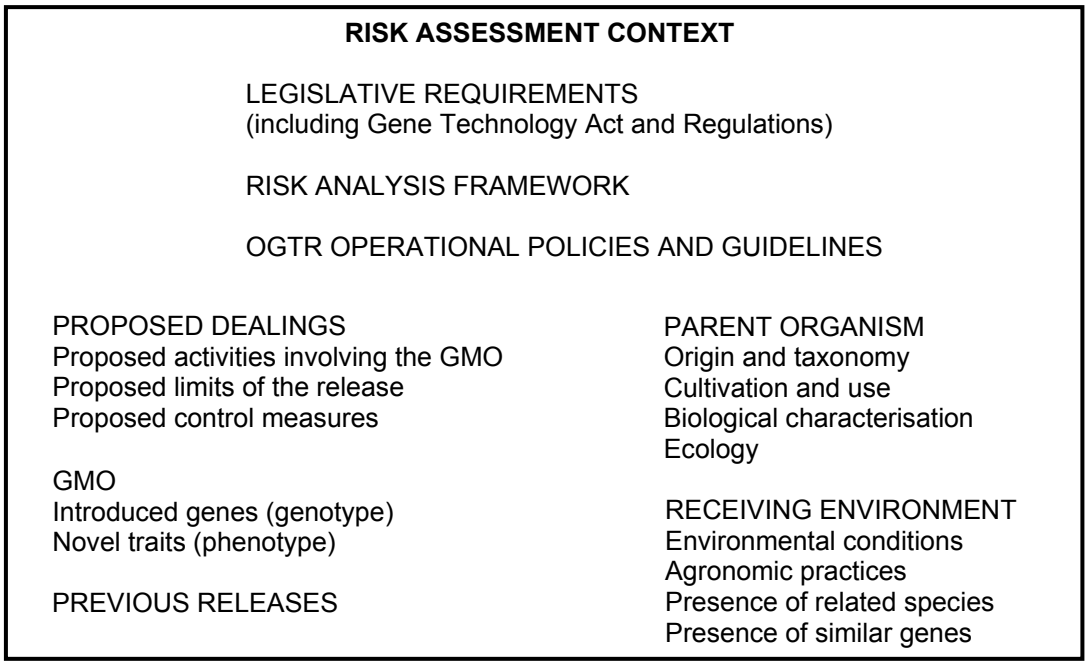

Figure 1. Parameters used to establish the risk assessment context

2. The risk assessment context is developed within the framework of the *Gene Technology Act 2000* (the Act) and Gene Technology Regulations 2001 (the Regulations, Section 2), the *Risk Analysis Framework*, and operational policies and guidelines available at the OGTR website <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 genetically modified organisms (GMOs), 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 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 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 on the size, location 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.

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, 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. Forty-four submissions were received from the public and their considerations are summarised in Appendix B.

7. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a 'significant risk' to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d). 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 118 lines[4] of genetically modified (GM) wheat and 40 lines of GM barley into the environment under limited and controlled conditions.

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

- conducting experiments with the GMOs
- propagating, growing, raising or culturing the GMOs
- breeding 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.

3.1 The proposed activities

11. The applicant has stated that the proposed field trial is an extension of the part of DIR 099 that is at present being conducted at the New Genes for New Environments (NGNE) facility at Merredin in WA (the licence for DIR 099 also allows for a trial at Narrabri, NSW). This facility is run by the Department of Agriculture and Food, Western Australia (DAFWA). Although some of the genetic constructs used to generate the GM plants are identical to those assessed in DIR 099 (and also DIR 092 and DIR 094), some are new. Nevertheless, the latter constructs are designed to induce the same biochemical changes to the GM plants as the previous constructs.

12. There are three objectives with the proposed field trial:

- to assess if the genetic modifications of wheat and barley result in increased biomass and yield in the GM plants
- to produce sufficient grain to allow replicated field trials in years two and three

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

- to provide sufficient grain to assess the impact of the genetic modifications on protein composition, and if there are any changes in grain composition, dough making properties and end product quality.

13. DAFWA intends the NGNE facility to be a multi-user facility where, subject to approval by the Regulator, various GM trials may take place simultaneously. This has been taken into account in establishing the risk assessment context.

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

14. The release is proposed to take place at one site, the NGNE facility, located in the shire of Merredin in WA. This facility has an area of 5 ha, but the applicant does not anticipate using more than 1 ha per year. The duration of the trial is proposed to be from May 2012 to June 2015.

15. Only trained and authorised staff would be permitted access to the proposed location(s) in the NGNE facility.

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

16. 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. These are:

- isolating the site (the NGNE facility) from any other wheat and barley by at least 200 m (with the exception of other GM wheat and barley approved under a separate licence by the Regulator)
- structuring the trial similar to the licences DIRs 092, 093 and 094, where the minimum distance between trials under different GMO licences within a single site is 4 m, and the entire site is surrounded by a monitoring zone
- the site is surrounded by a livestock proof fence and bird net
- implement a rodent control program
- harvest either by hand, using a small mechanical harvester or using a plot harvester, and cleaning equipment prior to removal from the site
- waste material derived from the harvesting will be left on site and tilled back into the soil, along with any stubble remaining from the harvest.
- monitoring the trial site after harvest at least once every 35 days for a period of 2 years, and destroying volunteer plants prior to flowering
- not allowing GM material to be used for human food or animal feed.

17. These controls (see Figure 2), and the limits outlined above, 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 3.1.1.

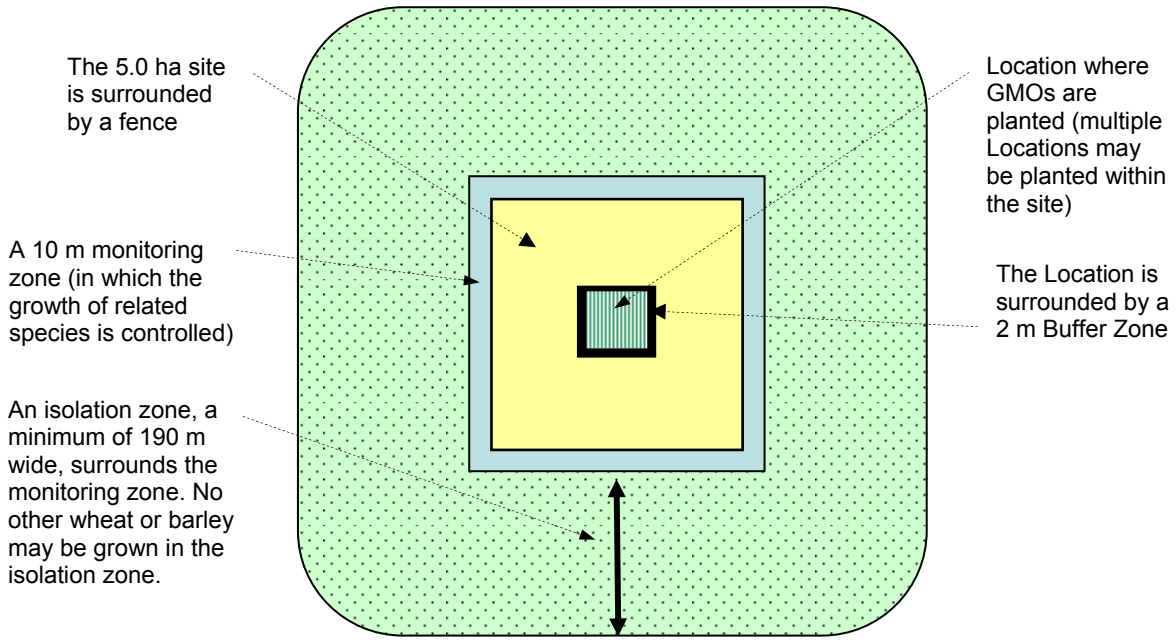

Figure 2. Schematic diagram of some of the proposed controls, reflecting those specified by the applicant and those in the licences for DIRs 092, 093, 094 (not drawn to scale)

Section 4 The parent organism

18. 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, Tasmania, southern South Australia and southern Western Australia (OGTR 2008b).

19. The wheat cultivars used to generate the GM wheat lines are Bobwhite, Frame, and Gladius. 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 genetically modify and has previously been used in conventional (non-GM) wheat breeding programs. The cultivars Frame and Gladius are commercially cultivated in Australia and have some degree of drought tolerance as they have been bred for Australian conditions.

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

21. 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)*, which were produced to inform the risk assessment process for

licence applications involving GM wheat and barley plants (OGTR 2008a; OGTR 2008b). These documents are available at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1.

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

5.1 Introduction to the GMOs

22. The applicant proposes to release up to 118 lines of GM wheat and 40 lines of GM barley into the environment under limited and controlled conditions. As mentioned above, some of the GM lines are the same as those assessed in DIR 092, DIR 094 and DIR 099. All of the GM lines have also been assessed in relation to application DIR 111, which authorises the release of these and other GM wheat and barley lines in the Australian Capital Territory.

23. Details of the GM wheat and barley lines are given in Table 1. Lines that are currently being trialled under other DIR licences are also identified.

24. The GMOs are classified into two groups, designated Group 1 and Group 2, on the basis of their genetic modifications and the respective desired traits. These groups are the same as Group 1 and Group 2 for application DIR 111.

Group 1:

Up to 23 of the GM wheat lines contain introduced partial sequences of the wheat glucan water dikinase (*GWD*) gene. *GWD* is involved in determining grain qualities (starch composition) important for dough making and human nutrition. The gene construct containing partial *GWD* gene sequences is designed to suppress the expression of the *GWD* gene in the endosperm, through a mechanism known as RNA interference (RNAi) (http://www.pi.csiro.au/RNAi; (Millar & Waterhouse 2005), thereby altering grain composition. In addition, these GM wheat lines contain the antibiotic resistance gene *nptII*, encoding the enzyme neomycin phosphotransferase type II, derived from *Escherichia coli*. The *nptII* gene confers resistance on the GM plant to antibiotics such as kanamycin or neomycin.

Group 2:

Up to 95 of the GM wheat and 40 of the GM barley lines contain an introduced alanine aminotransferase (*AlaAT*) 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. Most of the GM wheat lines contain either the *nptII* or *hpt* (hygromycin phosphotransferase) antibiotic resistance genes, while half of the GM barley lines contain the *hpt* gene. The remaining GM wheat and barley lines in this group have no antibiotic selection marker.

25. The partial *GWD* gene constructs in wheat would be expressed by a wheat promoter, while the *AlaAT* gene would be expressed by the use of a promoter from rice. The selection marker genes are expressed by promoters derived from maize or cauliflower mosaic virus (CaMV, a plant virus). Transcription termination regions for the introduced genes are derived from a CaMV and *Agrobacterium*. Regulatory elements are discussed further in Section 5.5 of this Chapter.

Table 1. Genes and constructs used to generate the GM wheat and barley lines proposed for release

Gene of interest (Source organism[b])	Anticipated effect of introduced gene	Construct	Parental cultivar	Max no of GM lines	Previous DIR licence
Group 1 - Glucan water dikinase RNAi					
RNAi targeting *GWD* (*Ta*)	Suppression of *GWD* gene in the endosperm leading to altered grain composition	pBX17GWDcasNOT	Bobwhite 26	3	092, 099
		pBX17GWDtwin	Bobwhite 26	10	New
		pBX17GWDdrb	Bobwhite 26	10	
Group 2 - Alanine aminotransferase					
AlaAT (*Hv*)	Increase plant biomass and yield through improved nitrogen use efficiency	pARC425	Bobwhite 26	3	094, 099
		pMDC/POsAnt1 HvAlat Nos	Bobwhite 26	15	094. 099
			Frame	2	094, 099
			Gladius	15	New
			Golden Promise	20	094, 099
		pARC316	Gladius	20	New
		pARC316drb	Bobwhite 26	20	
		pARC425drb	Bobwhite 26	20	
		pMDC-MF1/POsAnt1 HvAlat Nos	Golden Promise	20	

[a] *Hv=Hordeum vulgare; Ta=Triticum aestivum.*

5.2 The introduced RNAi constructs and their associated effects (Group 1)

26. In the current application, some wheat lines were genetically modified using RNAi constructs (Group 1, Table 1). The RNAi constructs contain fragments, rather than entire coding sequences, of the target gene. Expression of the RNAi constructs is designed to suppress the expression of the target gene. No proteins are encoded by the introduced RNAi constructs.

27. RNAi is a mechanism that occurs naturally in plants and other organisms and functions to control the expression of specific genes and remove aberrant RNA molecules (Agrawal et al. 2003). Systemic silencing is generally difficult to achieve; in the case of the GM wheat lines proposed for release, organ specific silencing is achieved through use of an endosperm specific promoter. In plants, RNAi constructs can also give rise to silencing of closely matching non-target sequences expressed in the same cells. Homology of 95% is generally required for any silencing to have an effect, and increases with greater stretches of homology to the non-target gene. Homology of as little as 20 nucleotides (nt) can give rise to non-target silencing, reviewed by Small (2007). Specific silencing of single genes of a gene family can be achieved if specific regions of the respective genes are targeted. Conversely, using highly conserved regions can result in effective silencing of several members of a gene family (Miki et al. 2005).

