Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan

Application for licence for dealings involving an intentional release into the environment

DIR 053/2004

Title: Field trial of genetically modified salt tolerant wheat on saline land

Applicant: Grain Biotech Australia Pty Ltd

April 2005

Abbreviations

ACT	Australian Capital Authority
APVMA	Australian Pesticides and Veterinary Medicines Authority
bp	Base pair
C	Carbon
cah	Gene encoding CAH
CAH	Cyanamide hydratase
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DIR	dealing involving intentional release
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
EMBL	European Molecular Biology Laboratory
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organisation of the United Nations
FSANZ	Food Standards Australia New Zealand
g	gram
GM	genetically modified
GMAC	Genetic Manipulation Advisory Committee
GMO	genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
ha	hectare
IgE	immunoglobulin E
kDa	Kilodalton
km	Kilometre
m	Metre
mRNA	messenger ribonucleic acid
N	Nitrogen
NSW	New South Wales
oat	Gene encoding OAT
OAT	Ornithine aminotransferase
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator
PCR	polymerase chain reaction
RNA	ribonucleic acid
SA	South Australia
T-DNA	transfer deoxyribonucleic acid
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
µg/g	micrograms per gram
WA	Western Australia
WHO	World Health Organisation

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EXECUTIVE SUMMARY

INTRODUCTION

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving intentional release of GMOs into the environment, in respect of application DIR 053/2004 from the Grain Biotech Australia Pty Ltd (GBA).

The *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001* (the Regulations) set out requirements which the Gene Technology Regulator (the Regulator) must follow when considering an application for a licence to intentionally release a genetically modified organism (GMO) into the environment.

For a licence to be issued, the Regulator must be satisfied that the release will not pose any risks to human health and safety and the environment that can not be managed. As part of the evaluation process, Section 51 of the Act requires the Regulator to prepare a risk assessment and risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and stakeholders.

Under Section 52 of the Act, the Regulator is required to seek comment on the RARMP from those consulted in its preparation and to invite submissions from the public. Matters raised relating to the protection of human health and safety or the environment are taken into account in finalising the RARMP, which then forms the basis of the Regulator's decision on whether, or not, to issue a licence, and if so, what conditions to impose.

The Act is designed to operate in a cooperative legislative framework with other regulatory authorities that have complementary responsibilities and specialist expertise. As well as enhancing coordinated decision making, this arrangement avoids duplication. The OGTR liaises closely with other regulators to ensure the identification, evaluation and management of risks that may be associated with development and use of gene technology.

THE APPLICATION

Grain Biotech Australia Pty Ltd (GBA) licence application number DIR 053/2004 requested approval for the intentional release, under limited and controlled conditions, of two genetically modified (GM) wheat lines[1]. The aim of the trial is to evaluate the salt tolerance and agronomic performance of the GM salt tolerant wheat on a site affected by different levels of salinity.

The release will take place during the winter growing season in Corrigin shire in Western Australia (WA) on a single site of 0.45 ha from Apri1 2005 to January 2006. Part of the release site includes a salt scald, ie an area affected by high salt levels. During the trial, the agronomic performance and salinity tolerance of the GM wheat will be compared with non-GM bread wheat, and non-GM salt adapted bread wheat.

The genetic modification consists of the introduction of two genes, the ornithine aminotransferase gene (*oat*) derived from the common plant species, *Arabidopsis thaliana* and the cyanamide hydratase gene (*cah*), from the soil fungus *Myrothecium verrucaria*.

[1] The term 'line' has been used throughout this RARMP to denote wheat containing a specific genetic modification derived from a single transformation event.

The *oat* gene encodes the enzyme[2] ornithine aminotransferase (OAT) which is part of a metabolic pathway that can lead to the production of the amino acid proline. Over-expression of OAT can increase the levels of the amino acid proline[3] in the plant. Proline is an unreactive compound that can serve as an osmoprotectant and enable plants to grow in the presence of elevated salt levels in soil.

The *cah* gene encodes the enzyme cyanamide hydratase (CAH) which confers tolerance to the herbicidal compound cyanamide. The *cah* gene was used as a selective marker in the selection of transformed plants in the laboratory.

GBA proposed a range of containment and inspection measures (detailed in Chapter 1), in part to limit the possible spread and persistence of the GM wheat lines, but also to maintain the integrity of the trial. The proposed measures were considered during the risk assessment of the application and in the preparation of the risk management plan.

None of the GM wheat plants from the release, or their by-products, would be used for animal feed or human food, and seed not required for possible future trials (subject to approval) or research would be destroyed. This GM wheat would require approval by Food Standards Australia New Zealand (FSANZ) before use for human food.

There have been no previous releases of GM wheat lines containing either the cah or oat genes in Australia. The GM wheat lines were originally developed in contained laboratories and glasshouses within the provisions of the Act, under NLRD 239/2002.

However, on 13 April 2005 the Regulator approved a limited and controlled field trial of a wheat genetically modified for altered grain starch (DIR054/2004).

In addition, under the former voluntary system that was overseen by the Genetic Manipulation Advisory Committee (GMAC) five field releases of other types of GM wheat were approved (see Chapter 1 of the risk assessment and risk management plan). There have been no reports of adverse effects on human health or the environment resulting from these releases.

THE EVALUATION PROCESS

A risk assessment and risk management plan (RARMP) has been prepared in relation to licence application DIR 053/2004 from GBA in accordance with the Act, the Regulations and the *Risk Analysis Framework*. This framework was developed as part of the establishment of the regulatory arrangements in consultation with the public, State, Territory and Australian Government agencies, key stakeholders and the Gene Technology Technical Advisory Committee[4].

Details of the process that the Regulator must follow, including the prescribed consultation process on the application, and the matters that she must consider in preparing a RARMP, are set out in Appendix 6 of the RARMP. The complete RARMP, a set of Questions and Answers on the decision on this application and a review document 'The Biology and Ecology of Bread Wheat (*Triticum aestivum* L. em Thell.) in Australia' (produced to further inform the risk analysis) can be obtained from the OGTR by contacting the Office on 1800 181 030 or from the OGTR's website at *www.ogtr.gov.au.*

[2] An enzyme is a protein that catalyses a specific biochemical reaction.
[3] Proline is one of the 20 amino acids that are the building blocks of all proteins.
[4] The *Risk Analysis Framework* has been recently revised (refer 'What's New?' at www.ogtr.gov.au) but was not applied to this RARMP as the consultation version was completed prior to the review's finalisation.

The risk assessment considered information contained in the application (comprising: information required by the Act and the Regulations on the GMO; on the parent organism, the proposed dealings, including proposed containment conditions; and potential impacts on human health and safety and the environment), current scientific knowledge, and submissions received during consultation with expert groups and authorities and the public (issues raised in submissions are summarised in Chapter 2 and Appendix 7 of the RARMP).

Through this process, potential hazards were identified to human health and safety or the environment that may be posed by the release of the two GM wheat lines. These have been carefully evaluated to determine whether risks might arise, based on the likelihood of each hazard occurring and the likely impact of each hazard were they to be realised.

The identified potential hazards relate to:

- **toxicity and** allergenicity to humans and other organisms: could these GM wheat plants be more toxic or allergenic to humans than non-GM wheat, or harmful to other organisms as a result of the novel gene products, altered proline content or because of unintended effects?
- weediness: could the genetic modifications be harmful to the environment by increasing the potential for these wheat plants to establish as problem weeds?
- transfer of introduced genes to other organisms: could there be adverse consequences from potential transfer of the introduced genes to non-GM wheat crops, naturalised wheats, or to other organisms?

CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator has concluded that the limited and controlled release of the two GM wheat lines over one season will not pose significant risks to human health and safety and the environment as a result of the genetic modification. The risk assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans and other organisms

The two GM wheat lines are unlikely to prove more toxic or allergenic to humans or other organisms than conventional wheat.

Neither the OAT protein nor the CAH protein are known to be toxic or allergenic, nor are they structurally similar to known protein toxins or allergens. Humans and other organisms are already exposed to both proteins.

The OAT enzyme forms part of a pathway for the metabolism of proline in many micro-organisms, plants and animals, including humans, and it is therefore widespread in the environment. The introduced OAT protein from *A. thaliana* is very similar to the OAT proteins of other plants, animals and microorganisms.

The *cah* gene was derived from the soil fungus *Myrothecium verrucaria* and genes thought to encode CAH enzymes have been identified in a number of other fungi and bacteria. As cyanamide is rapidly broken down by microbial activity in soil, CAH enzymes are likely to be widespread in the environment.

The effect of the introduction of the *oat* gene in the GM wheat lines is to increase levels of proline expression within the plants' cells. Under glasshouse conditions, the concentration of free proline in the GM wheat plants is increased approximately 3-fold. Proline is present in all organisms and is therefore ubiquitous in the environment and in food. It is not considered toxic even at high doses. Elevated levels of proline occur naturally in salt tolerant plant species, and proline levels can be increased in many plants in response to other environmental stresses. The level of free proline expected in the GM wheat plants is therefore highly unlikely to pose a risk of toxicity.

The level of occupational exposure to the proteins produced by the action of the introduced genes through working with the GM wheat is likely to be very low. Furthermore, exposure to the GM wheat would be limited as the release is limited in scale and licence conditions have been imposed to limit unintended exposure to the GMOs (refer to key licence conditions below).

The applicant does not intend to use any material produced in the release in human food or animal feed, thus limiting potential exposure. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment, and FSANZ approval would be needed before products from these GM wheats could be used in human food.

Weediness

The domestication of *Triticum aestivum* L. into what we now know as bread wheat resulted in the loss of most of the characteristics that contribute to successful weediness, such as competitive ability and seed heads that shatter at maturity. Wheat is not considered a problematic weed in Australia. The germination and persistence of non-GM wheats in Australia are limited by the availability of adequate soil moisture and nutrients, herbivory (vertebrate and invertebrate), fire, plant competition and/or frost.

The GM wheat has been modified to achieve salt tolerance through having increased levels of proline as a result of overexpression of the *oat* gene. The GM wheat lines grown hydroponically in the glasshouse under salt-stress conditions (150 mM NaCl) show a two-fold increase in tiller number, seed number and seed weight relative to non-GM wheat. Under these salt-stress conditions growth of non-GM wheat is severely impaired.

Elevated proline levels are also thought to confer tolerance to some other environmental stresses, including frost and moisture stress. The GM wheat might therefore have some advantage over non-GM wheat in response to frost or drought as well as saline environments. However, the GM wheat plants would still be limited by water availability and the range of other environmental factors which normally limit the persistence of wheat plants in Australia.

The parental cultivars of the GM wheat lines do not have any significant seed dormancy. While the genetic modifications may provide the GM wheat with an advantage in some environmental conditions relative to non-GM wheat, they are unlikely to increase other characteristics normally associated with intrinsic weediness.

The applicant has not observed any unintended or secondary effects in the GM wheat lines grown under glasshouse conditions and reports that the growth characteristics of the GM wheat lines are similar to those of conventional wheat. However, under non-saline growth conditions the GM wheat plants are slightly smaller than non-GM plants. Therefore it is

possible that there is a metabolic cost incurred through the overproduction of proline which may impact on the overall fitness of the GM wheat.

The *cah* gene introduced to the GM wheat lines confers tolerance to the herbicidal compound cyanamide. This tolerance was used to select transformed plants in the laboratory. Cyanamide is not registered for use as a herbicide in Australia and it would not be used during this release. Therefore the *cah* gene will not confer any advantage on the GM wheat plants.

The risk of the GM wheats establishing as problematic weeds in the release area is considered very low. The Regulator has imposed containment measures to minimise the spread and persistence of these GM wheats in the environment (refer to key licence conditions below).

Transfer of introduced genes to other organisms

Wheat is predominantly self-pollinating with rates of out-crossing to other cultivated wheat plants of less than 5% between adjacent rows. Wheat pollen is relatively heavy compared to grass pollen and does not remain viable for long periods (under field conditions, up to 30 minutes) and its dispersal is via wind, rather than by insects. Because wheat is primarily self-pollinating and pollen movement is mediated by wind, an isolation zone is a more suitable measure than a pollen trap to limit pollen escape from the release site.

Wheat can cross-pollinate with a number of species within the genus *Triticum* and related genera such as *Aegilops*, *Elytrigia*, *Hordeum* and *Secale*. Out-crossing to these species will not occur as these plants will not be present near the release site, except for those deliberately planted as part of the trial.

Non-GM bread wheat will also be planted as part of the trial. While outcrossing from the GM wheat plants to these plants is possible, these plants and resultant seed would be treated in the same manner as the GM wheat. Licence conditions have been imposed to minimise the risk of transfer of the introduced genes to plants outside the release site including the use of an isolation zone (refer to key licence conditions below).

The risk of transfer of the introduced genes to naturalised wheat is negligible due to geographic isolation. The likelihood of transfer of the introduced genes to other organisms is negligible because of genetic incompatibility. Even if such transfer occurred, it would be unlikely to pose any risk to human health and safety and the environment.

THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)

As part of the evaluation process for this licence application, a risk management plan has been developed (refer to Conclusion of the Risk Assessment, above). The applicant proposed a number of containment measures to minimise the spread and persistence of the GMOs and the introduced genes in the environment during the trial. The Regulator considered these proposals in selecting licence conditions that have been imposed to implement the risk management measures that will minimise the potential exposure of humans and other organisms and limit the likelihood of spread and persistence of the GMOs or the introduced genetic materials in the environment. The key licence conditions are outlined below.

Toxicity or allergenicity to humans and other organisms

Licence conditions have been imposed which require the applicant to:

- prevent the GMOs and products derived from the GMOs entering the human food supply;
- prevent GM wheat seed being used as stockfeed;
- limit the scale and duration of the release;
- limit exposure to humans and other animals;
- destroy all GM materials not required for any possible future trials or research;
- securely transport and store the GMOs; and
- report adverse effects to the Regulator.

Weediness

Licence conditions have been imposed which require the applicant to:

- limit the scale and duration of the release;
- locate the release site at least 50 m from natural waterways;
- contain the GM wheats with a 1.8 m fence to exclude rabbits and large animals;
- take measures to minimise rodent numbers, including mowing the 10 m monitoring zone around the trial site;
- use of bird proof netting to prevent birds entering site to minimise seed dispersal;
- securely transport and store the GM wheat material and seeds;
- clean the release site after harvest and equipment used at the site; and
- monitor the release site and the 10 m monitoring zone after harvest and destroy volunteers for at least 24 months.

Transfer of introduced genes to other organisms

Licence conditions have been imposed which require the applicant to:

- limit the scale and duration of the release;
- surround the GM wheat lines with a 500 m isolation zone in which no other wheat or sexually compatible plants are planted;
- monitor the release site and the 10 m monitoring zone after harvest and destroy volunteers for at least 24 months; and
- clean the release site after harvest and equipment used at the site.

General conditions

Any licence issued by the Regulator also contains a number of general conditions which are also relevant to risk management. These include, for example:

- identification of the persons or classes of persons covered by the licence;

- a requirement that the applicant allows access to the release site by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing; and
- a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to human health or safety or to the environment.

Chapter 2 of the RARMP provides a tabulated summary of assessment conclusions and corresponding management conditions. Full details of the imposed licence conditions are provided in Appendix 5.

Identification of issues to be addressed for future releases

The limited and controlled release is a small scale, single-site 'proof of concept' trial over one growing season, from April 2005 – January 2006, to test the efficacy of proline overexpression as an osmoprotectant in saline field conditions and to compare the GM wheats' field performance with conventional wheat. Hence, no research conditions have been imposed in the licence. However, the following information would be required from future applications, particularly to assess requests for larger scale releases of these GM wheat lines:

- the level of expression of the introduced genes and encoded OAT and CAH proteins, and the plant tissues (including pollen) and developmental stages in which they are being expressed;
- the level of free proline and metabolites present in various tissues at different developmental stages of the GM wheat plants under Australian conditions;
- genetic segregation and molecular characterisation of the introduced genes;
- the potential toxicity and allergenicity of the GM wheat, particularly the introduced OAT and CAH proteins;
- the magnitude of the tolerance of the GM wheat to salt and other abiotic stresses;
- agronomic characteristics of the GM wheats relating to fitness and potential weediness;
- the occurrence of gene flow from GM wheat to non-GM wheat under Australian field conditions; and
- any unintended or secondary effects resulting from the genetic modification.

It should be noted that provision of the above data during the release is not required to ensure the management of risks to human health and safety and the environment from this release. The risk management measures summarised in Chapter 2, Table 3 and given effect by the imposed licence conditions, will achieve this purpose

Monitoring and enforcement of compliance by the OGTR

As well as the legislative capacity to enforce compliance with licence conditions, the Regulator has additional options for risk management. The Regulator can direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment. The OGTR also independently monitors releases that the Regulator has authorised. At least 20% of all field trial sites will be inspected each year, in accordance with a monitoring and compliance strategy based on risk profiling (which takes into account biological, seasonal, geographical and ecological risk factors) to determine

whether licence holders are complying with the licence conditions, or whether there are any unforeseen problems.

FURTHER INFORMATION

Detailed information on the evaluation of the application, including the licence conditions, is available in the risk assessment and risk management plan document for this application, which can be obtained from the website of the Office of the Gene Technology Regulator (*www.ogtr.gov.au*), or by calling 1800 181 030 (please quote application number DIR 053/2004).

CHAPTER 1 BACKGROUND

1. This chapter provides background information about the application and previous releases of relevant genetically modified organisms (GMOs) into the environment.

SECTION 1 THE APPLICATION

2. The OGTR has received an application (licence application number DIR 053/2004) from Grain Biotech Australia Pty Ltd (GBA) for the intentional release of genetically modified (GM) wheat into the environment, on a limited scale and under controlled conditions. Key information on the application is given below:

Project Title:	**Field trial of genetically modified salt tolerant wheat on saline land**
Applicant:	Grain Biotech Australia Pty Ltd
Common name of the parent organism:	Bread wheat
Scientific name of the parent organism:	*Triticum aestivum* L.
Modified trait(s):	Salt tolerance, herbicide tolerance
Identity of the genetic elements responsible for the modified trait(s):	• Ornithine aminotransferase[5] (OAT) from *Arabidopsis thaliana* (salt tolerance) • Cyanamide hydratase (CAH) from *Myrothecium verrucaria* (selective marker, cyanamide tolerance)
Proposed Location(s):	Corrigin shire, Western Australia (WA)
Proposed Release Size:	0.45 ha
Proposed Time of Release:	April 2005 – January 2006

Section 1.1 The Proposed dealings

3. Grain Biotech Australia proposed the conduct of a small scale, limited and controlled release of GM wheat on one site covering an area of 0.45 hectares in the Corrigin shire of Western Australia. The release is planned for April 2005 to January 2006.

4. The applicant proposed to evaluate and compare the salt tolerance and agronomic performance of the GM salt tolerant wheat with non-GM wheats and other salt tolerant crops on a site affected by different levels of salinity.

Section 1.2 Parent organism

5. The parent organism is wheat (*Triticum aestivum* L.) which belongs to the family Poaceae (Graminae). The taxonomy of wheat is complex however it is thought that modern wheat varieties are probably derived from the einkorn lineage (*Triticum boeoticum*) hybridising with the emmer lineage (*T. dicoccides*) and incorporating

5 The ornithine aminotransferase is a δ-OAT, as distinct from an α-OAT which catalyses a different reaction. References to OAT throughout this RARMP are to δ-OAT.

germplasm from *Aegilops tauschii* (also called *T. tauschii* or *Ae. squarrosa*) (van Slageren 1994). Wild populations of these species originated and still exist in southeast Turkey, through to the Mediterranean and into the Middle East.

6. Bread wheat is exotic to Australia, but has been grown since European settlement in 1788. It is widely cultivated from southern Queensland, through New South Wales and Victoria to eastern South Australia and in the grain belt in the south of Western Australia. Planting can occur between early April and late June and is determined by soil moisture availability and whether the cultivar is a winter type or spring type. Harvest normally takes place between late November and late December.

7. More detailed information on bread wheat can be found in a review document 'The Biology and Ecology of Bread Wheat *(Triticum aestivum* L. em Thell.) in Australia' that was prepared in order to inform the risk assessment processes for licence applications involving GM wheats. This document is available at *www.ogtr.gov.au/.*

8. The field trial involves two commercial bread wheat cultivars, 'Westonia' and 'Carnamah' that have been genetically modified independently.

Section 1.3 Genetic modification and its effect

9. The genetic modification consists of the introduction of two genes, the ornithine aminotransferase gene (*oat*) derived from the common plant species *Arabidopsis thaliana*, and the cyanamide hydratase gene (*cah*), from the soil fungus *Myrothecium verrucaria*.

10. *M. verrucaria* is plant pathogen which produces a class of mycotoxins known as trichothecenes which have phytotoxic activity. Only the *cah* gene from *M. verrucaria* was introduced to the GM wheat. It represents only a small proportion of the *M. verrucaria* genome and is not involved in the production of the mycotoxins.

11. The *oat* gene encodes the enzyme[6] ornithine aminotransferase (OAT). OAT catalyses the conversion of ornithine into pyrroline-5-carboxylate (P5C), the subsequent conversion of P5C into the amino acid proline is catalysed by pyrroline-5-carboxylate reductase (P5CR). Over-expression of the OAT enzyme is capable of increasing free proline levels in the plant.

12. Proline is one of the 20 amino acids present in all organisms that are the building blocks of proteins. Proline itself is an unreactive compound that can serve as an osmoprotectant[7] that enables plants to grow in the presence of elevated salt levels in soil. The level of free proline is known to increase in many plants in response to saline conditions and a number of other environmental stresses such as frost and water stress. Many naturally occurring salt-tolerant plants (halophytes) have higher levels of free proline than other plants.

13. The *cah* gene encodes the enzyme cyanamide hydratase (CAH). CAH confers tolerance to the herbicidal activity of cyanamide by catalysing its conversion to urea. The *cah* gene was used as a selective marker in the selection of transformed plants in the laboratory. The trial does not involve the application of herbicide.

[6] An enzyme is a protein which catalyses a specific biochemical reaction.
[7] An osmoprotectant assists plants to regulate the pressure inside cells by adjusting the concentration of water and salts.

14. The expression of both the *oat* and *cah* genes is controlled by regulatory sequences obtained from maize (*Zea mays* L.) - the *Ubi*-1 promoter and the *zein* terminator.

15. Further details on the introduced genetic materials, their products and mechanism of action are provided in Appendix 1, Section 3.

Section 1.4 Method of genetic modification

16. The two GM wheat lines approved for release were selected through two generations of self pollination in the glasshouse. The GM wheat line 'Westonia' is designated 2490-1 and the GM wheat line 'Carnamah' is designated as 2721-1.

17. The genes were introduced into the wheat genome by microprojectile bombardment. Linear fragments of DNA containing the *oat* and *cah* genes and associated regulatory elements were coated onto gold particles and shot into wheat embryos using a helium pressure gun.

18. The successfully transformed embryos were selected by cultivation in the presence of cyanamide and regenerated into plantlets which formed the basis of the individual GM wheat lines.

SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

Section 2.1 Previous Australian releases of GM wheats

19. The GM wheat lines are derived from laboratory and glasshouse based research under the provisions of the Act under NLRD 239/2002. There have been no previous releases of these GM wheat lines in Australia.

20. A small scale limited and controlled field trial of GM wheat with altered starch characteristics to be conducted by CSIRO Plant Industry in ACT under Licence DIR054/2004 was approved for release on 13 April 2005.

21. In addition, under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), five field releases of other GM wheat with different genetic modifications were authorised as summarised in Table 1.

Table 1 Previous releases of GM wheat under the GMAC system

Reference No.	Date	Applicant	Introduced Trait †	Size of Release	Location
PR65	1996 – 1997	CSIRO Plant Industry	Herbicide tolerance	0.01 ha combined (325 plants for each release)	ACT
PR66			Altered grain starch composition / antibiotic resistance		
PR102	1998 – 1999	CSIRO Plant Industry	Altered grain protein composition / herbicide tolerance	0.04 ha per season (1500 plants per season)	ACT
PR102X	2000 – 2001				
PR107	1999 – 2000	University of Adelaide	Visual selectable marker / herbicide tolerance	0.04 ha (600 plants)	SA

† herbicide tolerance in this table refers to resistance to glufosinate ammonium.

22. There have been no reports of adverse effects on human health or the environment resulting from any of these releases under the former voluntary system.

Section 2.2 Approvals by other Australian government agencies

23. The OGTR is responsible for assessing the risks to human health and safety and the environment associated with development and use of gene technology. Other government regulatory requirements would also have to be met in respect of the release of the GMOs, including the requirements of Food Standards Australia New Zealand (FSANZ), if material from the GM wheats was proposed for use in human food.

2.2.1 Food Standards Australia New Zealand

24. FSANZ is responsible for human food safety assessment and food labelling, including GM food. Currently, the applicant has not applied to FSANZ for evaluation of material from the GM wheats proposed for release for use in human food and has proposed other measures to prevent its entry into the human food chain. FSANZ's approval would need to be obtained before such material could be used for this purpose.

25. Further information about food safety and food labelling is available from FSANZ:

Food Standards Australia New Zealand
PO Box 7186
Canberra Mail Centre ACT 2610
Phone (02) 6271 2222
Fax (02) 6271 2278
E-mail info@foodstandards.gov.au
http://www.foodstandards.gov.au

Section 2.3 International approvals

26. The two GM wheat lines approved for release under the current application were developed in Australia and have not been released in other countries. Table 2 lists the recent applications for field releases of GM wheat in Europe under limited and controlled conditions (from *gmoinfo.jrc.it/gmp_browse_geninf.asp*).