28. When an introduced RNAi construct is expressed, the self complementary (sense and anti-sense) segments of the derived RNA, corresponding in sequence to at least part of the target gene, anneal to each other to produce a double stranded RNA, and the intervening intron is spliced out. These duplexed regions are then cut into 21 nucleotide (nt) fragments, called short interfering RNAs (siRNAs) by the enzyme Dicer. One strand of a siRNA duplex is incorporated into a protein complex containing a ribonuclease, where it acts as a template to bind mRNAs with a complementary sequence and degrade them. Therefore, mRNAs of target genes are degraded, preventing their translation and leading to the "silencing" of these genes.

5.2.1 The Glucan Water Dikinase (GWD) gene

29. The Group 1 GM wheat lines have an introduced gene expressing siRNA targeting the *GWD* gene. The *GWD* gene encodes α-glucan water dikinase (GWD), a starch granule-bound enzyme, which catalyses the ATP dependent phosphorylation of α-glucans. Glucose residues of amylopectin can be phosphorylated on either the C3 or the C6 positions. Experiments with *Arabidopsis* have shown that GWD phosphorylates the C6 position, and a second enzyme, phosphoglucan water dikinase (PWD), phosphorylates the C3 position (Kotting et al. 2005; Ritte et al. 2006). However,

the activity of PWD is dependent on the preceding action of GWD, implying that any defect in the activity of GWD results in a reduction in the phosphorylation of both glucosyl positions. Phosphatase enzymes likely reverse the effects of these kinase enzymes (Hejazi et al. 2010). Phosphorylation of starch is thought to occur during both starch synthesis and starch degradation (Blennow et al. 2002).

30. Studies of *GWD* mutants in *Arabidopsis* and potato indicate that starch phosphorylation is an important part of starch degradation, as these mutants have a reduced rate of starch degradation, and accumulate excess leaf starch as a result (reviewed by Smith et al. 2005). The level of phosphorylation of starch varies among species, with potato tuber starch being relatively highly phosphorylated (0.5%) compared to cereal starches (less than 0.01%) (reviewed by Mikkelsen et al. 2004). Starch phosphorylation contributes to processing qualities such as pasting, gel strength and stickiness.

Effects of GWD silencing

31. The applicant has provided test results to show that for some GM wheat lines containing a GWD RNAi construct, GWD protein can no longer be detected. In these lines, the amount of phosphate in the starch is greatly decreased in comparison to the parental wheat cultivar. Except for the decrease in phosphate, no structural or quantitative modifications of the starch composition have been observed.

32. The applicant states that the reduction in starch phosphate in the GWD RNAi lines may give rise to altered processing properties, particularly in relation to pasting, for which starch phosphorylation is known to be an important factor (reviewed by Blennow et al. 2002). Nutritional value may be altered in wheat products derived from the GWD RNAi lines due to altered starch degradation. The changes observed in the GWD RNAi lines are largely within the range of starch phosphorylation values observed for wheat (Ral et al. 2008).

33. Data supplied by the applicant showed that GM wheat lines carrying the GWD RNAi construct display an increase in plant vigour at early growth stages compared to non-GM sibling plants, measured by leaf area (an increase between 40-80%) and dry weight of the above ground tissues (an increase between 20-50%) depending on the line. Mature GM plants exhibit various increases in biomass, grain weight and yield. Field trials for some of the GM wheat lines under licence DIR 092 further confirmed this increase in plant vigour at both early growth and mature stages under field conditions. However, the GM lines produced fewer tillers than the non-GM controls.

34. GWD RNAi lines also show an increase in seed production compared to the parental non-GM wheat variety, the result of both an increased number of heads per plant and increased seed weight. However, the increased seed weight is within the natural range observed in 372 diverse wheat lines studied by Bordes et al. (2008).

5.2.2 Toxicity/allergenicity associated with the introduced GWD RNAi constructs

35. In the GM wheat lines modified for grain composition, the use of RNAi has the direct effect of reducing the expression of endogenous transcripts of the target genes, without the expression of novel proteins. GWD RNAi lines have a decreased level of starch phosphorylation, resulting in the reduction of the amount of phosphate in starch.

36. 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 may need to be conducted if approval was sought for the GMOs or their products were to be considered for human consumption in Australia.

5.3 The introduced *AlaAT* gene, the encoded protein and associated effects (Group 2)

37. 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 provided in the RARMP for DIR 094 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>).

38. All GM wheat and barley lines in Group 2 contain an introduced barley *AlaAT* gene (Muench & Good 1994). The *AlaAT* gene encodes a cytoplasmic alanine aminotransferase (AlaAT), with a deduced amino acid sequence of 482 residues. AlaAT catalyses the reversible reaction of pyruvate and glutamate to alanine and 2-oxoglutarate (α-ketoglutarate). AlaAT has been well studied in animals, where it is most commonly associated with the liver. In plants, two to six AlaAT isozymes have been identified and localised to various subcellular locations.

39. The reaction catalysed by AlaAT, involving four major metabolites, acts as a link between primary carbon metabolism and the biosynthesis of a number of amino acids (Liepman & Olsen 2003). AlaAT also plays more specialised roles in the C4 pathway of photosynthesis (Son & Sugiyama 1992) and in plant responses to hypoxia (Good & Crosby 1989; Ricoult et al. 2006; Miyashita et al. 2007) and nitrogen stress (Muench et al. 1998). AlaAT has also been implicated in seed storage protein production in rice (Kikuchi et al. 1999). Although alanine is accumulated in response to drought in some plants, this accumulation does not coincide with an induction of AlaAT (Good & Zaplachinski 1994). Similarly AlaAT does not respond to salt, cold or heat stress in maize (Muench et al. 1998).

40. In *Brassica napus* (canola), over-expression of the barley *AlaAT* gene under the control of a canola root specific promoter resulted in an increased biomass and seed yield under low nitrogen conditions in both the field and laboratory (Good et al. 2007). In the field trials the increase in seed yield was 33-42%. When grown hydroponically, the GM canola plants over-expressing *AlaAT* had higher levels of alanine in the roots, and less glutamine and glutamate in the shoots, than control plants. In response to these altered amino acid levels, the GM canola plants increased the rate of nitrate influx (Good et al. 2007).

41. GM rice plants over-expressing the same barley *AlaAT* gene also showed an increase in biomass (30-34%) and grain yield (31-54%) in the laboratory (Shrawat et al. 2008). In this study, expression was driven by the *OsAnt1* promoter, which shows strong expression in roots, and plants were well supplied with nitrogen. The increase in biomass primarily depended on the accelerated formation of tillers. Hydroponically grown GM rice plants also showed more vigorous growth, produced bushier, finer and more branched root systems, showed changes in the amount of several amino acids, and had higher total nitrogen content than control plants. For example, glutamine, glutamate and asparagine levels were increased in both roots and shoots of the GM plants, whereas arginine levels were only increased in shoots. The increase in total nitrogen content was attributed to an increase in nitrogen uptake efficiency (Shrawat et al. 2008).

5.3.1 Toxicity/allergenicity associated with the introduced AlaAT *gene*

42. The introduced *AlaAT* gene was isolated from barley. *AlaAT* genes, and the encoded enzymes, are found in humans, animals, plants, fungi and archaea (Jing & Zhang 2011). Therefore people are widely exposed to the introduced gene, the encoded protein and their homologs. The introduced protein is not expected to be toxic or allergenic. However, *AlaAT* from the cephalochordate *Branchiostoma japonicus* has been shown to have toxic properties against some gram negative bacteria such as *E.coli* (Jing & Zhang 2011).

43. It is possible that the GM wheat and barley plants expressing the introduced *AlaAT* gene will produce altered levels of some metabolites in both below and above ground tissues. For example, levels of the metabolites involved in the reaction catalysed by AlaAT (pyruvate, glutamate, alanine

and 2-oxoglutarate) could be altered, as well as amino acids such as glutamine and asparagine. These metabolites are ubiquitous in nature and consumed widely by humans in both natural products and dietary supplements, although as with most substances, very high levels of intake are not recommended. In particular, high levels of glutamate (in the order of 1000 mg/kg body weight) have been associated with neurotoxicity in animals (FSANZ 2003; Olney & Ho 1970; Barinaga 1990). Glutamate is commonly added to processed foods in the form of mono-sodium glutamate (MSG), the safety of which has been debated for decades (Barinaga 1990). However, MSG still remains on the United States Food and Drug Administration list of additives generally regarded as safe[5].

5.4 The antibiotic resistance marker genes (*nptII* and *hpt)* and the encoded proteins

44. All of the GM wheat lines in Group 1 and up to 43 in Group 2 contain the antibiotic resistance gene *nptII*. This gene, encoding the enzyme neomycin phosphotransferase type II, was derived from *E. coli* and confers resistance on the GM plant to antibiotics such as kanamycin or neomycin.

45. Up to 32 GM wheat lines in Group 2, and up to half of the GM barley lines in Group 2, contain the *hpt* gene from *E. coli,* which confers resistance to the antibiotic hygromycin B. The *hpt* gene, also called *hph* gene in some literatures, encodes the hygromycin phosphotransferase (HPT or HPH) enzyme which catalyses the phosphorylation of the 4-hydroxy group on the hyosamine moiety, thereby inactivating hygromycin (Rao et al. 1983).

46. Both the *nptII* and *hpt* genes were used as selectable markers in the early laboratory stages of development of the plants to enable selection of plant cells containing the desired genetic modification.

47. In Group 2, a minority of the GM wheat lines (up to 20), and half of the GM barley lines have no plant selection marker.

48. The antibiotic selectable marker genes *nptII* and *hpt* were isolated from the common gut bacterium *E. coli*. These genes have been used extensively as selectable markers in the production of GM plants (Miki & McHugh 2004). As discussed in previous DIR RARMPs, regulatory agencies in Australia and in other countries have assessed the use of these genes in GM plants as not posing a risk to human or animal health or to the environment.

49. For the *nptII* gene, more detail can be found in the RARMPs for DIR 070/2006 and DIR 074/2007 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1> or by contacting the OGTR). 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).

50. For the *hpt* gene, more detail can be found in the RARMPs for DIR 073/2007 and DIR 077/2007 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>). 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] Source: http://www.cfsan.fda.gov/~dms/opa-appa.html, accessed 13 March 2009

5.5 The regulatory sequences

51. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct gene transcription. Also required for gene expression in plants is a transcription termination region, including a polyadenylation signal. Other sequences, such as introns, may contribute to the expression pattern of a given gene. Regulatory sequences used in the GM wheat and barley lines for controlling the expression of genes of interest are detailed below.

5.5.1 Regulatory sequences for expression of the RNAi constructs (Group 1)

52. Expression of the introduced RNAi constructs is driven by the Bx17 promoter, which derives from a wheat gene encoding a high molecular weight glutenin protein. During the middle to late stages of development of the grain, this type of storage protein is found exclusively in the endosperm tissue. This promoter is endosperm specific, providing an ideal candidate for the specific expression of genes in that tissue (Lamacchia et al. 2001; Oszvald et al. 2007a; Oszvald et al. 2007b).

53. Separation of the sense and antisense arms of RNAi constructs with a spliceable intron has been shown to increase the effectiveness of silencing (Smith et al. 2002). The intron used in the GWD RNAi constructs is from the rice starch branch enzyme I gene (GeneBank accession number D10838).

54. The mRNA termination region for the RNAi construct in the GM wheat of this Group is derived from the nopaline synthase (*nos)* gene from *Agrobacterium tumefaciens* (Depicker et al. 1982; Bevan 1984). The *nos* terminator has been used in a wide variety of constructs used for plant genetic modifications (Reiting et al. 2007).

5.5.2 Regulatory sequences for expression the AlaAT gene (Group 2)

55. In Group 2 GM wheat and barley lines, the *AlaAT* gene is under the control of a tissue specific promoter *OsAnt1*. This promoter is derived from a rice gene coding for a putative aldehyde dehydrogenase, the protein being designated antiquitin (Fong et al. 2006). Fusion of this promoter to the GUS reporter gene demonstrated that it drove expression mainly in the root epidermis of rice, but was also active in leaf vascular tissue (Shrawat et al. 2008). In the same study, Western blot analysis and enzyme assays of the expression of the barley *AlaAT* gene under the control of this promoter in rice showed it was active in root and shoot tissues. The *btg26* promoter, derived from a *Brassica* homologue of the rice gene, has been used to express the barley *AlaAT* gene in *B. napus*, likewise demonstrating expression mainly in the roots (Good et al. 2007).

56. As with the GWD gene constructs, the mRNA termination region is derived from the *nos* gene of *Agrobacterium tumefaciens.*

5.5.3 Regulatory sequences for expression of the selectable marker genes

57. Expression of the *nptII* gene in GM wheat plants is controlled by the CaMV 35S gene promoter (Odell et al. 1985), in combination with either the *nos* terminator or the CaMV 35S terminator.

58. Expression of the *hpt* gene in GM wheat and barley plants is controlled by either the CaMV 35S promoter or the maize ubiquitin-1 (*Ubi-1*) gene promoter (Christensen et al. 1992), in both cases in combination with the *nos* terminator.

59. Both the CaMV 35S promoter and the *Ubi-1* promoter are constitutive and direct the marker genes to be expressed in most plant tissues and throughout the plant lifecycle.

5.6 Method of genetic modification

60. Two different methods were used to generate the GM wheat and barley lines for the proposed release – biolistic transformation (some wheat lines in Groups 1 and 2) or *A. tumefaciens*-mediated transformation (some wheat lines in Groups 1 and 2, and all barley lines in Group 2).