27. None of the GM wheat types in the previous table are similar to those approved for release in this field trial.

28. The Canadian Food Inspection Agency (CFIA) website lists numerous field trials of plants with novel traits, including wheats, in recent years. Field trials of GM disease resistant and herbicide tolerant wheats have been conducted in Canada (*www.inspection.gc.ca/english/plaveg/bio/triesse.shtml*).

Table 2 Recent applications for field releases of GM wheat in Europe under limited and controlled conditions
(adapted from *gmoinfo.jrc.it/gmp_browse_geninf.asp*)

Date	Notification	Title	Introduced trait	Proponent
4/10/2002	B/DE/02/143	Fungal resistant wheat in Germany	FRG: gene of fungal origin conferring tolerance to Fusarium pathogens; PMI: Phosphomannose Isomerase gene isolated from E.coli, conferring tolerance to mannose	Syngenta GmBH
2/12/2002	B/GB/02/R34/4	To compare the pathogen infestation level and mycotoxin level of wheat modified to express an enhanced resistance to Fusarium pathogens with existing non-modified varieties, grown under standard agronomic conditions	FRG: gene of fungal origin conferring tolerance to Fusarium pathogens; PMI: Phosphomannose Isomerase gene isolated from E.coli, conferring tolerance to mannose	Syngenta Seeds Ltd
10/10/2003	B/DE/03/151	Fungal resistant wheat Germany 2004 (I)	FRG: gene of fungal origin conferring tolerance to Fusarium pathogens; PMI: Phosphomannose Isomerase gene isolated from E.coli, conferring tolerance to mannose	Syngenta GmBH
14/10/2003	B/DE/03/152	Fungal resistant wheat Germany 2004 (II)	FRG: gene of fungal origin conferring tolerance to Fusarium pathogens; PMI: Phosphomannose Isomerase gene isolated from E.coli, conferring tolerance to mannose	Syngenta GmBH
09/01/2004	B/ES/04/08-CON	Evaluation in field conditions of fungal resistant wheat	FRG: gene of fungal origin conferring tolerance to Fusarium pathogens; PMI: Phosphomannose Isomerase gene isolated from E.coli, conferring tolerance to mannose	Instituto de Agricoltura Sostenibile Consejo Superior de Investigaciones Cientificas
03/05/2004	B/IT/04/02	Study of the stability of the transgene and its heritability of genetically modified wheat under open field conditions	Gene for the sub-units Dx5B, Dy10A and Ax2 of glutenin each under transcriptional control of their endogenous promoters for tissue-specific expression in wheat endosperm	Metapontum Agrobios s.c.a.r.l.

CHAPTER 2 SUMMARY OF RISK ASSESSMENT AND RISK MANAGEMENT PLAN

29. The Act and the Regulations require that risks associated with dealings with GMOs are identified and assessed as to whether they can be managed to protect human health and safety and the environment (see Appendix 6). This chapter provides a summary of the finalised Risk Assessment and Risk Management Plan (RARMP) produced in response to application DIR 053/2004 for Grain Biotech Australia Pty Ltd (GBA).

SECTION 1 ISSUES RAISED IN SUBMISSIONS ON THE APPLICATION AND RISK ASSESSMENT AND RISK MANAGEMENT PLAN

30. Comments received in response to the consultation on the application DIR 053/2004 undertaken with expert groups and key stakeholders as required by Section 50 of the Act (see Appendix 6) and with the same stakeholders and the public on the RARMP under Section 52 of the Act (see Appendix 6), were very important in finalising the RARMP which then formed the basis of the Regulator's decision on the application.

31. Written submissions in relation to DIR 053/2004 received from the agencies and authorities and the public suggested that the following issues relating to the protection of human health and safety or the environment, should be addressed in the RARMP:

- effectiveness of whistling tape as a risk management measure to deter birds (Appendix 3 refers);
- selection of measures to minimise seed dispersal and gene transfer (Appendix 3, 4 refer);
- physical integrity of the fence which will surround the trial (Appendix 5);
- adequacy of post harvest measures to control volunteers (Appendix 3, 4 and 5 refer); and
- additional research for future trials (Section 3, Chapter 2).

32. The Regulator received three public submissions on this application. A summary of these submissions is provided in Appendix 7. The key issues raised that relate to risks to human health and safety or the environment are:

- stability of the genetic modifications (Appendix 1 refers)
- adverse effects on human and animal health due to the genetic modifications (Appendix 2 refers);
- potential for adverse environmental effects (Appendix 2 refers);
- potential for seed dispersal by water (Appendix 3 refers);
- potential for gene transfer (Appendix 4 refers) and
- adequacy of containment and cleaning measures (Appendix 2, 3 and 4 refer).

33. The public submissions also raised projected impacts on international markets, and segregation issues. However, the focus of the gene technology legislation is the protection of human health and safety and the environment and these matters are outside the scope of assessments the Regulator is required to conduct under the Act.

34. In accordance with Section 56 of the Act, the Regulator has taken into account all issues raised in written submissions that related to risks to human health and safety and to the environment in finalising the RARMP. These issues were considered carefully and weighed against the body of current scientific information in reaching the conclusions set out in this document.

SECTION 2 FINALISATION OF THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

35. The Regulator has conducted a risk assessment in relation to the proposed dealings and prepared a risk management plan in accordance with the Act and the Regulations using a *Risk Analysis Framework* as detailed in Appendix 6. The RARMP was finalised after consultation with expert groups and the public (see Section 2). The risk assessment process identified a number of hazards that may arise from the proposed dealings. The risks posed by these hazards were assessed as being either *'negligible'*, *'very low'*, *'low'*, *'moderate'*, *'high'* or *'very high'*[8] by considering:

- the likelihood of the hazard occurring; and
- the likely consequences (impact) of the hazards, were they to be realised.

36. The following table (Table 3) lists each of the potential hazards that were considered during the risk assessment process in the *Hazard Identification* column and summarises the assessment of each hazard under the column headed *Risk*. A comprehensive assessment of each identified hazard is provided in Appendices 2 to 4, as cross-referenced in the column headed *Summary of Risk Assessment*.

37. Where it is considered, on the basis of a combination of possible adverse impacts and likelihood of occurrence, that risk management may be required to protect the health and safety of humans and/or the environment, the *Risk Management* column identifies the methods selected to limit the potential for risk exposure and the reasons they were chosen. The risk management plan for the proposed dealings is given effect by specific conditions within the licence. These conditions are summarised in the final column, headed *Licence Conditions*, and detailed in Appendix 5.

SECTION 3 IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES

38. The limited and controlled release is a small scale, single-site 'proof of concept' trial over one growing season, from April 2005 – January 2006, to test the efficacy of proline overproduction as an osmoprotectant in saline field conditions in comparison with conventional wheat. Hence no research requirements have been imposed in the licence conditions. However, the following information would be required from future applications, particularly to assess requests for larger scale releases of these GM wheat lines:

- the level of expression of the introduced genes and encoded OAT and CAH proteins, and the plant tissues (including pollen) and developmental stages in which they are being expressed;

[8] This RARMP was prepared and consulted on prior to finalising a review of the *Risk Analysis Framework* which is progressively leading to the application of different terminology to characterise the different elements of risk assessment.

- the level of free proline and metabolites present in various tissues at different developmental stages of the GM wheat plants under Australian conditions;
- genetic segregation and molecular characterisation of the introduced genes;
- the potential toxicity and allergenicity of the GM wheat, particularly the introduced OAT and CAH proteins;
- the magnitude of the tolerance of the GM wheat to salt and other abiotic stresses;
- agronomic characteristics of the GM wheats relating to fitness and potential weediness;
- the occurrence of gene flow from GM wheat to non-GM wheat under Australian field conditions; and
- any unintended or secondary effects resulting from the genetic modification.

39. It should be noted that collection of the above data during the release is not required to ensure the management of risks to human health and safety and the environment from this release. The risk management measures summarised in Table 3 of this Chapter and given effect by the licence conditions will achieve this purpose.

SECTION 4 DECISION ON THE APPLICATION

40. Details of the matters that the Regulator must consider in making a decision are provided in Appendix 6. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings are able to be managed so as to protect human health and safety and the environment.

41. The finalised RARMP concludes that the limited and controlled release of the GM wheat lines does not pose significant risks to human health and safety or to the environment as a result of the genetic modifications. Detailed risk analyses based on the available scientific information are provided in Appendices 2 - 4 in support of this conclusion.

42. Therefore, the Regulator has issued licence DIR 053/2004 in respect of this application. The Regulator has imposed licence conditions to minimise potential exposure of humans and other organisms, and to limit the spread and persistence of the GMOs or the introduced genetic materials in the environment.

SECTION 5 TABULATED SUMMARY OF RARMP

Table 3 Summary of the risk assessment and the risk management plan (including licence conditions)

GM wheats: the genetically modified wheat lines approved for release.
OAT: Ornithine aminotransferase (enzyme), encoded by the ornithine aminotransferase gene (*oat*). Over-expression of this enzyme can increase proline levels in the plant. Proline is found in many plants. It is an inert compound that can enable plants to grow in the presence of elevated salt levels in soil.
CAH: Cyanamide hydratase (enzyme), encoded by the gene (*cah*), which enables plants to convert cyanamide to urea.
N/A Not Applicable

Hazard Identification	Risk Estimate (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
TOXICITY AND ALLERGENICITY FOR HUMANS: Food	Very low	See Appendix 2 • none of the GM wheat materials from the release will be used in human food or animal feed; • the introduced OAT protein is from the plant *Arabidopsis thaliana* and is very similar to OAT proteins from other plants. The OAT protein is naturally produced in micro organisms, plants and animals and is therefore widespread in the environment and present in food; • a range of common fungi and bacteria contain similar gene sequences to the introduced *cah* gene that was isolated from *Myrothecium verrucaria*. CAH proteins are therefore likely to be widespread in the environment; • the introduced proteins are not known to be allergenic, nor do they have properties characteristic of known allergenic proteins; • proline is approved for food use by FSANZ; and • the amino acid proline is not considered toxic at the concentrations found in plants and animals. Many naturally occurring salt tolerant plants also have elevated levels of proline. The level of free proline in the GM wheat plants under glasshouse conditions is approximately 3 fold that of non GM wheat, but still in the range encountered in plants and no toxic effects would be expected.	Yes	• Limit scale of release: decreases likelihood of exposure. • Fence: reduce probability of unauthorised access. • Prevent seed from entering human food supply: prevents exposure through food. • Destroy all plant material not required for possible future trials or research: prevents unintended exposure. • Ensure secure transport and storage of GM plant material: prevents unintended exposure. • Clean equipment used at the release site: prevents escape of viable GM plant material.	Yes	• Limit scale: restrict area to 0.45 hectares over one growing season. • Fence: contain GM wheats within a 1.8 m high fence. • Prevent seed from entering human food supply: no materials from the GMOs to be used in human food. • Destroy plant material: destroy all seed and plant material not required for possible future trials or research. • Secure transport and storage: the GM plant material must be transported in accordance with OGTR guidelines. • Clean equipment used at the release site: equipment must be cleaned before it is used for any other purpose.

Hazard Identification	Risk Estimate (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
TOXICITY AND ALLERGENICITY FOR HUMANS: Occupational exposure	Very low	See Appendix 2 • the limited scale of the trial limits the potential risk of the wheat pollen becoming an air-borne allergen; • while conventional wheat is known to have allergenic properties, the insertion of the new genes is unlikely to alter this effect; • the introduced proteins are already present in the environment, are not known to be toxic or allergenic, and do not have properties characteristic of known toxic or allergenic proteins; and • exposure to the introduced proteins through working with wheat plants would be very low.	Yes	• Limit scale of release: decreases likelihood of exposure. • Surround the release site with a fence: prevent unauthorised access. • Restrict access to authorised personnel: limit exposure. • Destroy all plant material not required for possible future trials or research: prevents unintended exposure. • Ensure secure transport and storage of GM plant material: prevents unintended exposure. • Report any adverse impacts on human health and safety: ensures identification of unexpected adverse impacts.	Yes	• Limit scale: restrict area to 0.45 hectares over one growing season. • Fence trial site: restrict access to the GM wheats. • Restrict access to authorised personnel. • Destroy plant material: destroy all seed and plant material not required for possible future trials or research. • Secure transport and storage: the GM wheat material must be transported in accordance with OGTR guidelines. • Report adverse impacts: any adverse impacts on human health and safety must be reported to the Regulator.
TOXICITY FOR OTHER ORGANISMS: Mammals and wildlife, including birds and fish	Very low	See Appendix 2 • all of the GM wheat grain will be harvested for further research purposes and none of the GM wheat lines from the release will be used in animal feed; • animals are naturally exposed to the introduced proteins. The introduced OAT protein is from the plant *Arabidopsis thaliana* and is very similar to OAT proteins from other plants. The OAT protein is naturally produced in micro organisms, plants and animals and is therefore widespread in the environment and present in food; • a range of common fungi and bacteria contain similar gene sequences to the introduced *cah* gene that was isolated from *Myrothecium verrucaria*. CAH proteins are therefore likely to be widespread in the environment; • Neither protein is known to be toxic to any organism; • the GM wheat is expected to have elevated levels of free proline. The amino acid proline is present in all organisms and is not considered to be toxic at concentrations in the physiological range; and • the release is small in size and limited in duration;	Yes	• Limit scale of release: decreases likelihood of exposure. • Surround the release site with a fence: prevents exposure of animals. • Bird proof netting: prevents birds entering site. • Prevent plant material from being used as stockfeed: prevents exposure of animals. • Destroy all plant material not required for possible future trials or research: prevents unintended exposure. • Ensure secure transport and storage of GM plant material: prevents unintended exposure.	Yes	• Limit scale: restrict area to 0.45 hectares over one growing season. • Fence: contain GM wheats with a 1.8 m high fence to exclude rabbits and large mammals. • Bird proof netting: to be installed from early seed set of the GMO to harvest. • Prevent plant material being used as stockfeed: no material from the GM wheat to be used in stockfeed. • Destroy plant material: destroy all seed not required for possible future trials or research. • Secure transport and storage: the GM wheat material must be transported in accordance with OGTR guidelines.

Hazard Identification	Risk Estimate (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
		• exposure of livestock and wildlife to the GM wheat lines would be very low.				
TOXICITY FOR OTHER ORGANISMS: Invertebrates, including beneficial insects; microbes	Very low	See Appendix 2 • the release is small in size and limited in duration; • the GM wheat is expected to have elevated levels of free proline. The amino acid proline is present in all organisms and is not considered to be toxic at the concentrations expected in the GM wheat; • the introduced OAT and CAH proteins occur naturally, are expected to be expressed at low levels and are not known to be toxic to any organisms; • OAT is produced in plants, bacteria and mammals. The introduced OAT from *A. thaliana* will be very similar to the native wheat OAT; and • CAH is likely to be common in the environment. *M. verrucaria* is a commonly occurring soil fungus and is likely to be present at the release site. Genes thought to encode CAH proteins have been identified in a number of other microbes.	Yes	• Limit scale of release: decreases likelihood of exposure. • Destroy all plant material not required for possible future trials or research: removes ongoing exposure. • Ensure secure transport and storage of GM plant material: prevents unintended exposure.	Yes	• Limit scale: restrict area to 0.45 hectares per season over one growing season. • Destroy plant material: destroy all seed not required for possible future trials or research. • Secure transport and storage: the GM wheat material must be transported in accordance with OGTR guidelines.
WEEDINESS	Very Low	See Appendix 3 • the release is small in size and limited in duration; • wheat has a low potential for dispersal by natural means; • conventional wheat does not possess characteristics commonly associated with weediness and is not known to be a problematic weed in any environment. The genetic modifications are unlikely to increase other characteristics normally associated with intrinsic weediness which wheat does not possess; • the parental cultivars of the GM wheat lines do not have any significant seed dormancy and the genetic modification is unlikely to increase other characteristics normally associated with intrinsic weediness. • major constraints on persistence of both GM and non-GM wheats are water availability, nutrient	Yes	• Limit scale of release: decreases likelihood of persistence. • Surround the GM wheat with a monitoring and an isolation zone: allows detection and removal of the GM wheat plants and related plant species beyond the release site • Ensure a mown area surrounding the trial and fence line are kept clean: decreases potential habitat for rodents around the location and therefore seed dispersal. • Fence trial: prevents access to trial site by rabbits and large animals to prevent seed dispersal. • Bird proof netting: prevents seed dispersal by birds. • Prevent planting close to natural waterways: prevents spread of seed beyond	Yes	• Limit scale: restrict area to 0.45 hectares over one growing season. • Monitoring and Isolation zones: the trial must be surrounded by a 500m isolation zone, which includes a 10 m monitoring zone. Sexually compatible plants must be removed from these zones. • Keep areas around the Location and fence line clean: the area within the surrounding fence must be mowed, and the fence line kept free of weeds and other material which may harbour rodents. • Fence trial: the trial site must be within a lockable fence capable of excluding rabbits and large animals. • Bird proof netting: to be installed from early seed set to harvest. • Separate trial site from natural waterways: ensure that the trial is at least 50 m from natural waterway. • Trial must be harvested: when trial is harvested it must be

Hazard Identification	Risk Estimate (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
		availability, temperature, plant competition, frost and disease. • expression of the *oat* gene results in increased levels of free proline which acts as an osmoprotectant, conferring tolerance to saline conditions; • increased proline may enhance the GM wheats ability to tolerate other stresses eg. persist in saline environments and to tolerate frost and dehydration relative to conventional wheat. • the GM wheat plants would still be limited by water availability and the range of other environmental factors which normally limit the persistence of wheat plants in Australia; • the GM wheat lines grown hydroponically in the glasshouse under salt-stress conditions (150 mM NaCl) show a two-fold increase in tiller number , seed number and seed weight relative to non-GM wheat. Under these salt-stress conditions growth of non-GM wheat is severely impaired. • under non-saline growth conditions the GM wheat plants are slightly smaller than non-GM wheat plants. There may be a metabolic cost incurred through the overproduction of proline which may impact on the overall fitness of the GM wheat. • the *cah* gene confers tolerance to cyanamide. However, cyanamide is not used as a herbicide in Australia and would not confer any selective advantage.		the release site through water. • Harvest: harvest by a method so that all seed is contained, to prevent seed spillage and dispersal. • Ensure secure transport of GM material: prevents escape of viable GM plant material outside the release site. • Clean Equipment (including clothing) used in connection with the GMOs: prevents escape of GM material beyond the release site. • Prevent GM material being used as stockfeed: prevents spread of seeds beyond the release site. • Destroy all plant material not required for further research: prevents accidental spread of seed beyond the release site. • Post-harvest inspection of release site: prevents spread and persistence of GM wheat plants.		harvested manually, or mechanically harvested with a screen in place to prevent the dispersal of GM plant material. • Secure transport and storage: material from the GMOs must be transported in accordance with OGTR guidelines. • Clean Equipment: equipment used in connection with the GMOs must be cleaned before being used for any other purpose. • Prevent use as stockfeed: no material from the GM wheat to be used in stockfeed. • Destroy plant material: destroy all plant material not required for further research. • Post-harvest inspection: after the GMOs have been grown, the release site and monitoring zone must be inspected at least once every month for at least 24 months, and any volunteer wheat plants destroyed before flowering.

Hazard Identification	Risk Estimate (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
GENE TRANSFER: Plants Other wheat plants	Very Low	See Appendix 4 • wheat pollen is predominantly self pollinating and outcrossing frequencies are low; • when outcrossing does occur, pollen is wind dispersed; • wheat pollen is heavy compared to grass pollen and only remains viable for limited periods (up to 30 minutes); • the introduced genes may confer a selective advantage in saline environments; • gene transfer to non-GM wheat grown on the trial site is possible; • gene transfer to sexually compatible wheat species (including cultivated and naturalised wheat) is unlikely to occur during the trial due to isolation from other wheat plants (e.g. at least 500 m from any other wheat crops, and no sexually compatible weeds at the release site); • gene transfer to other wheat would not pose any risks additional to the very low risks posed by the GM wheat lines themselves; and • The field trial will be small and of limited duration limiting the potential for the introduced genetic material to persist in the environment.	Yes	• Surround the GM wheat with a monitoring and isolation zone: minimises potential for spread of the introduced genes beyond the release site via pollen flow. • Limit scale of release: decreases potential transfer. • Ensure secure transport and storage of retained seed: prevents escape of viable GM plant materials outside the release site. • Clean equipment used at the release site: prevents escape of viable GM plant material. • Destroy all plant material not required for possible future trials or research: prevents unintended spread. • Monitor and destroy any volunteers: prevents persistence. • Treat non-GM plants as the GMO: prevents gene transfer, persistence and dissemination.	Yes	• Monitoring and Isolation zones: the trial must be surrounded by a 500m isolation zone, which includes a 10 m monitoring zone. Sexually compatible plants must be removed from these zones. • Limit scale: restrict area to 0.45 hectares over one growing season. • Secure transport and storage: the GM wheat seed material must be transported according to OGTR guidelines. • Clean equipment used at the release site: equipment must be cleaned before it is used for any other purpose. • Destroy seed: destroy all seed not required for possible future trials or research. • Destroy volunteers: the release site and monitoring zone must be monitored after harvest at least once every month for at least 24 months and any wheat volunteers destroyed before flowering. • Treat non-GM plants as the GMO: harvest and handle non-GM plants as above.
GENE TRANSFER: Plants In Triticeae	Very low	See Appendix 4 • As above • gene transfer to naturalised wheat populations and closely related species is unlikely due to geographic isolation.	Yes	• As above.	Yes	• As above.

Hazard Identification	Risk Estimate (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
GENE TRANSFER: Plants non-Triticeae	Negligible	See Appendix 4 • genetic incompatibility prevents successful cross-pollination with other plant species.	No	N/A	N/A	None Required
GENE TRANSFER: Micro-organisms	Negligible	See Appendix 4 • the introduced genes are already present in the environment and are readily available for transfer via demonstrated natural mechanisms; • limited probability of occurrence. The chance of interaction, uptake and integration of intact plant genes by microbes is extremely low, especially if it involves unrelated sequences (non-homologous recombination); and • gene transfer from plants to bacteria has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other sources of these genes.	No	N/A	N/A	None Required
GENE TRANSFER: Animals, including humans	Negligible	See Appendix 4 • the introduced genes in the GM wheat lines are already present in the environment; • limited probability of occurrence. The chance of interaction, uptake and integration of intact plant genes by animals is extremely low, especially if it involves unrelated sequences (non-homologous recombination); • natural events of horizontal gene flow from plants to distantly related organisms are extremely rare; • in the unlikely event of gene transfer occurring, human health and safety and the environment are unlikely to be adversely affected; and • products from the GM wheat lines are not intended for stockfeed or human food	No	N/A	N/A	None Required

APPENDIX 1 INFORMATION ABOUT THE GMOS

43. In preparing the risk assessment and risk management plan, the Regulator is required under Section 49 (2) of the Act to consider the properties of the parent organism and the effects of the genetic modification.

44. This Appendix addresses these matters and provides detailed information about the GMOs approved for release, the parent organism, the genetic modification process, the genetic materials that have been introduced and the new proteins that are expressed in the genetically modified (GM) wheats.

SECTION 1 SUMMARY INFORMATION ABOUT THE GMOS

45. In application DIR 053/2004, Grain Biotech Australia Pty Ltd (GBA) proposed to release two GM salt tolerant wheat lines to examine their salt tolerance under saline field conditions. The genetic modification consists of the introduction of two genes, the ornithine aminotransferase gene (*oat*) derived from the common plant species, *Arabidopsis thaliana* and the cyanamide hydratase gene (*cah*), from the soil fungus *Myrothecium verrucaria*. The regulatory sequences used to drive expression of the introduced genes were derived from maize (*Zea mays* L.).

46. The ornithine aminotransferase gene (*oat*) expresses the ornithine aminotransferase enzyme (OAT). Over-expression of this enzyme can increase the free levels of the amino acid proline in the plant. Proline can act as an osmoprotectant and confer tolerance to salinity and other abiotic stresses (discussed further below).

47. The *cah* gene produces the enzyme cyanamide hydratase (CAH) which confers tolerance to the herbicidal activity of cyanamide.

48. Gene regulatory sequences are DNA sequences that are important for the expression of genes but which do not encode functional products, such as proteins. Expression of each of the introduced genes is driven by the promoter and first intron of the *Ubi-1* ubiquitin gene (Christensen et al. 1992). The *Ubi-1* promoter is considered a constitutive promoter, but expression is highest in young active tissues. Terminator sequences which include polyadenylation signals are required for the production of complete and stable mRNA molecules. Transcription termination of both the *oat* and *cah* genes is provided by the *zein* terminator from maize (Lopes et al. 1994). The methods used to introduce the genes into wheat are discussed in Section 3 of this Appendix. The introduced genes, their encoded proteins and all other genetic elements present in the GM wheat lines are discussed in more detail in Section 4 of this Appendix.

49. There have been no previous approvals of GM wheat lines containing the *cah* or *oat* genes under the current regulatory system. The GM wheat lines are derived from laboratory and glasshouse based research under the provisions of the Act under NLRD 239/2002. Releases of other GM wheat are summarised in Chapter 1, Section 2.

50. Further details on the introduced genetic materials, their products and mechanism of action are provided in Section 3 of this Appendix.

SECTION 2 THE PARENT ORGANISM

51. The parent organism is bread wheat *(Triticum aestivum* L.) which is exotic to Australia and is grown as an agricultural crop in all states of Australia. Bread wheat is a significant crop in Australia. In Australia, bread wheat is grown commercially as a grain crop, however winter wheat varieties may also be treated as dual purpose wheats. They may be grazed before stem elongation and before grain is produced. In severe droughts where economic grain yields are not expected, livestock are allowed to graze crops.