61. Biolistic transformation (Pellegrineschi et al. 2002) involved coating very small gold particles with two transformation constructs (in the form of plasmid or DNA fragment), one containing a plant selectable marker and a second containing the gene of interest. The particles were then 'shot' into intact immature embryos from *T. aestivum* cultivars Bobwhite, Frame or Gladius. Genetically modified plant tissues were recovered by survival on tissue culture media containing one of the selective agents geneticin (G418; for *nptII* selectable marker) or hygromycin (for *hpt* selectable marker).

62. *A. tumefaciens*-mediated transformation was used to generate the GM barley lines, as well as some GM wheat 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* to the plant genome. 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).

63. To generate the GM wheat and barley lines in the current application, immature embryos from the wheat cultivars Bobwhite or the barley cultivar Golden Promise were infected with *A. tumefaciens* carrying the gene of interest (Tingay et al. 1997; Matthews et al. 2001). Following co-cultivation and callus induction steps, the wheat and barley calli were induced to form plantlets on media containing an antibiotic (such as Timentin) to eliminate *A. tumefaciens* and one of the selective agents G418 (for *nptII* selectable marker) or hygromycin (for *hpt* selectable marker).

64. 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.

5.7 Characterisation of the GMOs with RNAi constructs (Group 1)

5.7.1 Stability and molecular characterisation the GMOs

65. *Group 1 – GMOs modified for altered grain composition.* The polymerase chain reaction (PCR) has demonstrated that the inserted genetic construct is stably inherited over five generations. However, the copy number in plants has not been ascertained.

66. *Group 2 – GMOs modified for enhance nitrogen utilisation efficiency.* The presence of the genetic construct has been confirmed by the use of the PCR. However, as above, the copy number in plants has not been ascertained.

67. The number of gene copies integrated into a plant genome varies depending on the method of introduction. Copy number of an introduced gene following biolistic transformation usually varies from 1 to more than 20 (Pawlowski & Somers 1996), whereas 1-3 copies of introduced genes are commonly seen in GM lines obtained through *Agrobacterium*-mediated transformation (Arencibia et al. 1998). The genomic locations of the introduced DNA has not been characterised for any of the GM lines.

68. Ideally, *Agrobacterium* mediated transformation would deliver into a plant genome only the DNA fragment between the left and right border sequences (LB, RB) of the T-DNA. However, during such transformation, parts of the vector (especially beyond the left border of the T-DNA) can be inserted into the plant genome. The occurrence of vector sequences from outside the T-DNA region have been reported for a number of GM plants. These include rice (Kim et al. 2003), maize (Shou et al. 2004), wheat (Wu et al. 2006), and *Arabidopsis* and tobacco (De Buck et al. 2000). For

biolistic transformation, if whole plasmids are used rather than PCR-amplified fragments, insertion of vector sequences is likely. As such, in all of the GM wheat and barley plants there may be parts of the vector sequences. These have not been characterised.

5.7.2 Characterisation of the phenotype of the GM wheat and barley

69. *Group 1 – GMOs modified for altered grain composition.* Analysis of endosperm proteins in T_3 generation of GWD RNAi lines by western blotting showed that GWD protein was reduced to undetectable levels. Starch from T_1, T_2 and T_3 generations contains less than 40% of the level of phosphate as that of non-GM lines. Amylose content and chain length distribution of debranched amylopectin are unchanged. A secondary effect of GWD silencing has been observed in the T_3 generation in glasshouse: an increase in biomass of young wheat plants and an increase in seed production per plant. Significant increase in biomass, seed number per head and seed weight was also observed in field trial under the licence DIR 092. However, under the field conditions, the GWD RNAi plants produced fewer tillers than the control plants. Therefore, no significant difference in yield was observed. The data provided by the applicant also show that while the lines display early vigour and increased seed weight in comparison to the parental cultivar, the increase is within the normal range of phenotypes observed in conventionally bred wheat lines (Bordes et al. 2008).

70. *Group 2 – GMOs modified for enhance nitrogen utilisation efficiency.* The GM plant lines in this group are at an earlier stage of development than some of the Group 1 GMOs. Preliminary testing of some *AlaAT*-positive lines revealed no significant difference to *AlaAT*-negative transformants in seed number, average seed weight and total yield when growing the plants under high nitrogen conditions.

Section 6 The receiving environment

71. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes: any relevant biotic/abiotic properties of the geographic regions where the release would occur; intended agricultural 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).

72. The size, location and duration of the proposed release are outlined in Section 3.2. The location of the proposed dealing is in the shire of Merredin, WA. The NGNE facility is purposely built for the trialling of GM plants. Merredin is located approximately 250 km east north-east from Perth, in the central wheat belt region of WA.

6.1 Relevant abiotic factors

73. The abiotic factors relevant to the growth and distribution of commercial wheat and barley can be found in *The Biology of* Triticum aestivum *L. em Thell* (*Bread Wheat)* and *The Biology of* Hordeum vulgare *L. (Barley*) (OGTR 2008a; OGTR 2008b). The documents are available from the OGTR or from the website <http://www.ogtr.gov.au>.

74. The release is proposed to take place in WA with a temperate climate with dry hot summers (as defined by the Koeppen classification system used by the Australian Bureau of Meteorology, http://www.bom.gov.au/lam/climate/levelthree/ausclim/koeppen2.htm). This climate is often called a “Mediterranean” climate.

75. The proposed site is on land that is not subject to flooding and is 1.8 km away from the nearest waterway. The site is surrounded by a livestock-proof fence and covered with bird netting.

6.2 Relevant biotic factors

76. 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) and *The Biology* Hordeum vulgare *L.* (Barley) (OGTR 2008a; OGTR 2008b).

77. Of relevance to this proposed release are the following points:

- Some of the GM wheat and barley lines proposed for release in the application have already been released in the NGNE facility, under licence DIR 099.
- A large number of wheat varieties are cultivated in the Merredin area, including Gladius (Shackley et al. 2011). The conditions are considered excellent for the cultivation of wheat, which has been conducted in the area since the 19th century. Hence, unmodified wheat is cultivated in the general area of the proposed trial.
- Barley is also a major cereal crop in WA (Paynter et al. 2010) and is grown in the Merredin area.
- Invertebrates, vertebrates and microorganisms could be exposed to the introduced gene constructs, their encoded proteins and end products. In particular, native birds and rodents (either introduced or native) and kangaroos are known to consume cereals.

6.3 Relevant agricultural practices

78. It is not anticipated that the agronomic practices for the cultivation of the GM wheat and barley by the applicant will be significantly different from conventional practices for wheat and barley, with the exception that the applicant proposes to harvest either by hand, using a single row harvester, or a plot harvester. Conventional cultivation practices for wheat and barley are outlined in *The Biology of* Triticum aestivum *L. em Thell* (*Bread Wheat)* and *The Biology of* Hordeum vulgare *L. (Barley*) (OGTR 2008a; OGTR 2008b).

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

80. 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.

81. If the proposed release is approved the applicant anticipates planting the trial in May 2012. The trial is proposed to take place over three years.

82. Non-propagative plant material remaining at the field location after harvest (for example, residual stem stubble) would be tilled into the ground after the trial. The harvested areas would then be watered to encourage germination of any fallen seed, followed by treatment with herbicide to destroy volunteers, this process will be repeated twice more. The areas would then be sown with a break crop such as peas, chick peas or lentils, which will be monitored for volunteers.

83. To mitigate the problem of disease build up in soil, the applicant proposes that the same area will not be sown with the GM wheat and barley is successive years, but may be resown after a one year break. Due to a single growing "season" of wheat per calendar year, and the time limit of the proposed release, this would mean an area used in 2012 could conceivably be resown in 2014, but an area used in 2013 would not be resown as part of this proposed trial. The applicant proposes to sow a break crop in the year after the GMOs are grown.

6.4 Presence of related plants in the receiving environment

84. The GM wheat and barley lines proposed for release will be grown together at the field trial site. Barley and wheat are not known to hybridise with each other under natural conditions, see *The Biology of* Triticum aestivum *L. em Thell* (*Bread Wheat)* and *The Biology of* Hordeum vulgare *L. (Barley*)(OGTR 2008a; OGTR 2008b).

85. The applicant proposes to maintain a 200 m zone around the site in which there is no cultivation of wheat and barley for the duration of the trial. However, the NGNE facility is designed for concurrent trials of GM plants, which may be of the same species (as well as from different applicants). As such, in the future, other GM wheat and barley may be grown in the facility if both this trial and other GM wheat or barley trials are approved by the Regulator.

86. Wheat (*Triticum aestivum*) is sexually compatible with many species within the genus *Triticum*, and in closely related genera such as *Aegilops, Secale (rye)* and *Elytrigia*. *Triticum*, *Aegilops* and *Secale* all belong to the tribe Triticeae. Apart from commercially cultivated bread and durum wheat (*Triticum turgidum* subsp. Durum), other *Triticum* species are not known to be present in Australia. *Aegilops* spp are recognised as a quarantine weed species, but are not known to occur in Australia. However, both *Secale* and *Elytrigia* occur in WA.

87. Australasia possesses four native Triticeae genera – *Australopyrum*, *Stenostachys*, *Anthosachne* (*Elymus*) and *Connorochloa* (Barkworth & Jacobs 2011).

88. Barley is divided into three gene pools. The primary gene pool consists of *Hordeum vulgare ssp vulgare* (cultivated barley) and *H. vulgare ssp spontaneum* (the wild progenitor of cultivated barley). Wild barley is not present in Australia. However, species of the secondary and tertiary gene pools, such as *H. bulbosum*, *H. murinum*, and *H. marinum*, are widespread in Australia (Smith 1968; Mallett & Orchard 2002).

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

89. The introduced partial gene sequences used in the GWD RNAi constructs and *AlaAT* gene, were isolated from wheat and barley, respectively. Expression of the GWD RNAi constructs is driven by the wheat *Bx17*, and a rice intron is included. Expression of the AlaAT gene is driven by the rice *OsAnt1* promoter. All these cereals – and their genes – are widespread and prevalent in the environment and consumed by humans and animals.

90. The *nptII* and *hpt* 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). Depending on the construct, the *nptII* and *hpt* selection marker genes are driven by either the cauliflower mosaic virus (CaMV) 35S promoter or the maize ubiquitin promoter. Maize is a common cereal, and although the CaMV 35S promoter comes from a plant pathogen, it is not capable by itself of causing disease.

91. Termination sequences in all genes were isolated from either the soil bacterium *A. tumefaciens* or CaMV. Although these sequences derive from plant pathogens they comprise only a small part of the total genomes, and cannot by themselves cause disease.

Section 7 Australian and International Approvals

92. No GM wheat or barley has been commercially released in Australia or overseas.

7.1 Australian approvals of GM wheat and barley

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

93. Some of the GM wheat and barley lines proposed for release in this application have been approved for release in Australia under the following DIR licences:

- DIR 092 – GM wheat with altered grain composition in 1.0 ha in the ACT
- DIR 094 – GM wheat and barley with enhanced nutrient utilisation efficiency on 1.0 ha in the ACT
- DIR 099 – GM wheat and barley with enhanced nutrient utilisation efficiency and altered grain composition on a total area of 2 ha in NSW and WA.

94. The Regulator has also previously approved licences for field trials of various other GM wheat and barley lines on a limited scale under controlled conditions. The GM wheat licences are for plants genetically modified for: salt tolerance (Grain Biotech: licence DIR 053/2004); altered grain composition (CSIRO: licences DIR 054/2004 and DIR 092); drought tolerance (DPI Victoria: licences DIR 71/2006 and DIR 080/2007); and enhanced carbon assimilation in drought and heat prone environments (CSIRO: licence DIR 100). The GM wheat and barley licences are for plants genetically modified for: abiotic stress tolerance (University of Adelaide: licences DIR 077/2007 and DIR 102); altered grain composition (CSIRO: licence DIR 093); enhanced nutrient utilisation efficiency (CSIRO: licence DIR 094); and both enhanced nutrient utilisation efficiency and altered grain composition (CSIRO: licence DIR 099). No licence has been issued for a trial of GM barley plants on their own.

95. 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).

96. For further information on previous approval of the limited and controlled release of GM wheat, or wheat and barley in Australia, see the consultation RARMP for DIR 111 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>).

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

7.1.2 Approval by other government agencies

98. Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. However, dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration, National Industrial Chemicals Notification and Assessment Scheme and Australian Quarantine Inspection Service.

99. FSANZ is responsible for human food safety assessment and food labelling, including GM food. The applicant does not intend any material from the GM wheat and barley lines proposed for

release to be used in human food. All GM foods intended for sale in Australia must undergo a safety evaluation by FSANZ. Accordingly, the applicant is not required to apply to FSANZ for the evaluation of the GM wheat and barley lines. However, in the event of a commercial release, FSANZ approval would be required before materials or products derived from the GM wheat and barley lines could be sold for human consumption.

100. In addition, dealings authorised by the Regulator may be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

7.2 International approvals of GM wheat and barley

101. Some of the GM wheat and barley lines have been trialled in the United States under USDA notifications. A wheat trial is currently being conducted in Brawley, California, under notification #10-299-102N. Barley trials are currently being conducted at St. Thomas, North Dakota, and Brawley, California, these having the notifications #10-105-102N and #10-299-101N, respectively. Further wheat and barley trials are planned in 2011 at Minot, North Dakota, under notifications #11-104-110N and #11-060-110N, respectively.

102. Field trials of different GM wheat and barley plants have been approved internationally, including in the USA, Canada, Germany and the United Kingdom. The traits that have been modified include: novel protein production, disease resistance, insect resistance, altered grain properties and herbicide tolerance[6].