52. Bread wheat is the source of flour for breads, rotis, chapattis and semolina, biscuits and other confectionary products. Wheat grain is also used to manufacture alcoholic beverages. Bran from flour milling is used in livestock feed and the germ is a valuable addition to feed concentrate. Grains are fed to livestock whole or coarsely ground. Starch derived from bread wheat is used in pastes and sizing textiles.

53. Bread wheat is a member of the tribe *Triticeae* (subfamily Pooideae, family Poaceae (formerly Gramineae)). It is a segmental hexaploid (6x) which regularly forms 21 pairs of chromosomes (2n =42) during meiosis. These chromosomes are subdivided into 3 closely related (homoeologous[9]) groups of chromosomes, the A, B, and D genomes; each group normally contains 7 pairs of chromosomes (AABBDD). Sears (1966) established that each chromosome in hexaploid wheat has a homoeologue in each of the other 2 genomes. The effects of chromosomal relationships between bread wheat and its progenitors and wild relatives on gene transfer is discussed in Appendix 4. The level of homoeology that occurs between the chromosomes of bread wheat and other species influences the level of fertility that can occur in interspecific hybrids.

54. The wheat variety **'Westonia'** (Pedigree: CO1190-203/84W127-501) is an Agriculture Western Australia (AgWA) wheat variety and was the leading commercial wheat variety in WA in 1999 and 2000. It is described ('Wheat varieties in Australia 1968-2001', 2004) as early maturing and widely adapted to variations in rainfall and planting time in the medium to low rainfall zones of WA. It is also described as susceptible to pre-harvest sprouting. It is Plant Variety Rights (PVR) protection was granted in December 1998 and was terminated in December 2003.

55. The wheat variety **'Carnamah'** (Pedigree: Bolsena-1CH/[Siete Cerros/XBVT223//Awx011.G.48.2/XBVT221]) was released in 1996 by AgWA and PVR protection was granted in December 1997. It is a mid season spring wheat. It was the leading variety in WA in 2001. It is also susceptible to pre-harvest sprouting.

56. More detailed information on bread wheat can be found in a review document 'The Biology and Ecology of Bread Wheat (*Triticum aestivum* L. em Thell.) in Australia' that was produced in order to inform the risk assessment processes for licence applications involving GM wheat. This document is available at *www.ogtr.gov.au/.*

[9] Partially homologous, genetically and evolutionarily related chromosomes, but from different genomes.

SECTION 3 SALINITY AND PLANT RESPONSES TO SALINITY

Soil salinity in Australian agriculture

57. Pannell and Ewing (2004) reported that the majority of human-induced land salinisation in the world is associated with irrigation. In Australia, however, the majority of salt-affected land is due to land clearing and land management practices.

58. National Land and Water Resources Audit (2001) noted that the salt stores have developed because there is little capacity to drain the continent of salt and water. The salt stores stretch in an arc from northern Australia, south by the Great Dividing Range and then across the Riverina and Mallee regions. They are also found in south western WA (Figure 1).

59. In Australia, the National Land and Water Resources Audit (2001) estimated the area of land in Australia with 'a high potential to develop dryland salinity' to be 5.7 m ha. The audit also concluded that Western Australia had the greatest area at risk and forecast that by 2050 the proportion of agricultual land at risk in Western Australia would exceed 30 per cent. At least 1500 plant species are likely to suffer from dryland salinity. Nearly one third of these have been identified at risk of extinction.

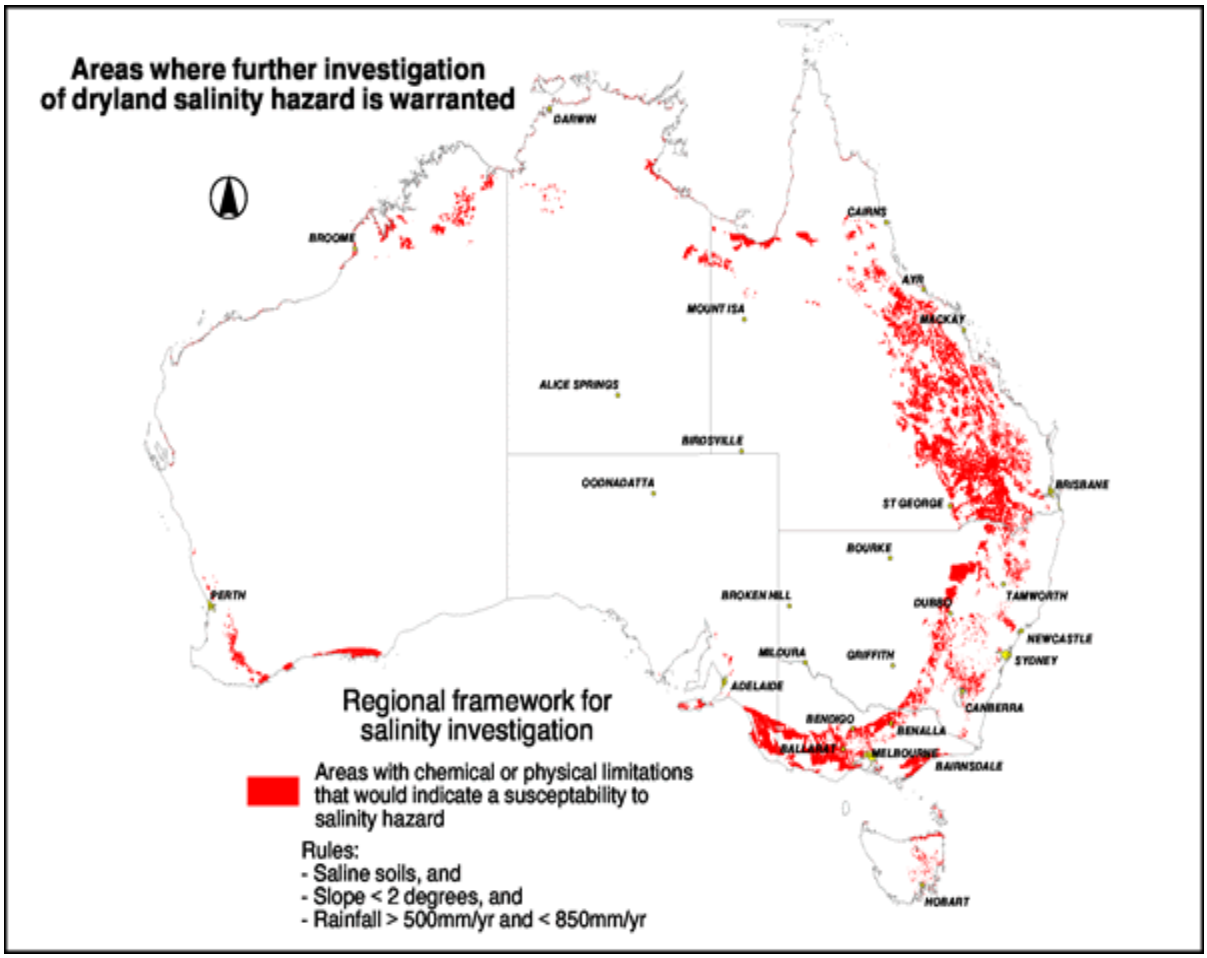


Figure 1. Extent of dryland soil salinity in Australia (Bureau of Resource Sciences 2004).

60. Even in more responsive groundwater flow systems, the net output of salt may take 150 years to flush from the system (The National Land and Water Resources Audit 2001).

61. Areas at risk include the wheat-sheep belt in south west WA and the crop-pasture zones of SA, NSW and Victoria. Broadacre crops and traditional pasture species do not tolerate salt and crop losses occur when salt concentrates within the root zone.

Effects of salinity on wheat production and quality characteristics

62. Richards (1987) stated that 'salinity adversely affects plant growth by decreasing the availability of soil water to the plant and because the major ions contributing to salinity are toxic at high concentrations'.

63. A two phase response to salinity in wheat and barley was described by Munns et al. (1995). In the first phase, growth reduction is due to the osmotic strength of the salt solution outside the roots and thus affects all genotypes similarly. The second phase commences only after salt has accumulated to toxic levels in enough leaves to cause a large amount of injury and reduce the supply of nutrients to growing regions of the plants.

64. Rawson et al. (1988) found that a good indicator of high absolute tolerance to salinity in winter cereals was large area of seedling leaves. The authors defined physiological tolerance as a small relative reduction in growth due to salinity and absolute tolerance as an intrinsic high growth rate of the genotype in and out of salinity.

65. Grewal et al. (2004) recently evaluated the responses of 15 wheat cultivars to subsoil salinity. There was a range in responses between varieties, however there was no evidence of tolerance to high saline subsoils.

Plant responses to salinity - osmoprotectants

66. Protection from abiotic stresses, such as osmotic stress resulting from saline conditions, is conferred by compounds known as compatible solutes or osmoprotectants in plants, bacteria, marine algae and animal cells (Rathinasabapathi 2000) Compatible solutes are small, electrically neutral molecules that are non-toxic at molar concentrations; they stabilise and protect proteins and membranes against the deleterious effects of high concentrations of salt and other harmful solutes. They are accumulated in response to osmotic stress and even at high concentrations do not inhibit enzyme activity (Rathinasabapathi 2000).

67. Osmoprotectants are widespread amongst plant species (Rontein et al. 2002). There are a number of compounds which are known to function as osmoprotectants, including: proline, glycinebetaine (eg in spinach, beet and also *E.coli*), sorbitol (eg in apple) and D-ononitol (eg in ice plant, M*esembryanthemum crystallinum* L.) (Rontein et al. 2002). In a review of the mechanisms of salt tolerance in non-halophytes, Greenway and Munns (1980) identified glycinebetaine, sucrose and proline as organic solutes that increase in concentration in the plant cytoplasm at high salinity.

68. In plants the osmoprotectants are normally confined to the cytosol and mitochondria that occupy 20 % or less of the volume of the mature cells. Natural osmoprotectant concentrations in the mitochondria can reach or exceed 200 mM (Rhodes & Samaras 1994). Such concentrations are osmotically significant and are important for the maintenance of cell turgor and for driving the gradient for water uptake under stress. Natural levels of

osmoprotectants in plants range from 5 -50 μmol/g fresh weight and levels are highest during exposure to osmotic stress.

Proline as an osmoprotectant

69. L-Proline is one of the 20 amino acids from which the proteins present in all organisms are synthesised. Proline is strictly known as an imino acid, and the side chain is bonded to both the amino group and the α-carbon to form a cyclic structure.

70. Proline is considered to be an unreactive compound and has been reported as an important osmoprotectant in many plants, and elevated levels of free proline can enable plants to grow in the presence of elevated salt levels in soil (2002). Proline has also been identified as an effective osmoprotectant in several species of bacteria (Csonka 1989). The applicant expects that the introduced OAT protein will increase the level of free proline in the GM wheat approximately 2-fold higher than in the parent (no-GM) material under saline conditions in the field.

71. Alia (2003) reported that in plants proline constitutes less than 5 % of the total free amino acids under normal conditions but that "under various forms of stress proline concentration increases up to 80 % of the total amino acid pool". Greenway and Munns (1980) reported that most available evidence suggested that the production of proline was related to survival rather than the maintenance of growth. Nearly all salt-sensitive and salt-tolerant species contained substantial concentrations of proline (>2 μg/ g fresh weight) only when growth was severely reduced. They also hypothesised that proline only accumulates when phosphate availability and/ or growth is reduced. More recent reviews (Hasegawa et al. 2000a; Hasegawa et al. 2000b) have confirmed the importance of proline as an osmoprotectant.

72. Many plants that are naturally adapted to high salt environments (halophytes) exhibit high levels of proline (approximately 100 μmol/ g fresh weight), and this level can increase up to 10-fold (up to 1500 μmol/ g fresh weight) in response to salt stress (eg Thomas & Bohnert 1993).

Proline in cereal plants

73. Proline level is regulated up and down in response to a range of environmental stimuli (Hellmann et al. 2000). Proline accumulation has been reported as a response to water stress (Chu et al. 1978; Vajrabhaya et al. 2001; Nabizadeh et al. 2004) and to increased levels of both $CaCl_2$ and $MgCl_2$, but leaf free proline decreased when NaCl increased (Chauhan et al. 1983). The application of ultra-violet light also increased levels of proline in both radicle and coleoptile tissues of wheat plants (Demir 2000).

74. Gusta and Chen (1987) reported that total free amino acids increase in water stressed leaves of wheat and the increase in proline concentration was the most pronounced. The observed proline accumulation in water stressed plants was attributed to (i) stimulated synthesis due to the loss of feedback inhibition; (ii) inhibited oxidation, due to effects on mitochondria; (iii) impaired protein synthesis.