[6]< http://www.aphis.usda.gov/brs/status/relday.html>, <http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx> accessed 20 October 2011.

Chapter 2 Risk assessment

Section 1 Introduction

103. 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 3). 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.

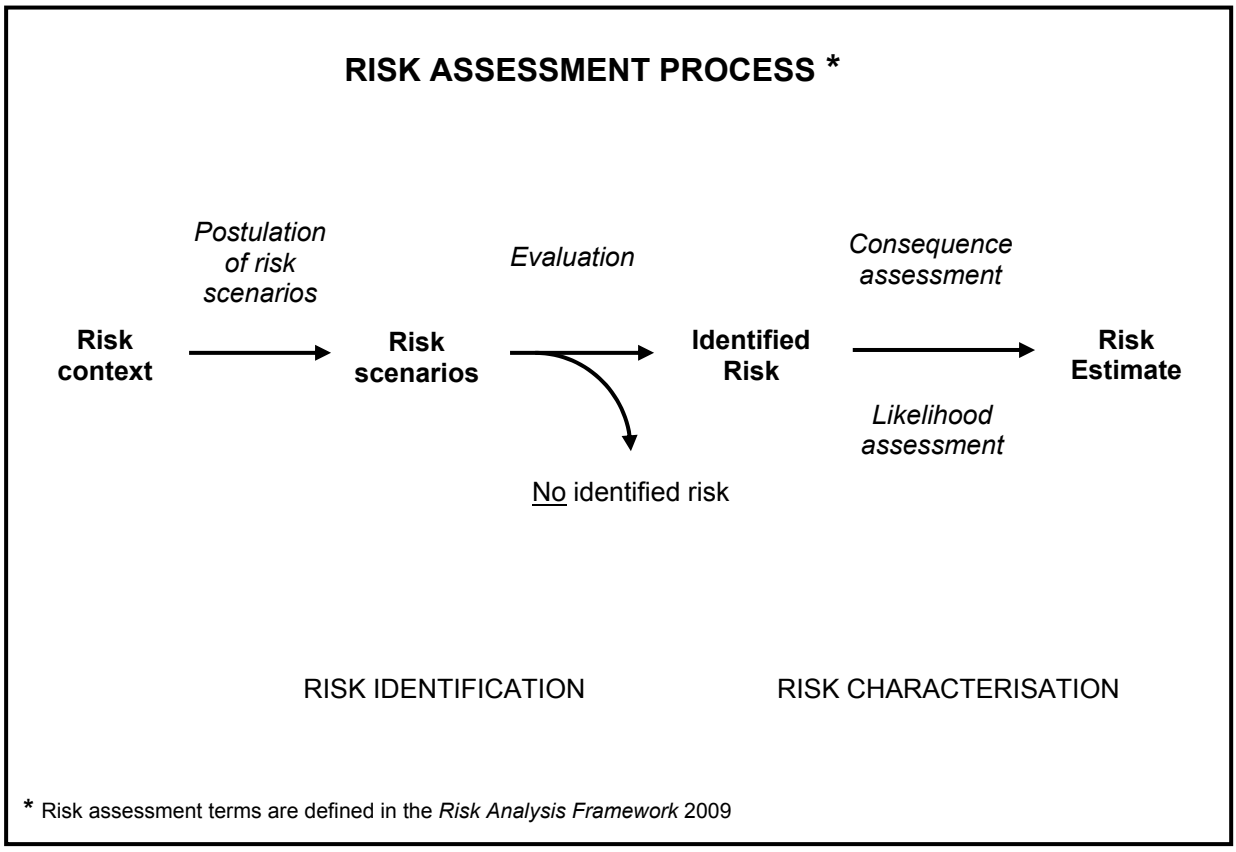

Figure 3. The risk assessment process.

104. 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).

105. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified for further assessment 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.

106. 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.

107. Identified risks (*i.e.* those identified for further assessment) 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

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

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- the proposed limits
- the proposed controls
- characteristics of the parent organism(s)
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- agronomic management practices for the GMOs.

109. Six risk scenarios were postulated and evaluated. These are summarised in Table where circumstances that share a number of common features are grouped together in broader risk categories. In the context of the control measures proposed by the applicant and considering both the short and long term, none of the risk scenarios were identified as a risk that could be greater than negligible. Therefore, they did not warrant further detailed assessment. More detail of the evaluation of these scenarios is provided later in this Section.

110. As discussed in Chapter 1 Section 5.1 and Chapter 1 Section 5.4, the GM wheat and barley lines contain the selectable marker genes *nptII*, and *hpt*. These genes and their products have already been considered in detail in previous RARMPs (for example, DIR 070/2006 and DIR 074/2007 for *nptII*; DIR 073/2007 and DIR 077/2007 for *hpt*) and by other regulators (EFSA 2007; EFSA 2004; CERA 2011). Since none of these genes has been found to pose risks to either people or the environment, their potential effects will not be further assessed for this application.

111. All of the introduced regulatory sequences are derived from common plants, bacteria and viruses. Similar regulatory elements are naturally present in wheat and barley, and the introduced elements are expected to operate in similar ways to endogenous ones. Therefore, although the transfer of introduced regulatory sequences to other sexually compatible plants could result in unpredictable effects, the impact is not likely to be greater than that arising from transfer of endogenous regulatory elements. Hence, these potential effects will not be further assessed for this application.

112. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in literature (Keese 2008) as well as assessed in many previous RARMPs. HGT was most recently considered in the RARMP for DIR 108, while HGT was considered for GM wheat and barley with similar genetic modifications in the RARMPs for DIR 092 and 099. These RARMPs are available at http://www.ogtr.gov.au or by contacting the OGTR. No risk was identified as the gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, HGT will not be assessed further.

Table 2. Summary of risk scenarios from dealings with GM wheat and barley genetically modified for altered grain composition or nutrient utilisation efficiency

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 **Production of a toxic or allergenic substance**	1. Unintended exposure to GM plant material containing the introduced RNA or AlaAT protein or its end products .	Allergic reactions in people or toxicity in people and other organisms	No	• The introduced RNAi constructs do not express novel protein. • Adverse effects from siRNA intake by animal or human through ingestion of GM wheat or barley with RNAi constructs are unlikely, and any effects would be transient. • The introduced *AlaAT* gene is derived from barley. The encoded protein occurs naturally in the environment and is not known to be toxic or allergenic to people or toxic to other organisms. • Plant material from the GMOs would not be used for human food or animal feed. • 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 (weediness) of the GM wheat and barley plants in the environment**	2. The genetic modifications increasing the ability of the GMOs to persist at the proposed trial site beyond the proposed release	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Many abiotic and biotic factors restrict the spread and persistence of wheat and barley in Australia, for example low intrinsic competitive ability, nutrient availability, pests and diseases. • The limits and controls proposed for the release would restrict persistence of the GM wheat and barley plants at the trial site.
	3. The genetic modifications increasing the ability of reproductive GM plant material to spread and/or persist outside the proposed release site	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Dispersal would be minimised by the proposed limits and controls, which include locating the trial site away from waterways, measures to exclude livestock and control rodent numbers, and transporting material 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 RNAi constructs or *AlaAT* gene in commercial wheat and barley plants or in other sexually compatible plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Pollen-mediated gene transfer in wheat and barley occurs at low rates, and generally over short distances. • The proposed limits and controls would restrict gene flow between the GM lines and other sexually compatible plants.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.4 Unintended changes in biochemistry, physiology or ecology	5. Changes to biochemistry, physiology or ecology of the GM wheat and barley plants resulting from expression, or random insertion, of the introduced RNAi constructs or *AlaAT* gene	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Obvious unexpected alterations are likely to have been detected and eliminated during production and glasshouse trials of the GM wheat and barley lines. • Unintended adverse effects, if any, would be minimised by the proposed limits and controls. • The licence holder must report any unintended effects of the dealings.
Section 2.5 Unauthorised activities	6. Use of the GMOs outside the proposed licence conditions (non-compliance)	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	• 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 toxic or allergenic substance

113. 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).

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

115. A range of organisms may be exposed directly or indirectly to the RNA or proteins encoded by the introduced genes, and their end products or associated effects. Workers cultivating the GM wheat and barley would be exposed to all plant parts. Organisms may be exposed directly to the RNA or proteins 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) or indirectly through the food chain.

Risk Scenario 1. Unintended exposure to GM plant material containing the introduced RNA or AlaAT protein encoded by the introduced genes, or their end products

116. Expression of the introduced RNAi constructs or *AlaAT* gene could potentially alter the expression of endogenous wheat and barley proteins and/or result in the production of novel toxic or allergenic compounds in the GM wheat and barley lines. 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.

117. In the context of the proposed dealings, both of the following requirements would have to be met for GM wheat and barley to have any increased toxic or allergenic effect:

- the genetic modification would have to result in either (i) the increased production of recognised endogenous toxic or allergenic molecules, (ii) the elevated production of endogenous molecules, that normally induce no toxic or allergenic reactions, to a level such that they elicit a toxic or allergenic reactions, or (iii) the production of one or more molecules novel to wheat and/or barley that elicit toxic or allergenic reactions, and

- human or other organisms would have to be exposed to the GM wheat and barley plants through contact, ingestion or inhalation.

118. In general, non-GM wheat and barley are not known to be toxic to humans or other organisms, though wheat has been identified as one of a number of crop plants that are capable of accumulating nitrogenous products such as nitrate that, when consumed in large amounts by some ruminants, can be converted to toxic nitrites. Non-GM wheat and barley flour can produce allergic and other immune responses in susceptible individuals on inhalation or ingestion. Several types of allergic and immune reactions to wheat and barley products have been recorded, with baker's asthma and celiac 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). Celiac disease is an inflammatory disorder of the small intestine triggered by consumption of the prolamin fraction of the storage protein complex, gluten, which results in poor nutrient absorption (reviewed by Sollid 2002). These properties are not expected to be altered in the GM wheat and barley lines proposed for release because the introduced genes are not related to the metabolic pathways associated with these factors.

119. No toxicity studies have been performed on the GM wheat and barley plant material or the isolated encoded proteins. However, the partial sequences in the RNAi constructs and the *AlaAT* gene were isolated from wheat and barley, respectively, which are already widespread and prevalent in the environment and consumed by humans and animals. Other than short RNA fragments, it is not expected that any novel products would be produced as a result of the expression of the introduced RNAi constructs.

120. The introduced RNAi constructs are designed to silence or reduce the expression of the targeted endogenous GWD gene in wheat. This occurs via short sequences of RNA (siRNAs), derived from expression of the RNAi construct, matching the target gene sequences. siRNAs fall under a general category of plant small RNAs that also includes microRNAs (miRNAs); siRNAs and miRNAs are common in both plants and animals and are believed to play regulatory roles in many biological processes. As discussed in Risk Scenario 5, RNAi constructs (via siRNAs) can give rise to off-target silencing effects within the plant, leading to changes other than the intended effects. In addition, a recent publication (Zhang et al. 2011) has reported evidence that natural plant miRNAs can be absorbed by mammals through food intake, and have the potential to modulate gene expression in animals. A particular plant miRNA, highly abundant in rice and other plants, was detected in sera from healthy Chinese women and men whose main diet was rice, as well as in the sera of animals. In a study on mice, this plant miRNA was found to modulate expression of a mouse gene having a near-perfect sequence match to the miRNA sequence. The effect on the mouse gene by the plant miRNA ceased when rice was no longer included in the food intake.

121. The possibility exists that similar effects could occur from the novel small RNA molecules expressed in Group 1 GM wheat lines, although no orthologues of *GWD* have been found in humans or animals. Plants within our diet contain numerous micro and siRNAs, often with perfect homology to some of our genes (Ivashuta et al. 2009). Further, the experience from traditional plant breeding in wheat (and other) species, is that the concurrent introduction of many genes into their genomes represents a negligible risk to human health (Kuiper et al. 2001). Such breeding undoubtedly involves the introduction of genes involved in the production of microRNAs and siRNAs (Della Vedova et al. 2005; Tuteja et al. 2009).

122. Recently, RNAi has been developed as a potential mechanism of insect control (Huvenne & Smagghe 2010; Mito et al. 2011). The digestion by insects of plant material containing dsRNA molecules targeted to selected host insect genes has resulted in developmental defects and even mortality in the pests. The relative success of these experiments perhaps reflects that invertebrates more easily uptake nucleic acids, such as dsRNA, as opposed to vertebrates (Parrott et al. 2010).

123. Even if novel small RNAs are taken up by people or animals, to have any effect a number of conditions would have to be met: the siRNA-containing wheat would need to constitute a large proportion of the diet, the siRNA would need to be expressed at high levels in the wheat material consumed, match a target sequence of a human or animal gene and be taken up by specific human and animal cells expressing that gene. Lastly, it is likely that even if the siRNAs were acquired through food intake and did affect the expression of mammalian genes, such an effect would be transient as was reported by Zhang et al. (2011).

124. The introduced *AlaAT* gene encodes a protein normally present in wheat and barley, as well as humans, animals, fungi and archaea. No information has been found to suggest that the barley AlaAT protein is toxic or allergenic to people or toxic to other organisms (Chapter 1, Section 5.3.1), or could affect the production of endogenous wheat and barley toxins and allergens. Therefore, exposure to GM plant materials from these lines is not expected to adversely affect the health of humans or other organisms.

125. The applicant has not proposed any means for segregating the GM wheat lines or GM barley lines from each other while growing in the field, so the potential exists for crossing between the GM groups and lines. This could lead to stacking of GM traits, which could potentially lead to increased toxicity or allergenicity. However, as outlined above, the introduced genes and partial gene sequences are derived from wheat and barley, and exposure to GM plant materials with the introduced RNA or AlaAT protein is unlikely to lead to toxic or allergenic effects. No information has been found to suggest that these proteins are toxic or allergenic to people or toxic to other organisms (Chapter 1, Sections 5.2 & 5.3), or could affect the production of endogenous wheat and barley toxins and allergens. In addition, staff working on the GMOs in the glasshouse have not reported adverse reactions to the plant material. There is no reason to expect the stacking of any of these genes will substantially alter the risk of increased toxicity or allergenicity above that assessed for any of these genes inserted on its own. None of these genes act in a pathway known to affect the biosynthesis of a toxin or allergen, so their concurrent expression is also unlikely to lead to increased toxicity or allergenicity. Additionally, the experience from traditional plant breeding in wheat and barley, which often involves the concurrent introduction of many genes (for example, crossing wheat and rye to produce *Triticale*), is that such plants do not present an increased risk to human health.

126. The NGNE facility is designed as a multi-user multi-trial facility. In such a facility it is possible that the other genetic modifications, approved under a future licence, could be transferred into (*ie* hybridisation), or mixed with (*ie* mixing of seed), the GM material from this proposed trial. Without the knowledge of the other genetic modification, a full risk assessment cannot be conducted at this stage but would be required in relation to any relevant future application. Any identified risk would be addressed in the risk management plan for the later application. Nevertheless, potential for mixing genetic modifications would be restricted by the use of a buffer zone between different field trials, as applied in licences DIR 092, 093 and 094.

127. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of unintentional exposure of the general public and other organisms to GM plant materials. No GM material is intended to enter the food supply, so there is little potential for exposure of the general public to GM plant material via ingestion.

128. ***Conclusion***: The potential for allergic reactions in people or toxicity in people and other organisms, as a result of unintended exposure to the introduced RNAi constructs or *AlaAT* gene and their products, in the context of the control measures proposed by the applicant and considering both the short and long term, is not identified as a risk that could be greater than negligible. Therefore it does not warrant further assessment.

2.2 The potential for spread and persistence of the GM wheat and barley plants in the environment

129. This section addresses the question of whether or not the proposed dealings with the GMOs may lead to harm to human health and safety or the environment as a result of an increased potential for spread and/or persistence due to the genetic modification.

130. All plants have the potential to lead to harm in certain environments. Harms that may arise from a certain plant species in a particular environment include:

- adverse effects on the health of people and/or animals
- reduction in the establishment, yield and/or quality of desired plants
- restriction in the physical movement of people, animals, vehicles, machinery and/or water
- adverse effects on environmental health, such as adverse changes to strata levels, nutrient levels, fire regime, soil salinity, soil stability, or by providing food and/or shelter to pests, pathogens and/or diseases.

131. For the purpose of this document, plant species causing significant levels of one or more of these harms are called 'weeds'. A plant species may be weedy in one or more land uses, such as dryland cropping or nature conservation.

132. Characteristics that influence the spread (dispersal of the plant or its genetic material) and persistence (establishment, survival and reproduction) of a plant species impact on the degree of its invasiveness. These characteristics include the ability to establish in competition with other plants, to tolerate standard weed management practices, to reproduce quickly, prolifically and asexually as well as sexually, and to be dispersed over long distances by natural and/or human means. The degree of invasiveness of a plant species in a particular environment gives an indication of the likelihood of its weediness in that environment. In addition to local experience, a history of weediness overseas can be used as an indicator for weediness in Australia.

133. Baseline information on the weediness of wheat and barley, including factors limiting the spread and persistence of non-GM wheat and barley plants, 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). 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 and 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).

134. Scenarios relating to altered spread and/or persistence of the GM wheat and barley, compared to non-GM wheat and barley, include:

- the genetic modification enabling the GM wheat and barley to persist at the release site beyond the proposed dealings, leading to an increased level of harm relative to non-GM wheat and barley varieties
- the genetic modification enabling reproductive GM plant material to spread outside the proposed release site, and to persist in the environment leading to an increased level of harm relative to non-GM wheat and barley varieties.

Risk Scenario 2. The genetic modifications increasing the ability of the GMOs to persist at the proposed trial site beyond the proposed release

135. If the genetic modifications (either individually or in combination) were to provide the GM wheat and barley plants with a selective advantage relative to non-GM wheat and barley varieties,

and if they were to persist at the proposed trial site after the trial, this would increase exposure of the environment, including people and other organisms, to the GMOs. This may give rise to an increase in the level of one or more of the potential harms associated with weeds relative to non-GM wheat and barley varieties. Persistence may also provide increased opportunity for the GMOs to be dispersed beyond the release site.

136. An increase in the level of harm relative to commercially grown wheat and barley varieties could only occur where a plausible pathway to harm exists. For this to occur in the context of the proposed dealings, both of the following requirements would have to be met:

- the genetic modification would have to provide the GMOs with a selective advantage relative to commercially grown wheat and barley varieties
- the GM plants would have to persist at the proposed trial site after the trial, leading to some harm.

137. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of the proposed dealings has been considered in Risk Scenario 1 and was not identified as a risk that warrants further assessment.

138. While the impact of the genetic modifications on survival of the GM wheat and barley lines is uncharacterised, a number of predictions can be made based on knowledge of the individual gene functions and their predicted effects, as well on observed phenotypes of other GM plants expressing the same gene. It is noted that some of the genetic modifications are intended to increase the productivity of the GMOs, and this may also enhance their ability to persist in other land uses (such as nature conservation). Relevant characterisation of the different groups of GM wheat and barley are described below.

Group 1: GWD RNAi lines

139. Data supplied by the applicant shows that GM wheat lines carrying a GWD RNAi constructs display an increase in plant vigour at early growth stages, observed in both glasshouse and field trial (see Chapter 1, Section 5.2.1). This may promote the persistence of the GM wheat lines in all land uses. Early vigour is advantageous because increased rooting depth leads to better nutrient capture, and faster leaf growth leads to quicker canopy closure, reducing evaporation from the soil (Richards et al. 2007). These characteristics typically contribute to increased grain production, which was observed in lines carrying the GWD RNAi construct, both in terms of seed weight and seed number per head. However, the GM wheat lines also display a decrease in tiller number under field conditions. The increases in seed number and seed size compensates for the decrease in tiller number as no significant difference in yield was observed compared to the non-GM parents. Furthermore, data provided by the applicant shows that the increase in early vigour and seed weight are within the range of values observed for wheat and barley bred using conventional breeding techniques.

Group 2: AlaAT lines

140. The involvement of *AlaAT* in plant responses to hypoxia has been well documented (Liepman & Olsen 2003). It is thus possible that the expression of the introduced *AlaAT* gene in the GM wheat and barley lines may confer enhanced tolerance to hypoxic conditions such as waterlogging. In an environment in which oxygen availability was the main factor limiting the persistence of wheat and barley, expression of the *AlaAT* gene could result in increased persistence of the GM wheat and barley lines.

141. In other GM plants, expression of the *AlaAT* gene has resulted in increased biomass and seed yield resulting from more vigorous growth, accelerated tillering and altered root structure (see Chapter1, Section 5.3). It is possible that the GM wheat and barley lines may also show these phenotypic changes, which could impact on the persistence of the GM wheat and barley plants at the trial site.

142. As discussed in Risk Scenario 1, as the NGNE facility is designed as a multi-user multi-trial facility, it is possible that the genetic modifications approved under a future licence could be mixed with the GM material from this proposed trial. Without the knowledge of the other genetic modification, a full risk assessment cannot be conducted at this stage but would be required in relation to any relevant future application. Any identified risk would be addressed in the risk management plan for the later application. Nevertheless, potential for mixing of genetic modifications could be restricted by the use of a buffer zone between different field trials, as applied in licences DIR 092, 093 and 094.

143. In summary, some of the genetic modifications could enhance the tolerance of the GM wheat and barley to particular environmental factors. Observed and expected phenotypes for the GM wheat and barley include increased vigour and tolerance to water logging. Stacking of such traits could increase the potential for spread and persistence of the GM wheat and barley at and beyond the trial site. However, even in the event of successful crossing between lines, spread and persistence would be likely limited by factors such as lack of seed shattering, low competitive ability and other factors that normally limit the spread and persistence of wheat and barley plants in Australia. Survival of wheat in particular is limited by a number of factors including temperature, competitive ability, nutrient availability, pests and diseases (Slee 2003; Condon 2004). Modern wheat and barley cultivars, some of which are bred for high vigour, are not recognised as significant weed risks in Australia, and there have been no reports of bread wheat or barley becoming an invasive pest in Australia or overseas.

144. Even if there were any significant advantages conferred to the GM wheat and barley lines as a result of the genetic modification, the proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of persistence of the GM wheat and/or 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, post harvest irrigation of the site to encourage germination of remaining seed followed by herbicide treatments to destroy volunteers and post harvest monitoring of the release site.

145. ***Conclusion:*** The potential for an increase in the level of harm as a result of the genetic modification increasing the ability of the GM wheat and barley plants to persist at the trial sites, in the context of the control measures proposed by the applicant and considering both the short and long term, is not identified as a risk that could be greater than negligible. Therefore it does not warrant further assessment.

Risk Scenario 3. The genetic modification increasing the ability of GM wheat and barley to spread and/or persist outside the proposed release site

146. If the GM wheat and barley lines were to be dispersed from the release site, and persist in the wider environment, this could increase exposure of the environment, including people and other organisms. Such exposures could lead to an increase in the level of one or more of the potential harms associated with weeds, relative to non-GM wheat and barley varieties.

147. To realise any increase in the level of harm relative to non-GM wheat and barley as a result of spread and persistence of the GMOs outside the trial site in the course of the proposed dealings, both of the following requirements would have to be met:

- the GMOs would have to be able to spread from the trial site, with or without persistence in the wider environment
- the presence of the GMOs in the wider environment would have to lead to some harm.

148. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of the proposed dealings has been considered in Risk Scenario 1. Additionally, risks that

may arise through gene flow via pollen are not considered in this risk scenario as they are addressed in Risk Scenario 4.

149. If the expression of the introduced RNAi constructs or *AlaAT* gene were to provide the GM wheat and barley with a significant selective advantage over non-GM wheat and barley plants and they were able to establish and persist in favourable non-agricultural environments this may give rise to undesirable changes in species composition in these environments.

150. The traits associated with the introduced RNAi constructs or the *AlaAT* gene are not expected to enhance the ability of the GM wheat and barley to spread and persist. As discussed in Risk Scenario 2, tolerance to some environmental factors may be increased in the GM plants, but spread and persistence would still be limited by a range of factors that normally limit the spread and persistence of these plants in Australia.

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, the activity of people, or through extremes of weather such as flooding or high winds. Seed yield and number of seeds per head may be increased in the GM wheat and barley lines.

152. 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). These seed dispersal characteristics are not expected to be altered in the GMOs. Seed yield may be increased in the GM wheat and barley lines, but other seed production and dispersal characteristics, such as grain number per spike, are not expected to be altered compared to non-GM parental cultivars.

153. 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). Preliminary evidence has suggested that when fed mature seed, corellas and galahs dehusk barley seeds prior to ingestion and thus viable seeds are not excreted; however, corellas were shown to excrete some viable wheat seeds, although the proportion is extremely low (Woodgate et al. 2011). Nonetheless, birds tend to favor the green plant parts to the seed and dispersal of viable GM wheat and/or barley seed is likely to be low. The proposed trial site is covered by bird netting, which would prevent access by birds. However, there has been no evidence of seed dispersal from other GM wheat and barley trials licenced by the Regulator and conducted without bird netting (for example under DIR 077/2007 and DIR 099).

154. Kangaroos, rabbits and mice are known pests of wheat and barley crops, and cattle or sheep may graze cereals. The proposed release site will be surrounded by a fence with a locked gate, limiting the possibility of seed dispersal by any large animals such as kangaroos, cattle and sheep, or by unauthorised people accessing the site. 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 (Welch 1985; Wicklow & Zak 1983).

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). The applicant has proposed to implement a rodent control program at the trial site, which will discourage dispersal by rodents.

156. Dispersal by authorised people entering the proposed trial site would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing. Additionally, equipment used at the NGNE facility is dedicated for use in that facility only and would be washed and remain within the facility, this should prevent spread of the GMOs from the facility via equipment or vehicles. All GM plant material would also be transported in accordance with the Regulator's transport guidelines which would minimise the opportunity to disperse the GM material.

157. Dispersal of the GM wheat and barley seed via water run-off from irrigation or rainfall would be minimised because the site is reasonably flat and irrigation of the site or rainfall would produce minimal water run-off. Further, the site is 1.8 km from the nearest waterway.

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 site away from natural water ways to prevent dispersal in the event of flooding, and having an isolation zone in which no other wheat or barley crops would be grown and related plants are controlled.

159. As discussed in risk scenario 2, some of the genetic modifications could enhance the tolerance of the GM wheat and barley to particular environmental factors. Observed and expected phenotypes for the GM wheat and barley include increased vigour and tolerance to water logging. Stacking of such traits could increase the potential for spread and persistence of the GM wheat and barley at and beyond the trial site. However, even in the event of successful crossing between lines, spread and persistence would be likely limited by factors such as lack of seed shattering, low competitive ability and other factors that normally limit the spread and persistence of wheat and barley plants in Australia. Survival of wheat in particular is limited by a number of factors including temperature, competitive ability, nutrient availability, pests and diseases (Slee 2003; Condon 2004). Modern wheat and barley cultivars, some of which are bred for high vigour, are not recognised as significant weed risks in Australia, and there have been no reports of bread wheat or barley becoming an invasive pest in Australia or overseas.