75. Chu et al. (1978) found that proline accumulation at low temperatures in the first leaves of barley and wheat was light dependent. Proline accumulation, in response to water stress was not light dependent at 20°C, but was light dependent at 5°C.

76. Tkachuk (1979) examined free amino acids in wheat germinated at 3 different temperatures (10°C, 16.5°C and 25°C). Five days after germination free amino acid content was respectively 4x, 10x and 7x the content of sound wheat at 0 hours. Proline increased 100 fold during the 122 hour period at 16.5°C.

77. Nabizadeh et al. (2004) observed a three fold increase in free proline concentration in (non-GM) wheat plants subjected to incremental water deficits. Vajrabhaya et al. (2001) measured proline content in rice subjected to water stress for drought susceptible and drought tolerant (somaclonal variants of the susceptible parent). They observed a 3 – 4 fold increase in free proline in the susceptible plants and a 7 – 14 fold increase in tolerant plants.

78. Increased proline concentration has been correlated with increased salt tolerance in conventionally bred salt tolerant wheat varieties (Shahbazi & Doust 1996; Gupta & Srivastava 1990; Kafi et al. 2003).

SECTION 4 THE INTRODUCED GENES AND THEIR PRODUCTS

Section 4.1 The *oat* gene and encoded protein

79. The *oat* gene is derived from the common plant, *Arabidopsis thaliana* and encodes the enzyme δ-ornithine aminotransferase (δ-OAT) (Roosens et al. 1998). Roosens et al. (2002) showed that the overexpression of the *A. thaliana* δ-OAT in *Nicotiana plumbaginifolia* resulted in elevated levels of free proline and increased osmotolerance.

80. Proline may be synthesised in plants, and other organisms, from either glutamate (the 'glutamate pathway') or ornithine (the 'ornithine pathway') (Delauney & Verma 1993). The various biochemical steps and enzymes involved in proline production and catabolism are illustrated in Figure 2.

81. δ-OAT catalyses the δ-transamination of ornithine to produce Δ^1-pyrroline-5-carboxylate (P5C, see Figure 2) (Delauney et al. 1993). The subsequent conversion of P5C into proline is catalysed by pyrroline-5-carboxylate reductase (P5CR). The *A. thaliana* δ-OAT enzyme shares significant sequence homology with δ-OAT enzymes produced by other plants, animals and microorganisms (Roosens et al. 1998).

82. The level of free proline in plants is controlled by synthesis, catabolism and transport and these processes are highly regulated by cellular proline concentration and abiotic stress (Hellmann et al. 2000; Nanjo et al. 2003). Proline represses the expression of the enzyme pyrroline-5-carboxylate synthase (P5CS) that catalyses the conversion of glutamate to P5C (see Figure 2) and induces the expression of proline dehydrogenase (ProDH) that catalyses the degradation of proline to P5C (Verbruggen et al. 1996; Peng et al. 1996). Salt stress acts as an antagonist, overriding proline-dependent regulatory mechanisms (Nakashima et al. 1998).

83. The δ-OAT enzyme is distinct from α-OAT which catalyses the α-transamination of ornithine to α-keto-δ-aminovalerate (Delauney & Verma 1993; Delauney et al. 1993). References to OAT throughout this RARMP are to δ-OAT.

84. The *oat* gene from *A. thaliana* encodes a protein with a 40 amino acid mitochondrial transit peptide at its N-terminus (Roosens et al. 2002). The OAT enzyme is localised in mitochondria in both plants and animals (Delauney et al. 1993; Roosens et al. 2002; Inana et

al. 1986). The transit peptide directs the OAT protein to the mitochondria where it is cleaved to give rise to the mature enzyme.

85. Transit peptides occur naturally in plants and facilitate the transport of nuclear encoded proteins to the chloroplast or mitochondria. The pre-protein (containing a transit peptide) is transported into mitochondria or chloroplast stroma where the transit peptide is cleaved and rapidly degraded leaving the mature enzyme (Bartlett et al. 1982; della-Cioppa et al. 1986).

86. Potential hazards relating to the toxicity and allergenicity of OAT and an increased level of free proline are discussed in Appendix 2 and those of weediness and gene transfer in Appendices 3 and 4 respectively.

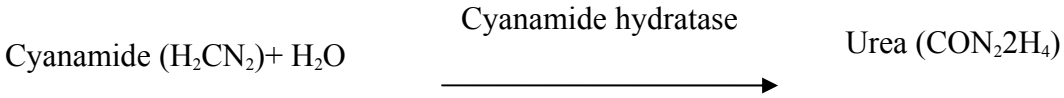
Section 4.2 The *cah* gene and encoded protein

87. The GM wheat lines also contain the *cah* gene from the soil borne fungus *Myrothecium verrucaria*. The *cah* gene was used as a selectable marker in the development of the GM wheat plants in the laboratory(Weeks et al. 2004; Damm 2003).

88. ***M. verrucaria*** is plant pathogen which produces a class of mycotoxins known as trichothecenes which have phytotoxic activity (Andolfi et al. 2005; Abbas et al. 2002). Only the *cah* gene from ***M. verrucaria*** was introduced to the GM wheat. It represents only a small proportion of the ***M. verrucaria*** genome and is not involved in the production of the mycotoxins.

89. The *cah* gene encodes the enzyme cyanamide hydratase (CAH). CAH confers tolerance to the herbicidal activity of cyanamide by catalysing the hydration of the nitrile group of cyanamide to urea as shown in Reaction 1. The CAH protein has a high specificity for the nitrile cyanamide, and chemically related compounds, ie other nitriles, do not act as substrates (Maier-Greiner et al. 1991).

Reaction 1:



90. Worldwide, cyanamide has had a number of applications in agriculture as a herbicide, fungicide, pesticide and nematicide. (Kamo et al. 2003); as a fertiliser (Klasse 1996); and as a growth regulator to control bud burst in fruit trees (Williams & Tax Tzoc 1990; APVMA 2005a). In Australia, cyanamide is only registered for use as a growth regulator in kiwifruits and grapevines (APVMA 2005b; APVMA 2005a)

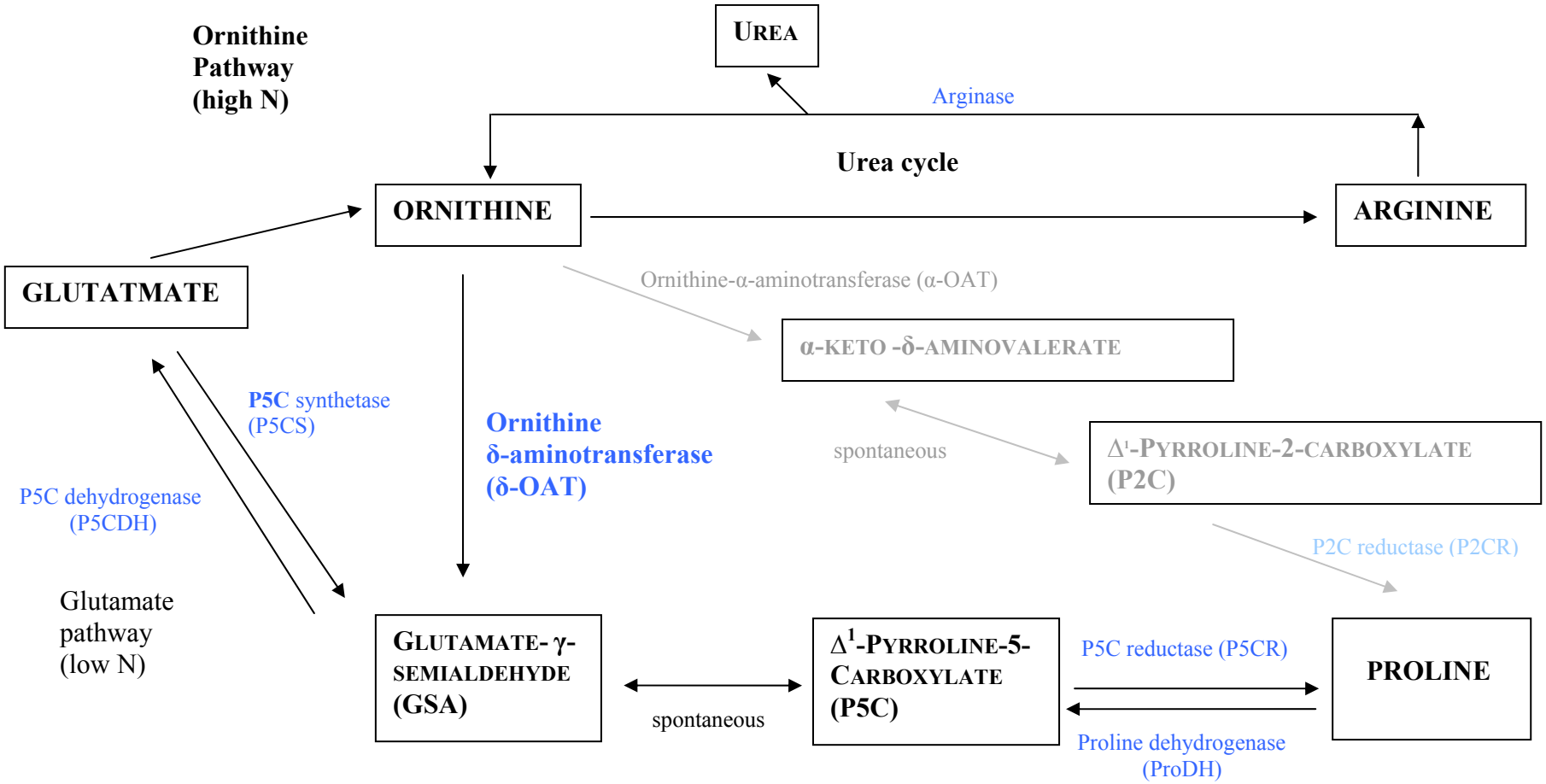


Figure 2. Interrelated pathways of proline and arginine biosynthesis in plants (adapted from Delauney & Verma 1993).

The ornithine pathway is generally considered to predominate under conditions of high nitrogen whereas under low nitrogen the glutamate pathway operates.

91. Biochemical confirmation of CAH enzyme activity has not been demonstrated in other species. However, the applicant advised that sequence analysis of the genomes of the fungi *Aspergillus nidulans*, *Gibberella zeae*, *Neurospora crassa* (red bread mould) and in the bacteria *Streptomyces*, *Photorhabdus luminescens* and *E. coli* and the yeast *Saccharomyces cerevisiae* has identified putative genes which would encode proteins with significant homology to the CAH from *M. verrucaria*. It is well known that cyanamide is degraded by microbial activity in soil (Estermaier et al. 1992) and the identification of putative *cah* genes in these species strongly suggest that CAH enzymes do exist in other microbial species.

92. Potential hazards relating to the toxicity and allergenicity of CAH are discussed in Appendix 2 and those of weediness and gene transfer in Appendices 3 and 4 respectively.

Section 4.3 Regulatory and non-coding sequences

4.3.1 The *Ubi-1* promoter and intron

93. The expression of the *oat* and *cah* genes are each under the control of the *Ubi*-1 promoter and first intron derived from maize (*Zea mays* L.)(Christensen et al. 1992). The intron is a non-coding nucleotide sequence which is transcribed into RNA as part of the RNA transcript and subsequently removed by RNA splicing. The presence of an intron in an introduced gene can improve expression of that gene in plants (Wilmink et al. 1995) and the intron in the Ubi-1 promoter is known to enhance expression levels (Christensen & Quail 1996).

94. The *Ubi*-1 promoter provides strong constitutive expression of introduced genes in transgenic monocots (Christensen et al. 1992) and has been widely used for this purpose (Rooke et al. 2000). Although the *Ubi*-1 promoter is considered constitutive, it has been observed to have the strongest activity in young, metabolically active tissues and in pollen grains, and the activity decreases in older tissues. In wheat some of the highest levels of expression from the *Ubi*-1 promoter have been found in meristematic tissues such as immature inflorescences as determined by expression of GUS (Stoger et al. 1998).

95. However, performance of the *Ubi*-1 promoter can vary between individual GM wheat lines (Rooke et al. 2000). Stoger et al. (1998) found that *Ubi*-1 promoter activity in anthers was restricted to pollen grains.

4.3.2 The *zein* terminator

96. Terminator sequences, including polyadenylation signals are required for the production of complete and stable mRNA molecules. The expression of the *oat* and *cah* genes is terminated by the *zein* terminator derived from maize (Lopes et al. 1994) .

SECTION 5 METHOD OF GENETIC MODIFICATION

97. The genes were introduced into the wheat genome by microprojectile bombardment.

98. The *oat* and *cah* gene constructs were each constructed in separate plasmids, pGBA2OAT and pGBA2CAH. Linear fragments of DNA containing the *oat* and *cah* genes and associated regulatory elements were excised from the plasmids, mixed together, coated onto gold particles and shot into wheat embryos of varieties, 'Carnamah' and 'Westonia' using a high pressure Helium gun.