160. ***Conclusion:*** The potential for an increased level of harm due to the spread of reproductive GM plant material and persistence of the GMOs outside the trial site , in the context of the control measures proposed by the applicant and considering both the short and long term, is not identified as a risk that could be greater than negligible. Therefore it does not warrant further assessment.

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

161. 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 & Hegde 2003). For GM crops, vertical gene flow could therefore occur via successful cross-pollination between the crop and neighbouring crops, plants, related weeds or native plants (Glover 2002).

162. It should be noted that vertical gene flow *per se* is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome. For an increased potential for adverse effects to arise as a result of gene flow of the introduced genetic elements from the GM wheat and barley to sexually compatible plants, both of the following steps must occur:

- transfer of the introduced genetic elements to sexually compatible plants
- increased potential for adverse effects, such as toxicity of the recipient plants, due to expression of the introduced genes.

163. 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 2008b) and *The Biology of* Hordeum vulgare *L. (Barley)* (OGTR 2008a). 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 RNAi constructs or AlaAT gene in commercial wheat and barley plants or other sexually compatible plants

164. Transfer and expression of the introduced RNAi constructs or *AlaAT* to other wheat and barley plants could alter the allergenic and/or toxic potential, or increase the weediness potential, of the resulting hybrid plants.

165. All of the introduced partial gene sequences in RNAi constructs and *AlaAT* were isolated from wheat and barley, respectively, so transfer of these genes to other wheat or barley does not introduce new proteins, although it may result in altered protein localisation, protein levels or end product content.

166. 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 constructs and *AlaAT* gene. This will be the same if the introduced RNAi construct or the *AlaAT* gene is transferred to other wheat or barley plants.

167. Both wheat and barley are predominantly self-pollinating (94-99%) and any outcrossing occurs through wind pollination (reviewed in OGTR 2008a; OGTR 2008b). Gene flow generally occurs over much shorter distances from small scale experimental releases than from the commercial scale, although gene flow levels are highly variable. The majority of gene flow from small scale fields of wheat occurs within 10 m 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).

168. Studies under Australian field conditions (in 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).

169. The applicant proposes to prevent cultivation of other wheat and barley within 200 m of the trial site. Isolation from other wheat and barley cultivation will greatly restrict the potential for pollen flow and gene transfer.

170. As discussed in Chapter 1, Section 6.4, wheat is sexually compatible with many species within the genus *Triticum*, and in closely related genera such as *Aegilops, Secale (rye)* and *Elytrigia*. Durum wheat (other than bread wheat, the only other *Triticum* species present in Australia) can cross with wheat, although there are no reports of gene flow beyond 40 m (Matus-Cadiz et al. 2004). Hybrids between wheat and *Secale cereale* are sterile, but treatment with colchicine doubles the chromosome number and results in a fertile plant, commercially known as Triticale, which is grown in Australia (Knupffer 2009). Natural hybridisation between wheat and Triticale rarely occurs (Ammar et al. 2004; Kavanagh et al. 2010), at least partly due to both species being largely self-fertilising (Acquaah 2007). To facilitate hybridisation, breeders usually resort to hand pollination (Chaubey & Khanna 1986; Hills et al. 2008). Usually viable F1 seeds are produced only when Triticale is the female parent, but *in vitro* embryo rescue can be used to produce hybrid lines when Triticale is the male parent. *Elytrigia repens* does occur as an introduced plant in Australia, but a review of possible means of pollen-mediated gene flow from GM wheat to wild

relatives in Europe concluded that there was a minimal possibility of gene flow from wheat to *Elytrigia spp*. (Eastham & Sweet 2002). Species of *Aegilops* are not known in Australia.

171. There has been no concerted investigation of natural hybridisation of the four native Australasian Triticeae genera with wheat. However, based on experience of hybridising wheat with most other members of the Triticeae, it is likely it never occurs under natural conditions. Purposeful breeding of species of *Anthosachne* (*Elymus*) with wheat has only yielded results with hormone application and embryo rescue (Torabinejad & Mueller 1993).

172. *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). Hybridisation of *H. bulbosum* (secondary gene pool of barley) and cultivated barley usually results in the elimination of the genome of *H. bulbosum* and the formation of barley haploids (Pickering & Johnston 2005). Although hybrids can be formed between cultivated barley and members of the barley tertiary gene pool, due to infertility these have not proven useful in the introgression of germplasm into barley.

173. More discussion of the hybridisation of wheat and barley can be found in the RARMPs for DIRs 100 and 102 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>).

174. Other than (bread) wheat and barley themselves, durum wheat, rye and other species of *Hordeum* (such as *H. leporinum, H. marinum*) and *Elytrigia* have all been recorded in WA.

175. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for gene transfer to non-GM wheat and barley plants, and other sexually compatible plants. In particular, the applicant proposes to isolate the GMO planting from commercially grown wheat and barley crops by at least 200 m. As in licences for DIRs 092, 093 and 094, related species would be controlled in a monitoring zone around the trial site. The applicant also proposes to perform post harvest monitoring and to destroy any volunteer plants found at the site to ensure that no GM wheat and barley remains.

176. ***Conclusion:*** The potential for allergenicity in people, toxicity in people and other organisms or increased weediness due to the expression of the introduced genes and regulatory sequences in commercial wheat and barley plants or other sexually compatible plants as a result of gene transfer, in the context of the control measures proposed by the applicant and considering both the short and long term, is not identified as a risk that could be greater than negligible. Therefore it does not warrant further assessment.

2.4 Unintended changes in biochemistry, physiology or ecology

177. All methods of plant breeding can induce unanticipated changes in plants, including through pleiotropy[7] (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 unintended 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 protein encoded by the introduced gene changing chromatin structure, affecting methylation patterns or modulating/influencing signal transduction and transcription
- increased metabolic burden associated with high level expression of the introduced gene

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

- novel traits arising from interactions between the products of the introduced gene and endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in the biochemical pathway associated with the activity of the protein encoded by the introduced gene.

178. Unintended 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 5. Changes to biochemistry, physiology or ecology of the GM wheat and barley plants resulting from expression, or random insertion, of the introduced RNAi constructs or AlaAT gene

179. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced RNAi constructs or the *AlaAT* gene, have already been discussed in Risk Scenarios 1 to 4, and were not considered identified risks.

180. Various biochemical pathways of the GM wheat and barley plants could be changed by the expression of the introduced RNAi constructs or the *AlaAT* gene, resulting in the production of novel or higher levels of endogenous toxins, allergens or anti-nutritional compounds, or in other unpredictable effects.

181. For GM wheat lines modified using RNAi constructs, expression of the partial gene sequences is under the control of an endosperm-specific promoter (see Chapter 1, Section 5.5.1). The applicant has not tested other plant tissues to confirm this specificity. In GM wheat lines containing a GWD RNAi construct, a significant increase in the activity of α-amylase in the endosperm was observed and the activity is specifically upregulated in the aleurone and pericarp layers. This phenomenon is similar to wheat genotypes affected by late maturity α-amylase (LMA) (Mares & Mrva 2008). LMA is a characteristic that renders wheat unsuitable for high value end products.

182. 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. An inadvertent outcome could therefore be the cross silencing of unrelated genes. Potential off-target silencing may be predicted if the sequence of the host genome is known, but this is not yet the case for wheat and barley. Similar to the effect of random insertions discussed below, any strong off-target silencing effect is likely to be detrimental to the plant, so likely to be detected during production and glasshouse trials of the GM wheat lines. This allows for elimination of those lines.

183. The outcome of random insertion of an introduced gene in the recipient's genome, leading to disruption of endogenous genes, 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. This could also include higher levels of endogenous toxins, allergens or anti-nutritional compounds. Non-GM wheat can be toxic to some ruminant 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 2008b) and *The Biology of* Hordeum vulgare *L. (barley)* (OGTR 2008a).

184. Unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003). While the GM wheat and barley 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 in the glasshouse. None of the GM lines proposed for release have led to adverse reactions in staff developing these lines.

185. The range of possible unintended effects produced by genetic modification is not likely to be greater than that from accepted traditional breeding techniques (Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health 2004; Bradford et al. 2005). Breeders utilising either traditional hybridisation, mutagenesis or somaclonal variation have rarely used molecular or cytogenetic methods to characterise the new varieties they have generated. As such, the numbers or the positions of mutations, or the chromosomal segments that are translocated, are not known. New varieties may have altered expression of some genes or novel fusion proteins produced. Nevertheless, the generation of traits that are undesirable for human health, safety or the environment has rarely been a problem (Hajjar & Hodgkin 2007). Phenotypic analysis conducted by breeders is accepted across the world, and has led to the commercialisation of numerous new plant varieties.

186. The likelihood of any unintended outcomes of the genetic modifications causing adverse effects would be minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2 and 3.3. In particular, the scale of the trial would minimise the potential for adverse effects.

187. ***Conclusion:*** The potential for an adverse outcome as a result of altered biochemistry, physiology or ecology, in the context of the control measures proposed by the applicant and considering both the short and long term, is not identified as a risk that could be greater than negligible. Therefore it does not warrant further assessment.

2.5 Unauthorised activities

Risk Scenario 6. Use of the GMOs outside the proposed licence conditions (non-compliance)

188. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM wheat and barley 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 for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

189. ***Conclusion:*** The potential for an adverse outcome as a result of unauthorised activities is not identified as a risk that could be greater than negligible. Therefore it does not warrant further assessment.

Section 3 Risk estimate process and assessment of significant risk

190. 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 parent organism and within the context of the receiving environment.

191. Six risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if it occurred were also assessed.

192. A risk is only identified when a risk scenario is considered to have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

193. The characterisation of the six risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the limits and control measures proposed by the applicant and considering both the short and long term, did not give rise to any identified risks that could be greater than negligible and required further assessment. The principal reasons for this include:

- limits on the size, location 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
- the genetic modifications are unlikely to give rise to adverse affects on human health and safety or the environment
- widespread presence of the same and similar genes and gene sequences in the environment and lack of evidence of harm from them
- limited ability and opportunity for the GM wheat and barley plants to transfer the introduced genes to for the GM wheat and barley to spread and persist is restricted by a range of environmental factors that restrict non-GM wheat and barley
- none of the GM plant materials or products would be permitted to enter human food or animal feed supply chains.

194. Therefore, any risks to the health and safety of people, or the environment, from the proposed release of the GM wheat and barley plants into the environment is 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 environment[8].

Section 4 Uncertainty

195. 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.

196. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability[9]. 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.

197. For DIR 112, the primary purpose of which is to undertake research, uncertainty is noted particularly in relation to the characterisation of:

- Risk Scenario 1, regarding potential increases in toxicity or allergenicity as a result of the introduced genes
- Risk Scenario 2, associated with the potential for increased persistence of the GMOs

[8] 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.

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

- Risk Scenario 5, associated with the potential for any unintended effects as a result of changes to biochemistry, physiology or ecology of the GM wheat and barley plants.

198. Additional data, including information to address these uncertainties, may 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.

199. Chapter 3, Section 4 discusses information that may be required for future release.

Chapter 3 Risk management plan

Section 1 Background

200. 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 informs the Regulator's decision-making process and is given effect through imposed licence conditions.

201. 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.

202. All licences are 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 allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors 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.

203. The licence is also 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 Risk treatment measures for identified risks

204. The risk assessment of 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. These risk scenarios were considered in the context of the scale of the proposed release (a maximum area of 1 ha on one site between May 2012 and June 2015), the proposed containment measures (Chapter 1, Section 3), and the receiving environment, and considered both the short and the long term. 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. Therefore, no conditions are proposed to treat these negligible risks.

Section 3 General risk management

205. Licence conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and limit the release to the size, location and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are detailed in the licence and summarised in this Chapter.

3.1 Imposed licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by CSIRO

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

207. The trial would be confined to a maximum area of 1 ha per year within the 5 ha fenced NGNE facility between 2012 and 2015. This facility, recently opened in WA (4 October 2011), is planned

to be a multi-user multi-trial facility for the trialling of GM plants. Only authorised personnel would have access to the facility. These measures will minimise the potential for unintentional exposure of humans, vertebrates and other organisms to the GMOs (Risk Scenario 1).

208. The NGNE facility is surrounded by fence capable of restricting access to the site by people, grazing livestock and large wildlife, further minimising the potential for both exposure to GM plant material and dispersal outside the proposed release site (Risk Scenario 3). A licence condition requires maintenance of the fence.

209. The facility also has a dedicated wash-down area for the air and water cleaning of equipment used to deal with GMOs, the water from this area being channelled into an evaporation dam. Residue from evaporation will be collected and destroyed with a hammer mill. Use of this facility will assist in the management of the spread and persistence of the GMOs (Risk Scenarios 2 and 3). Licence conditions require records of cleaning of the wash-down facility to be kept.

210. The NGNE facility is also covered with bird netting (Chapter 1, Section 3.3) and this has the potential to further reduce the likelihood of dispersal of plant material from the trial site. The possibility of dispersal of GM plant materials by birds was considered in detail in the RARMP for DIR 071/2006 which is available from the OGTR or from the website, and is discussed in *The Biology of* Triticum aestivum *L. em Thell (Bread Wheat*) (OGTR 2008b). Barley seed dispersal by birds has been considered in *The Biology of* Hordeum vulgare *L. (Barley)* (OGTR 2008a). Preliminary evidence has suggested that corellas and galahs do not excrete viable barley seed, but corellas were shown to excrete viable wheat seeds (Risk Scenario 3). Nonetheless, birds tend to favour the green plant parts to the seed and dispersal of viable GM wheat and/or barley seed is likely to be low. There has been no evidence of seed dispersal from other GM wheat and barley trials licensed by the Regulator and conducted without bird netting (for example under DIR 077/2007 and DIR 099). Therefore, no conditions relating to bird netting of the site have been imposed.

211. The applicant has stated that the NGNE facility is located approximately 1.8 km from the nearest permanent waterway, which would reduce the likelihood of plant material being washed away from the site. It is a standard DIR licence condition that trial sites be located at least 50 m from waterways to limit the dispersal of viable GM plant material in the event of flooding. This condition is not proposed for this licence since the location of the trial site already exceeds this separation. A licence condition has been imposed requiring immediate notification of any extreme weather conditions affecting the site during the release. This will minimise scope for the GM wheat and barley to disperse and establish outside the proposed release site (Risk Scenario 3).

212. CSIRO proposes that conditions similar to those in licences DIR 092, DIR 093 and DIR 094 be applied to this proposed trial. These licences allow planting of the different GM wheat and barley lines in the same fenced area, separated by a 4 m buffer zone (4 m representing the addition of the two 2 m buffer zones surrounding each individual trial). Applications DIR 092, DIR 093 and DIR 094 were assessed in parallel, allowing consideration of the potential combination of different genetic modifications, and all three licences are held by CSIRO. Other control measures imposed in these licences include:

- surrounding the area of the three trials with a monitoring zone and isolation zone
- controlling rodents within the site
- cleaning of equipment
- cleaning of areas where GMOs were harvested
- inspecting the area during growth and after harvest of the GMOs and
- not permitting the use of GM plant material in human food or animal feed.

213. These controls are considered below.

Monitoring zone

214. In analogy to licences DIR 092, DIR 093 and DIR 094, a 10 m wide monitoring zone around the site is proposed. A monitoring zone around GM wheat and barley trials, kept free of sexually compatible plants while any GMOs within the site are flowering, serves a number of purposes:

- limiting the potential for spread of the introduced genetic material to nearby sexually compatible plants (gene flow; Risk Scenario 4).
- limiting the possibility for spread of the GMOs outside the area authorised for release, for example by accidentally dispersed via movement of equipment or during harvest (Risk Scenario 3).
- by being maintained so it does not attract rodents, minimising exposure to the GMOs while at the same time minimising the possibility for dispersal by rodents (Risk Scenarios 1 and 3).

215. Licence conditions require a 10 m wide monitoring zone, in which related species must be prevented from flowering and which must be managed so as to not attract or harbour rodents. Additionally, in keeping with the previous DIR licences (above), appropriate measures must be implemented to control rodent numbers in the trial site. These may include, but are not limited to, traps and/or poison bait within and/or surrounding the trial site while GMOs are being grown and until the site has been cleaned.

Isolation zone

216. The applicant has proposed to maintain a distance of at least 200 m between the NGNE facility and other wheat and barley crops. The potential for pollen movement and gene flow between GM wheat or barley and other sexually compatible species has been addressed at some length in the RARMPs for DIR 092, DIR 093, DIR 094, DIR 100 and DIR 102. On the basis of the evidence detailed there, including scientific literature on gene flow, international containment measures for GM wheat and barley trials, and the rules for producing basic and certified seed, 200 m isolation is considered adequate to minimise gene flow from the GM wheat and barley plants to other wheat and barley crops (Risk Scenario 4). Similar to DIR 092, DIR 093 and DIR 094, a licence condition is imposed to maintain a 190 m isolation zone (in addition to a 10 m monitoring zone), in which no other wheat or barley may be grown and in which sexually compatible plants must be controlled while any GMOs are flowering within the site.

Buffer zone and cleaning of equipment

217. During sowing and harvesting seed may be dispersed into the area immediately around the trial. An environment assessment prepared for a field trial of GM wheat in the United States concludes that a distance of 20 feet (approximately 6.1 m) is sufficient to minimise "mechanical mixing" of GM and non-GM wheat plants (USDA-APHIS 1994). The South Australian Seed Certification Manual (Smith & Baxter 2002) states that production areas for cereals must be separated from other cereals by at least 2 m (or by a physical barrier such as a fence) to prevent the mixture of seed during harvest. The accepted levels of contamination are 0.1% for basic seed and 0.3% for certified seed. Similar to licences DIR 092, DIR 093 and DIR 094, a 2 m buffer zone is required which must be cleaned and monitored following harvest, along with the planting location (see below). Further conditions require that if any GM plant material is dispersed beyond the buffer zone during harvest, this area must also be included in post-harvest cleaning and monitoring. These measures will assist in management of persistence of the GM wheat and barley within the trial site (ie. NGNE facility; Risk Scenario 2).

218. The applicant has proposed to harvest the seed by hand, or by the use of either a small mechanical harvester or a single row harvester. Using hand harvesting or small mechanical equipment is expected to limit the potential for spread and persistence of GM material during

harvesting (Risk Scenarios 2 and 3). A condition specifying these harvesting methods is included in the licence.

219. Cleaning of equipment is an accepted method to minimise spread of GM plant material and a condition to require cleaning of equipment before use for any other purpose is imposed in the licence (Risk Scenario 3). This will also assist in management of persistence within the trial site (Risk Scenario 2). Additionally, equipment used at the NGNE facility is dedicated for use in that facility only and would be washed and remain within the facility, this should prevent spread of the GMOs from the facility via equipment or vehicles.

220. Maintaining buffer zones and cleaning of equipment would also help to minimise potential for mixing of different GMOs in the event that trials of other GM plants are approved to occur in the NGNE facility in the future. However, any assessment of a future licence application to grow GMOs in the NGNE facility would consider risks associated with concurrent trialling within the NGNE facility of different GM plants authorised under separate licences (as discussed in Risk Scenarios 1 and 3). Additional separation or other measures may be imposed if warranted by future risk assessments and risk management plans, but are not included in the proposed licence as no related risk currently exists.

Post-harvest management

221. The applicant has proposed a number of measures to minimise the persistence of any GM wheat or barley plants and seeds in the seed bank at the release site after harvest of the GMOs (Risk Scenario 2). These measures include tillage and irrigation to promote germination of remaining seed, and monitoring of the trial site at least every 35 days for two years. Volunteer plants that emerge would be destroyed by tilling or herbicide treatment before flowering. Waste material from the harvest would be tilled back into the soil. These measures are part of the licence conditions.

222. Viable wheat seeds have been detected in the soil over longer periods under dry conditions than under moist conditions, and wheat seeds present as un-threshed ears have longer dormancy than that of loose seeds (Komatsuzaki & Endo 1996). The minimum level of moisture necessary for germination of wheat seeds is 35 to 45% of the kernel dry weight (OGTR 2008b). In a Canadian field study of wheat, volunteer seedlings were still emerging 16 months after harvest and occasionally seedlings were observed 3 years after harvest (Anderson & Soper 2003; Harker et al. 2005). Dormancy of cereals is reduced in warmer temperatures (reviewed by Pickett 1989), so dormancy is expected to be reduced in Australian field conditions compared to western Canada. Australian barley crops do not generally show strong dormancy due to favourable environmental conditions and the varieties grown (Woonton et al. 2001).

223. There is a difference in germination rates between buried grain and grain lying on the surface; grains remaining on the surface, for example following shallow tillage after harvest, can generally easily germinate and become established (Ogg & Parker 2000). Shallow tillage after harvest, combined with irrigation, will germinate much of the seed lying on the surface (Ogg & Parker 2000). However, deep cultivation in certain soil types can reduce seed viability but can also encourage prolonged dormancy in seeds as a result of a cool, moist low oxygen environment (Pickett 1989; Ogg & Parker 2000).

224. It is therefore considered that under Australian conditions three irrigations, combined with appropriate tillage, and monitoring for and destruction of volunteers for at least 24 months, and until no volunteers are found for at least six months, would effectively manage survival and persistence of viable wheat seeds in the soil. The initial irrigation should take place within 60 days of harvest, which will encourage surface seed to germinate. The remaining two irrigations should take place at a minimum of 4 week intervals, with the last irrigation occurring during the final six months of the monitoring period. Tillage should not occur within 4 weeks after harvest, to promote after-ripening of seed. At least one tillage, to no deeper than the original sowing depth, must occur prior to the final required irrigation. All tillages must be to a depth no greater than the original depth

of sowing. These treatments will ensure seeds are exposed to sufficient moisture and placed at an appropriate depth for germination, as well as encouraging the microbial decomposition of any residual seed.

225. These measures will manage the persistence of the GMOs in the trial site (Risk Scenario 2) and are licence conditions, applying to the area where the GMOs have been grown and the surrounding buffer zone (see above). Deep tilling is not permitted.

Use of break crops within the site

226. The applicant has proposed that areas within the NGNE facility that are not being used for growing the GM wheat and barley may be sown with break crops. These crops would likely be one or more of peas, chickpeas, or lentils, all legumes that would help remediate the soil. Spraying of these crops with a selective herbicide that targets wheat and barley would minimise the possibility of GM volunteer plants reaching maturity, flowering and setting seed. Licence conditions allow growing of break crops within the site, however such plants must be handled and controlled as if they are the GMOs or Plant Material from the GMOs.

Measures to restrict the spread of the GMOs during transport and experimentation

227. 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, storage and disposal 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 people and other organisms (Risk Scenario 1), dispersal into the environment (Risk Scenario 3) and gene flow/transfer (Risk Scenario 4). This is imposed as a licence condition.

228. Experimental analysis may take place outside the NGNE facility within a laboratory certified by the Regulator under appropriate authorisation. Should a place other than a certified facility be used, CSIRO must first obtain the Regulator's approval to use that place.

GM plant material is not permitted for use as human food or animal feed

229. The applicant does not propose using any of the plant material for human consumption. In the future, if the phenotypes of any of the GM plant lines are as desired, it is possible that these lines will eventually be developed for commercial release, including human consumption. FSANZ conducts mandatory premarket assessments of GM products in human foods. As the GM wheat and barley have not been assessed by FSANZ, a licence condition prohibits material from the trial from being used for human or animal feed.

230. In addition, since the NGNE facility is designed to be a multi-user facility, other sexually compatible GM plants may be grown there in the future (subject to separate authorisation by the Regulator). Therefore licence conditions state that if other sexually compatible GMOs are grown in the facility concurrently with the GMOs in this application, seed produced in the NGNE facility from this trial must not be used in the development of cultivars for commercial release.

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

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

- limit the release to a total area of up to 1 ha per growing season at one site in the fenced NGNE facility between May 2012 and June 2015
- surround the site by a 10 m monitoring zone in which sexually compatible plants must be destroyed before flowering or prevented from flowering

- surround the monitoring zone with a 190 m isolation zone in which no other crops of wheat and barley may be grown, and where sexually compatible species plants must be destroyed before flowering or prevented from flowering
- the monitoring zone must be maintained in a manner that does not attract or harbour rodents, and if rodent activity is detected in the site, measures must be implemented to control the rodents
- harvest the GM wheat and barley plant material separately from other crops
- clean the areas and equipment after use
- apply measures to promote germination of any wheat and barley seeds that may be present in the soil after harvest, including irrigation and tillage
- monitor 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
- all material from plants, whether GM or non-GM, grown within the site of the trial must be treated as if is GM
- destroy all GM plant material not required for further analysis or future trials
- if other sexually compatible GMOs are later approved and grown in the site, seed derived from concurrent trials must not be used for later commercial development
- not allow GM plant material to be used for human food or animal feed
- transport material from the GMOs in accordance with the Regulator's guidelines.

3.2 Other risk management considerations

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

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting structures
- a requirement that the applicant allows access to the trial site and other places for the purpose of monitoring or auditing.

3.2.1 Applicant suitability

233. 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 capacity of the applicant to meet the conditions of the licence.

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

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

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

3.2.2 Contingency plan

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

238. 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 is required within 30 days of the issue date of the licence.

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

239. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to growing the GMOs, CSIRO is also required to provide a list of people and organizations who are covered, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

240. 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.

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

- location of trial site
- expected and actual dates of planting
- actual dates of harvest and cleaning after harvest.

3.2.5 Monitoring for Compliance

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

243. 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.

244. 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 4 Issues to be addressed for future releases

245. 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 toxicity and allergenicity of plant materials from the GM wheat and barley lines
- additional phenotypic characterisation of the GM wheat and barley lines, particularly with respect to traits that may contribute to weediness, including tolerance to environmental stresses and disease susceptibility
- additional molecular and biochemical characterisation of the GM wheat and barley lines.

Section 5 Conclusions of the consultation RARMP

246. The risk assessment concluded that this proposed limited and controlled release of the GM wheat lines and barley lines on a maximum total area of 1.0 ha per year over three growing seasons in the shire of Merredin (WA), poses negligible risks to the health and safety of people or the environment as a result of gene technology.