99. The wheat embryos were cultured on Murashige and Skoog (MS) media with successively higher concentrations of cyanamide and surviving plantlets transferred to soil. These plantlets were then screened by ELISA for the CAH protein and Polymerase Chain Reaction (PCR) for the *oat* gene.

SECTION 6 CHARACTERISATION OF THE INSERTED GENETIC MATERIAL AND STABILITY OF THE GENETIC MODIFICATION

100. The T_1 and T_2 generations of the two GM wheat lines were obtained from primary transformants (T_0) by self-pollination and were shown to contain the *oat* gene and showed salt tolerance characteristics. Only the T_0 generation was screened with cyanamide to confirm the presence of the *cah* gene.

101. The T_1 and T_2 generation of these two GM wheat lines contained the *oat* gene and salt tolerance characteristics. The applicant reports that the inserted genes have been inherited as dominant Mendelian traits over at least two generations in the glasshouse.

102. Insertion copy number can be determined by Southern blot, using probes of either *oat* or *cah*. The applicant reports that copy numbers in the GM wheat lines are still being determined. Analysis of other *oat*-containing wheat lines (not proposed for release) indicates an average copy number of six transgenes per line. The applicant expects a similar number of transgenes in the GM wheat lines approved for release.

103. Preliminary Southern Blot data for the 'Westonia' GM wheat line 2490-1 approved for release indicate that there are 5-7 copies of the *oat* gene present.

104. The applicant has advised that the exact location of the inserted genes within the bread wheat genome is not known and has not been determined as the research with the GM wheat is at an early stage.

105. The applicant has not thoroughly investigated the stability of the genotypes of the GM wheat lines approved for release other than to demonstrate the presence of the *oat* gene over several generations. Cyanamide was applied only to the T_0 generation. The applicant reports that previous results have indicated that the *cah* gene and *oat* gene are linked.

106. The GM wheat plants approved for release under this application will be from the T_4 generation.

SECTION 7 EXPRESSION OF THE INTRODUCED PROTEINS

Section 7.1 OAT expression

107. Quantification of the level of OAT protein expression has not been determined. However, OAT activity is inferred from increased tolerance to saline conditions.

108. From the characteristics of the *Ubi*-1 promoter it can be predicted that expression will be strongest in young, metabolically active tissues and in pollen grains, and the activity decreases in older tissues (Rooke et al. 2000). Even though *Ubi*-1 directs comparatively strong expression, the level of OAT protein expression would still be expected to represent only a fraction of total protein expression in the plants.

Section 7.2 CAH expression

109. Similarly, CAH protein expression would be strongest in young, metabolically active tissues and be expected to represent only a fraction of total protein expression in the plants.

Section 7.3 Free proline levels and salt tolerance

110. The applicant has advised that for hydroponically grown plants under salt-stress conditions (150 mM NaCl) the free proline concentration in extracts from the GM wheat plants was two (per g fresh weight) to three (per mg protein) times that in non-GM 'Westonia' (see Table 4). No difference was detected between the proline content of GM and non-GM wheat in the absence of salt stress, however the applicant advised that only very preliminary data were available.

Table 4 Proline content of GM wheat under salt stress (150 mM NaCl)

	μmol proline/ g Fresh weight	μmol proline/mg protein
OAT transgenic wheat	0.31	0.0062
'Westonia'	0.18	0.0019

111. In GM *Nicotiana plumbaginofolia* plants overexpressing the OAT enzyme from *A. thaliana* free proline was increased approximately 3-fold relative to the wild type in non-stressed plants (2002). In osmotically stressed plants the proline levels were higher for both GM and non-GM, but the relative increase in the GM plants was only 1.5-fold (Roosens et al. 2002).

112. The increases in proline achieved to date by single genetic modifications have generally been low and the increases in stress tolerance relatively small (Rontein et al. 2002). Even in GM plants modified to achieve elevated proline, the effects of feedback regulation on the proline synthesis and catabolism still effect control over the pool of free proline (Blumwald et al. 2004). This homeostatic control may provide some explanation for the apparently small increase in free proline levels in the GM wheat in the absence of salt stress.

SECTION 8 PLEIOTROPIC EFFECTS OF THE GENETIC MODIFICATION

113. A single plant gene can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Single genes inserted into a plant by genetic modification can also result in pleiotropy. It is therefore necessary to evaluate GM plants for unintended pleiotropic effects, such as changes in agronomic characteristics, which may be a consequence of the gene insertion.

114. No unintended or secondary effects have been observed in the GM wheat lines grown under glasshouse conditions. The applicant reports that the growth characteristics of the GM wheat lines are the same as for conventional wheat. However, the applicant has advised "that while plants do not look different, the size of the GM plants is slightly smaller than the non-GM plants" under non-saline conditions.

115. It can be postulated that constitutively increasing the level of the amino acid proline would result in a metabolic drag on plant growth because less C and N can be directed to other amino acids and hence might affect total protein synthesis.

SECTION 9 RESEARCH REQUIREMENTS

116. The release is a small scale research trial designed to evaluate the salt tolerance and agronomic performance of two GM salt tolerant wheat lines on a site affected by different levels of salinity.

117. If the applicant makes an application for any particularly larger scale releases of these GM wheat lines, more data will be required on:

- the level of expression of the introduced gene(s) and encoded protein(s), and the plant tissues (including pollen) and developmental stages in which they are being expressed;
- levels of free proline in different plant tissues and at different developmental stages under field conditions;
- stability of the introduced genes and modified traits;
- genetic segregation and molecular characterisation of the introduced genes; and
- unintended effects of the genetic modification.

APPENDIX 2 TOXICITY AND ALLERGENICITY TO HUMANS AND OTHER ORGANISMS

118. Under Section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan (RARMP). This Appendix considers potential hazards that may be posed to the health and safety of humans and other organisms as a result of any toxicity or allergenicity of the GMOs or their novel proteins.

119. It should be noted that other GM wheats have been trialed in Australia under the previous voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC). There have been no reports of adverse effects on the health of humans or other organisms from dealings with these GMOs.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

120. A toxic response to a chemical is shown by the cascade of reactions resulting from exposure to a dose of chemical sufficient to cause direct cellular or tissue injury, or otherwise inhibit normal physiological processes (Felsot 2000).

121. Allergic responses are immune system reactions resulting from stimulation of a specific group of antibodies known as IgE or sensitisation of specific tissue bound lymphocytes (Taylor & Lehrer 1996; FAO 2003). Allergic responses have a well-defined etiology (i.e. biochemical cause) that is quite different from toxicity. An allergic response can have severe consequences for an individual. Anaphylaxis, for example, is a shock syndrome caused by a massive release of histamine and other allergic mediators from even minute exposures to an allergen in a sensitised individual. Food proteins are common causes of anaphylaxis, especially peanut and shell fish (Frick 1995).

122. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases (Astwood et al. 1996). This is because it is necessary that a protein be sufficiently stable to pass through the stomach and cross the mucosal membrane for it to stimulate an allergenic response following oral ingestion.

123. The GM wheat lines approved for release differ from non-GM wheat in the expression of two additional proteins, the OAT and CAH proteins and having an increased level of the amino acid proline. The potential for these wheats to be toxic or allergenic to humans or other organisms due to expression of these proteins, or due to unintended effects of the genetic modification, is considered in this Appendix.

124. If the GM wheat lines are toxic to humans and other organisms, the potential hazard could result in adverse effects on:

- people (e.g. through food products, or working with the GM wheats);
- livestock and wildlife, including mammals, fish and birds;
- invertebrates, including beneficial insects (parasitoids/predators of insect pests); and
- microorganisms, particularly soil microorganisms, with direct impact on growth of crops on farms.

SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

125. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of the GM wheat lines on the health and safety of humans and other organisms, the following factors were considered:

- the inherent toxicity and allergenicity of non-GM wheat;
- the potential toxicity and allergenicity of the introduced proteins expressed in the GM wheats;
- the potential exposure to the GM wheat lines, to their products and to the introduced proteins (CAH and OAT) which are expressed in the GM wheat;
- the potential exposure to the CAH and OAT proteins from other sources in the environment; and
- the potential toxicity and allergenicity of the GM wheat lines.

Section 2.1 Toxicity and allergenicity of non-GM wheat

126. Bread wheat is an established agricultural field crop with a long history of safe use as human food. A number of anti-nutritional factors occur in bread wheat and in extreme cases these have a toxic effect. A comprehensive review of non-GM wheat including information on its toxicity and allergenicity is provided in the document 'The biology and ecology of bread wheat (*Triticum aestivum* L. em Thell.) in Australia' (OGTR 2005) which was produced in order to inform the risk assessment process for licence applications involving GM wheat lines. This document is available at www.ogtr.gov.au. Information on non-GM wheat is included here to establish a baseline for comparison with the GM wheat lines being considered in this risk assessment.

127. There is no evidence of any direct toxicity associated with the use of wheat grain as a food crop for humans. Wheat grain does contain low levels of some anti-nutritional chemicals such as phytic acid, phenol derivatives or tannins, protease inhibitors, and lectins (Simmonds 1989; Kent & Evers 1994; Garcia-Carreno et al. 2000). However, these anti-nutritional factors are not present in sufficient concentrations to have any effect upon humans but may reduce the nutritional quality of food for animals if wheat grain constitutes a large part of their diet (Simmonds 1989; Garcia-Carreno et al. 2000).

Allergenicity

128. The Farrp allergen database (www.allergenonline.com) lists several known food allergens in wheat including the gluten proteins, α-amylase inhibitors, and agglutinin (lectin). Consequently, it is well established that the protein fraction of wheat grains induces all known allergic responses and all allergies to wheat arising from both ingestion and inhalation are induced by specific proteins.

129. Hypersensitivity to cereals causes a number of recognised allergic reactions in people. These include baker's asthma, IgE-mediated food allergy, coeliac disease, non-IgE mediated enteropathy, wheat-dependent exercise-induced anaphylaxis and atopic dermatitis (Armentia et al. 2002; Sampson 2001). Approximately 5% of children under five develop food based allergic reactions. However wheat hypersensitivity has an estimated prevalence of 0.15% of

infants (Hill et al. 1997), which is lower than the prevalence of hypersensitivity to egg, cow's milk or nuts, but higher than that to soy or fish in children under 2 years (Hill et al. 1997).

130. Humans can suffer from severe bronchial irritation caused by the inhalation of wheat dust. Simmonds (1989) reported that several types of allergic responses to wheat have been reported. Wheat grain, dust and the milled products can cause a range of allergic reactions. Symptoms range from mild rhinitis to asthma.

131. Cereal flour is well established as a cause of baker's asthma associated with inhalation of flour dust. Several IgE binding proteins from wheat, barley and rye are associated with flour allergy (Armentia et al. 2002). These allergens are 12 to 16 kD in size with homology to the α-amylase/trypsin inhibitor family. Such protease inhibitors are primarily produced by plants as antinutritional substances (toxins) to discourage feeding by invertebrates and attack by microorganisms. They do not occur at high enough levels to be toxic to humans although they may cause allergic reactions (Ryan 1990; Lawrence & Koundal 2002; Kranthi et al. 2002; Ussuf et al. 2001). The wheat α-amylase inhibitor is associated with wheat allergies both through ingestion and inhalation (Armentia et al. 2002; De Leo et al. 2002; James et al. 1997). In addition, other 20 and 47 kD proteins have been implicated as antigens in food based wheat allergies (Jones et al. 1995).

132. The starch fraction of wheat is considered to be harmless (Armentia et al. 2002; Kent & Evers 1994 pp 297; Palosuo et al. 2001; Stone 1996)

Section 2.2 Exposure of people to the GM wheats

133. The applicant will destroy the GM wheat material and seed produced in the trial, apart from some seed which will be retained for research or for possible future trials (subject to further approvals). Since it is not intended that any product of the release will be used in human food or animal feed, there will be no opportunity for human exposure to these GM wheat lines through food. If products from these GM wheats were proposed to be used in food, the applicant would need to obtain approval from FSANZ.

134. There will be no opportunity for humans to be exposed to the GM wheats through flour, or animal feed such as hay or greenfeed. Therefore, potential risks to humans as a result of such exposure to GM wheat products will not be discussed.

135. Potential exposure of people to the GM wheats will be by means of:

- working with the GM wheat (e.g. on farms); and
- living in or near the area where the GM wheat lines are grown (general environmental exposure, e.g. people breathing wheat pollen).

136. The release site is in the Corrigin shire in Western Australia. The applicant has advised that the distance from the release to the nearest farm buildings is approximately 800 m and the GM wheat will be located within a securely fenced area. Hence, human exposure to the wheat plants would be limited to those people working within the trial sites. Exposure to GM wheat pollen would depend on wind conditions. Measurements of pollen dispersal have shown dispersal distances of about 50 m with wind speeds of 3 m/s (D'Souza 1970). The small size of the trial site (maximum area of 0.45 ha) and the limited duration of the trial (April 2005- January 2006) will restrict the amount of pollen produced and thus decrease the exposure risk.