247. 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, location 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 authorities[10] on the consultation RARMP for DIR 112

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

Summary of issues raised	**Comment**
Agree with the overall conclusions of the RARMP.	Noted
Due to the multi-user multi-trial nature of the NGNE facility, it is noted that the genetic material from one trial could contaminate that of another trial. Therefore, advises that no viable GM material should leave the NGNE facility, and if it does leave the facility is must be subject to destructive analysis. Further, advises that any grain samples leaving the facility should be ground or kibbled to destroy itheir viability.	Licence conditions require that plant material collected or harvested from a Location may be used for experimentation or analysis or storage provided this is conducted in the Location or a facility approved by the Regulator, and compliance with the Regulator's guidelines for transport, storage and disposal, which manages the risk of dispersal of the GMOs leaving the facility.
Claims that the RARMP is inconsistent on whether or not feeding trials would be conducted on humans and animals. Requests that this is clarified.	Using the GM wheat and barley, or products derived from them, in human or animal nutritional trials was not proposed by the applicant for the current DIR. This is explicit and consistent throughout the RARMP and is given effect through imposed licence conditions. The word commercial has been deleted when referring to human food trials.
Advises that the risk of allergenicity of the glucan water dikinase enzyme is low but should be clarified.	Levels of the enzyme are lower in the GMOs than in non-GM wheat, and may be undetectable in the GMOs (see Ch1 subsection 5.2.1). Further information on toxicity or allergenicity of the GMOs had been identified as a future requirement in Ch3 of the RARMP (section 4).

[10] GTTAC, State and Territory Governments, Australian Government agencies, LGAs and the Minister for the Environment.

Summary of issues raised	Comment
Advises that more detailed examination of the potential effects of combining the traits within this trial or with traits from other current trials is needed. This should inform data collection requirements. Advises that prior to larger scale trials it would be prudent to conduct a fore-sighting exercise on all wheat and barley trials in Australia to determine data gaps and whether the interaction of these crops with the Australian environment may change.	More detail was added on the potential effects of the combination of the introduced traits on toxicity and allergenicity (Risk Scenario 1), and on spread and persistence of plants (Risk Scenario 2). The limits and controls minimise the likelihood of other GM traits combining with those in the GMOs to be release in this trial. The RARMPs provide broad guidance on data requirements for possible future larger scale or commercial releases. The OGTR also strongly encourages potential applicants to discuss details of data required prior to submission of such applications. If sufficient data is not presented in a future application, the application may not be accepted, more data may be requested from the applicant, risk treatment measures imposed or a licence refused if the risks cannot be adequately managed.
In risk scenario 2, characteristics of non-GM wheat and barley are used to justify the conclusion that certain abiotic and biotic factors would limit spread and persistence of the GM wheat and barley. However the modifications have the potential to increase the persistence of the GMOs. Therefore any conclusions on the importance of these factors to the GMOs should be made after the trial data are available. However, the trial containment conditions adequately manage any risk from this pathway.	The purpose of some of the genetic modification is to produce plants that are more productive in agricultural environments than non-GM wheat or barley plants. These changes could also result in greater persistence in agricultural and other land uses, Risk Scenario 2 has been modified to better reflect this. However, modern wheat and barley cultivars, some of which are bred for high vigour, are not recognised as significant weed risks in Australia, and there have been no reports of bread wheat or barley becoming an invasive pest in Australia or overseas. The limits and controls of the trial will restrict spread and persistence of the GM plants.
While substantial phenotypic changes would have been detected during development of the GMOs, there may be unidentified subtle changes. The statement (paragraph 180) that "unintended changes that occur as a result of gene insertions are rarely advantageous to the plant" does not appear to be supported by available evidence. Notes that Bollgard II cotton, genetically modified for insect resistance, also has reduced water utilisation. Positive changes in plant performance will be selected for.	While some counter examples may be found, most unintended changes are not of any advantage to the plant. For GM Bollgard II cotton, the intended change is resistance to a major insect pest. As a result of reduced insect attack the plants tend to grow faster. Improved water use efficiency is also likely due to reduced insect attack, and so results directly from the intended change. The limits and controls of the trial will restrict spread and persistence of the GM plants.
The copy number of the RNAi construct has not been verified. Due to complex compensation mechanisms occurring in the plant, the result of those modifications cannot be precisely predicted. Only observations during the field trial can show if there are subtle phenotypic changes which are advantageous to the plant.	The absence of information on inserted gene copy number is noted in Chapter 1, section 5.7.1. The issue of unforeseen results of the modifications is addressed in Risk Scenario 5. Any unforeseen phenotypic changes will likely be observed during the trial. The range of possible unintended effects produced by genetic modification is not likely to be greater than that from accepted traditional breeding techniques. The likelihood of any unintended outcomes of the genetic modifications causing adverse effects would be minimised by the proposed limits and controls.
Until the effects of a particular genetic modification are characterised in a commercial product [i.e. an elite Australian wheat or barley variety] it is not possible to give an assurance of safety that the phenotype will be within the known range.	The limits and controls of the trial will restrict the spread and persistence of the GM plants to be trialled. If a commercial release application is received in the future, the risks associated with the release of the commercial product would be assessed prior to approval.
The future research requirements identified in the RARMP are appropriate. However, more detailed guidance would ensure that more specific data are available to assess the risks arising from any larger scale or commercial release of these GMOs, particularly with respect to traits which may	The RARMPs provide broad guidance on data requirements for possible future larger scale or commercial releases. The OGTR also strongly encourages potential applicants to discuss details of data required prior to submission of such applications. If sufficient data is not presented in a future application, the application may not be accepted, more data

Summary of issues raised	Comment
contribute to weediness. The following additional data are suggested: • relative growth rates and tolerance to conditions such as water logging • biomass accumulation and harvest index • effect on tillering, grain size, root structure and root depth • comparison of potential weediness of the GMOs to elite cultivars	may be requested from the applicant, risk treatment measures imposed or a licence refused if the risks cannot be adequately managed. The applicant has been advised of these suggestions.
The Regulator should consider clarifying arrangements for buffer zones between the trials at the location.	The buffer zone requirement has been clarified in chapter 3, subsection 3.1.1. Licence conditions require that Locations are surrounded by a buffer zone. The wording in the RARMP and licence related to buffer zones and the use of seed harvested from the site if there were multiple trials of the compatible GMOs was also raised in the pre-meeting comments and are addressed above. These have been clarified in the RARMP and licence.
The Regulator should consider clarifying the wording in the RARMP relating to handling of GM and non-GM material grown at the location.	Licence conditions have been clarified to make it clear that if GMOs are grown in the NGNE facility under this licence concurrently with other sexually compatible GMOs, approved under a separate licence, the seed from the GMOs of this licence can not be used in the future development of cultivars for commercial release. Use of seed from the GMOs from any other licence would be addressed in the relevant RARMP and licence.

Appendix B Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 112

The Regulator received forty-four submissions from the public on the consultation RARMP. The issues raised in these submissions are summarised in the table below. All issues raised in submissions that related to risks to the health and safety of people and the environment 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.

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

Issues raised: E: Environmental risk; **H**: Human health; **RA**: Risk analysis; **S**: Segregation; **W**: Weediness

Other abbreviations: Act: *Gene Technology Act 2000*; **Ch**: Chapter; **FSANZ**: Food Standards Australia New Zealand; **GM**: Genetically Modified; **GMO**: Genetically Modified Organism; **LC**: Licence Conditions; **RAF**: Risk analysis framework; **RARMP**: Risk Assessment and Risk Management Plan.

Sub. No:	View	Issue	*Summary of issues raised*	Comment
1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 39, 40, 41, 42, 43, 44	x	E, S	Contamination of non-GM plant material and the environment by GM plant material (including the risks posed to Australia's international wheat markets).	The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. The current application is not for a large scale or commercial release of GM wheat or barley. The RARMP concludes that proposed field trial poses negligible risks to people and the environment. However, a range of licence conditions have been imposed to limit the release to the size, location and duration requested by the applicant as these were important considerations in the assessment process. As well as limits on the scale of the release, control measures have been imposed to restrict the spread and persistence of the GMOs and their introduced genetic material. When deciding whether or not to issue a licence, matters that relate to marketing and trade, including coexistence of GM and non-GM crops, are outside the legislative responsibility of the Regulator. These matters are addressed by the States and Territories and industry.
1, 4, 5, 8, 9, 11, 13, 14, 15, 16, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 39, 40, 41, 42	x	H	GM foods are dangerous to human health, or have not been tested for their effects on human health.	The current application is for a limited and controlled release (field trial) of GM wheat and barley. No material from the trial will be used for human food or animal feed. FSANZ approval would need to be obtained before materials from these GMOs could be sold as food.
10	x	E, W	GM wheat may cross with "couch or other grasses" and produce a "super weed".	The issue of hybridisation of wheat with other plants has been considered in Risk Scenario 4 of the RARMP. Wheat will not hybridise with couch grass. In WA, where the proposed trial would take place, the only plants with which wheat could hybridise, and produce fertile progeny, are other wheat plants. Conditions are imposed to restrict gene flow from the trial to related species.

Sub. No:	View	Issue	*Summary of issues raised*	Comment
17, 18, 39	x	E, W	Association of GM crops with the use of chemicals. Concern over the development of "super weeds" from the use of herbicides on GM crops, and a supposed link between a decline in bee numbers and the use of chemical sprays on GM crops.	The genetic modifications to the GM plants are not associated with the use of any agricultural chemicals (most obviously, these modifications do not produce herbicide tolerance). There is no scientific evidence to support a link between decline in bee numbers (colony collapse disorder or CCD) and GM crops. Current scientific evidence suggests that CCD is likely to be linked to a combination of factors contributing to the stress of honey bees.
36	x	E, S	Animals and/or farm equipment may move GMOs away from the trial site.	Licence conditions impose a range of measures to restrict the spread of the GMOs from the trial site, including fencing, cleaning of equipment, and transport of material according to the Regulator's guidelines. The NGNE facility is also covered by bird netting.
38	y	E, RA	Support the risk assessment and believe that the proposed containment conditions mean that the trial would not pose a risk to the environment.	Noted
40	x	E	The GM plants may have deleterious effects upon animals, birds, bees and fish.	The RARMP concludes that the proposed field trial poses negligible risks to people and the environment. The field trial is of small scale (1 ha) and short duration (3 years). In addition, control measures have been imposed to restrict the spread and persistence of the GMOs and their introduced genetic material, which will restrict exposure of animals to the GMOs. The genetic modifications are not expected to alter the toxicity of the plant material compared to non-GM wheat and barley. Plant material from the GMOs will not be used for food or animal feed.
43	x	E	Soils will become "degraded and useless" if sown with GM plants.	The RARMP concludes that the proposed field trial poses negligible risks to people and the environment.
44	x	E, S	Concern about seed dispersal by water runoff, wind, and soil absorption.	Risk Scenarios 2 and 3 deal with spread and persistence, including seed dispersal by water runoff and extreme weather. The RARMP concludes that proposed field trial poses negligible risks to people and the environment. However, a range of licence conditions have been imposed to limit the release to the size, location and duration requested by the applicant as these were important considerations in the assessment process. As well as limits on the scale of the release, control measures have been imposed to restrict the spread and persistence of the GMOs and their introduced genetic material. For example, measures to promote germination of seeds in the soil are imposed.
39	x	H, RA	Information necessary to conduct "adequate pre-clinical evaluation" is withheld as CCI.	The information that is declared as CCI pertains to the DNA sequences of oligonucleotides that may be used to identify GM plants, and is not relevant to the risk assessment. CCI is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.
39	x	S	The location of the trial site should be publicised in order that local producers are aware of its presence.	The Gene Technology Act requires public notification of the consultation RARMP on the OGTR website, in the Australian Government Gazette, and a newspaper circulated generally in all States. The Regulator also published notifications in the *West Australian* and *Farm Weekly*. The location of the trial is provided in the RARMP. Trial site details will also be included on the Maps page on the OGTR website once the trial has been planted.

Sub. No:	View	Issue	*Summary of issues raised*	Comment
2, 27, 30	x	S	Australia's regulatory system is not solid enough to protect farmers who use non-GM crops from contamination with GM plant material.	The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. The RARMP concludes that the proposed field trial poses negligible risks to people and the environment. As the current application is for a limited and controlled release (field trial), a range of licence conditions have been imposed to limit the release to the size, location and duration requested by the applicant. As well as limits on the scale of the release, stringent control measures have been imposed to restrict the spread and persistence of the GMOs and their introduced genetic material. When deciding whether or not to issue a licence, matters that relate to marketing and trade, including coexistence of GM and non-GM crops, are outside the legislative responsibility of the Regulator. These matters are addressed by the States and Territories and industry.
8	x	RA	The Regulator "rubber stamps" the approval of the trials of GM plants.	The Regulator prepared a comprehensive RARMP, in accordance with the requirements of the Act, and concluded that the proposed field trial poses negligible risks to people and the environment. RARMPs apply a *Risk Analysis Framework* based on the internationally recognised Australia-New Zealand Standard on Risk Management (AS/NZS 4360:2004) and include a comprehensive and critical assessment of data supplied by the applicant, together with a thorough review of other relevant national and international scientific literature. Advice on risks to human health and safety and the environment from experts, other government agencies and authorities is also taken into consideration prior to making the decision.
37	x	E	The trial would contravene the clause of the Gene Technology Act that states the object of the Act is to "protect the health and safety of people and to protect the environment" (Part 1 - Preliminary).	The RARMP concludes that the proposed field trial poses negligible risks to people and the environment.
7, 10, 11, 21, 26, 40	x	H, E	Biotechnology companies are more concerned with profits than the environment and the welfare of people	The RARMP concludes that the proposed field trial by CSIRO poses negligible risks to people and the environment.