137. Humans working in the field with wheat plants would be exposed primarily to the outer waxy cuticle layer at the plant surface and to the seed coat, all of which are essentially free of proteins. Exposure to proteins (including the introduced proteins expressed in the GM wheats), or to other cellular components of the wheats, will only occur if plant cells are ruptured.

138. Even if the cells rupture, exposure to the introduced proteins expressed in the GM wheat will be very low as these proteins are expected to be present at relatively low levels in all GM wheat tissues including wheat pollen.

139. At the commercial scale, the primary processing of wheat seed at silos and flour mills can create and stir up fine dust and flour particles. Use of personal protective equipment by exposed workers is commonplace in such facilities to prevent respiratory irritations. However, processing of the GM wheat seed will only occur on a very small scale, in a PC2 facility, for preparation of seed for possible future trials and milling to determine flour and bread making characteristics.

140. As specified above, the applicant has proposed containment measures to minimise contact between humans and the GM wheat lines. Conditions in the licence require these and additional containment measures.

Section 2.3 Exposure of livestock and wildlife, including mammals, birds and fish, to the GM wheats

141. None of the wheat plants from the release or their by-products will be used as stockfeed. The applicant will destroy all materials produced in the release, apart from some plant tissue and wheat seed for use in research.

142. The likelihood of small animals, such as mice, gaining access to the trial sites and consuming plant material and seeds depends greatly on the density of the animals in the area at the time of the trial. Mouse numbers fluctuate every three to four years on average in Australia, and after a mouse plague can remain very low for up to two years (Brown & Singleton 2002). The applicant has proposed to monitor the trial site to determine the extent of mouse activity. In the event that mouse or rat activity is observed, traps will be deployed around the perimeter of the trial site.

143. Wheat seed or pollen does not enter aquatic habitats in any significant quantity (OGTR 2004) and therefore the level of exposure of aquatic species to the GM wheats will be very low. Exposure is also limited by the small size of the trial and the requirement that it be at least 50 m from the nearest natural waterway.

144. The exposure of livestock and wildlife to the GM wheat lines will be limited due to the location and nature of the release site. The applicant will contain the GM wheat in a 1.8 m high fence which will exclude rabbits and large animals. Bird proof netting will prevent access to birds at seed-setting stage of the GM wheat.

145. The small scale (0.45 hectares) and limited duration (April 2005-January 2006) of the release will further limit the potential for exposure of stock and wildlife to the GM wheat.

Section 2.4 Exposure of invertebrates, including beneficial insects, to the GM wheats

146. Invertebrates could be exposed to the GM wheat lines and to the introduced protein directly, through feeding on the plants, seeds or pollen, or via the soil when wheat tissues

decompose. Exposure of soil invertebrates to the introduced protein could also occur as a result of root exudations. Exposure could also occur indirectly, through consumption of other organisms that have fed on the GM wheat plants.

147. Relative exposure would be greatest for herbivorous species (such as locusts) feeding on the GM wheat plants. Sap feeders (such as aphids) would have minimal exposure to the introduced proteins as the sap is primarily composed of sugars and mineral salts dissolved in water. Insects such as ants could remove seeds or plant material for consumption and be exposed through this method. There are no known insect pollinators of wheat florets in Australia (information supplied by the applicant). Neither the OAT or CAH protein or proline is known to be toxic to any organism.

148. However, the small size and limited duration of the release will limit the potential for exposure of invertebrates to the GM wheats.

Section 2.5 Exposure of microorganisms, particularly soil microorganisms, to the GM wheats

149. Microorganisms, particularly soil microorganisms, will be exposed to the GM wheat plants and the introduced proteins during growth and decomposition of plant material. Exposure of soil microorganisms to the introduced proteins and elevated levels of free proline may occur during the season as a result of root exudations. Root exudation has been observed in some GM plants: Bt corn expressing Cry1Ab (Saxena et al. 1999; Stotzky 2000) and INGARD® cotton expressing Cry1Ac (Gupta et al. 2002). Root breakage could also lead to the release of the introduced proteins into the soil.

150. After the wheat seed is harvested, the applicant proposes that the remaining plant material would be collected and incinerated on site. However, the licence conditions also include the possibility of incorporation of stubble into the soil. The initial level of expression of the introduced proteins is low and exposure is likely to decrease with time, as a result of their degradation in the soil. Neither the OAT or CAH protein or proline is known to be toxic to any organism.

151. After harvest, soil micro organisms would be likely to be exposed to the introduced proteins and free proline while the plant residues are broken down. Data on the persistence of the introduced proteins in soil are not available.

152. The release is small in size and limited in duration, which will limit exposure of microorganisms to the GM wheats.

Section 2.6 Other sources of OAT and CAH proteins and proline in the environment

OAT Protein

153. The OAT protein is widespread in the environment and has been detected in numerous microbial, plant and animal species (Roosens et al. 1998). In mammals, OAT enzyme activity occurs in a number of tissues including liver, kidney and retina (Inana et al. 1986; Mitchell et al. 1988; Dekaney et al. 2001).

154. OAT proteins from different organisms have a conserved amino acid sequence. Delauney et al. (1993) reported that moth bean (*Vigna aconitifolia*) OAT was highly homologous to mammalian and yeast δ-OATs. The OAT enzyme introduced to the GM

wheat is from *A. thaliana* and it shares significant homology with other OAT proteins from plants, animals and microbes (Roosens et al. 1998).

155. The OAT protein is therefore widespread in the environment and is commonly encountered by plants and mammals.

CAH Protein

156. The CAH protein occurs naturally in the fungus *Myrothecium verrucaria*. It has not been found in plants. *M. verrucaria* is found in both tropical and temperate soils (Drenth & Guest 2004). Two accessions of *M. verrucaria* were sourced from the rhizosphere of Australian wheat fields. The fungus is widely distributed in the environment, and likely to be present at the release site (information supplied by the applicant).

157. It is well established the breakdown of cyanamide to urea in soil involves microbial activity (Estermaier et al. 1992). While the enzyme from the *M. verrucaria* is the only CAH described in the literature to date, and biochemical confirmation of the production of CAH enzyme activity has not been demonstrated in other species, it is likely that CAH is present in other soil microorganisms.

158. Putative *cah* genes have been identified from gene sequencing projects in the fungi *Gibberella zeae* PH-1, *Aspergillus nidulans* FGSC A4, *Neurospora crassa* (red bread mould) and in bacteria *Streptomyces*, *Photorhabdus luminescens* and *E. coli* and in *Saccharomyces cerevisiae* (bakers yeast). The hypothetical protein sequences predicted from these putative genes share significant homology with CAH from *M. verrucaria* and on this basis are predicted to encode CAH proteins. It is therefore likely that CAH is commonly encountered in the environment.

Proline

159. Proline is present in all organisms and is therefore ubiquitous in the environment and in is present in food either as free proline or incorporated into proteins. Elevated levels of proline occur naturally in salt tolerant plant species, and proline levels can be increased in many plants in response to osmotic and other environmental stresses (Delauney & Verma 1993; Hasegawa et al. 2000b; Hellmann et al. 2000).

Section 2.7 Toxicity and allergenicity of the introduced proteins and increased proline content.

160. Neither OAT nor CAH proteins have been implicated in toxic or allergenic responses.

2.7.1 Toxicity

Toxicity of proline

161. The altered phenotype of the GM wheat conferred by the genetic modification is likely to produce elevated proline levels approximately two to three times that of non GM wheat. This is still within the range encountered in other plants.

162. L-proline is one of the 20 amino acids present in all organisms and is ubiquitous in the environment. In humans, proline is a non-essential amino acid that constitutes the bulk of collagen and is a normal component of the human diet. Proline is not considered toxic at the levels in normal dietary exposure nor is it considered toxic even at high doses (Kampel et al.,

1990, cited in Anon 1992) and it is used as a dietary supplement (Food Standards Australia New Zealand 2003).

163. Hayasaka et al. (1985) reported no adverse effects in human patients administered between 3 - 10 g of L-proline per day for between two and four years . No adverse effects, (including histological inspection of liver and kidneys) were noted in rats administered a dose of L-proline of 50 mg/kg body weight/day for one month (Kampel et al., 1990, cited in Anon 1992).

Increased proline concentration

164. The level of free proline in the GM wheat under salt-stress (150 mM NaCl) was approximately three times that in non-GM wheat. No difference was detected between the proline content of GM and non-GM wheat in the absence of salt stress (see Appendix 1 for details).

165. Many plants naturally produce elevated levels of proline in response to osmotic stress. The levels are typically 5-50 μmol/g fresh weight (approx. 6 – 60 mM on plant water basis) and are highest during exposure to osmotic stress (Rontein et al. 2002). A number of plants displaying salt tolerance are utilised as human food or animal feed including: *Acacia* spp.; barley (*Hordeum vulgare* L.); pearl millet (*Pennisetum typhoides*); quinoa (*Chenopodium quinoa*); wild rice (*Zizania aquatica*); and beets (*Beta vulgaris*) (Biosalinity Awareness Project 2005).

166. The level of free proline in the GM wheat even under conditions of salt stress would be less than that encountered in many naturally occurring salt tolerant plants. There are no reports of toxicity associated with animals or humans ingesting plants with naturally high proline levels.

167. Plants which completely lack the key enzyme in the pathway for proline degradation, ProDH, manifest toxicity symptoms in response to exogenous proline (Mani et al. 2002; Nanjo et al. 2003). However the observed toxicity does not appear to be mediated by a toxic effect of proline *per se*, but a range of other factors (Nanjo et al. 2003) and may involve the intermediate P5C (Mani et al. 2002). None of the key enzymes for proline synthesis and degradation will be impaired as a result of the genetic modification. Even with the overexpression of the *A. thaliana* OAT, proline levels would not be expected to accumulate excessively in the GM wheat plants, but would rather be constantly metabolised by the intact pathway (Delauney & Verma 1993; Delauney et al. 1993).

168. It is important to note that the increases in proline achieved to date by single genetic modifications have generally been low and the increases in stress tolerance relatively small (Rontein et al. 2002). Even in GM plants modified to achieve elevated proline, the effects of feedback regulation on the proline synthesis and catabolism still effect control over the pool of free proline (Blumwald et al. 2004).

169. The level of free proline expected in the GM wheat plants is therefore highly unlikely to pose a risk of toxicity.

OAT protein

170. There are no reports of the OAT enzyme acting as a toxin. Wheat naturally contains the OAT protein. The introduced OAT protein from *A. thaliana* and will be very similar to the native wheat OAT protein and would have similar properties with respect to toxicity.

171. The level of OAT protein in the GM wheat lines will be greater than for non GM wheat. Based on the pattern of expression normally associated with the *Ubi*-1 promoter, expression is expected to be highest in rapidly growing plant tissues (Stoger et al. 1998). Nevertheless, the level of OAT expression would be expected to represent only a small fraction of the total protein in the GM wheat plants.

CAH protein

172. The CAH protein will be expressed in the GM wheats. Details on the level of expression are not yet available. However, based on the pattern of expression normally associated with the *Ubi*-1 promoter, expression will be highest in rapidly growing plant tissues. Nevertheless, the level of CAH expression would be expected to represent only a small fraction of the total protein in the GM wheat plants. There are no reports of the CAH enzyme acting as a toxin.

173. The *cah* gene is derived from *M. verrucaria* which is a plant pathogen that produces a class of mycotoxins known as trichothecenes (Andolfi et al. 2005; Abbas et al. 2002). However, only the *cah* gene from *M. verrucaria* was introduced to the GM wheat. It represents only a small proportion of the *M. verrucaria* genome and is not involved in the production of the mycotoxins.

2.7.2 Allergenicity

174. Although there are no predictive assays available to assess the allergenic potential of proteins, much is known about the biochemical events associated with allergic reactions, as well as the kinds of proteins that cause problems (Metcalfe et al. 1996; Taylor & Lehrer 1996).

175. Predictions of allergenicity have been based on sequence and structural and biochemical comparisons with known allergens. Protein allergens usually share a number of characteristics (Davies 1986; Flavell et al. 1992; Fuchs et al. 1993b; Fuchs et al. 1993a; Taylor 1995, Monsanto Unpublished; Fuchs & Astwood 1996; Metcalfe et al. 1996; Kimber et al. 1999; ANZFA 2001), including the following:

- molecular weight ranges between 15-70 kD;
- typically glycosylated;
- stable in the mammalian digestive system;
- stable during high temperatures involved in cooking or processing; and
- present as the major protein component in the specific foods.

176. Neither the OAT nor CAH protein has been reported as an allergen. The mature OAT protein from *A. thaliana* and CAH protein from *M. verrucaria* have predicted molecular weights of approximately 48 kD and 27 kD respectively, within the size range of allergens. No data is available on whether OAT from *A. thaliana* or other organisms, or the CAH protein are stable in mammalian digestive systems or at high temperatures.

177. OAT is not recognised as a major protein component in plant tissues. Although the *A. thaliana* OAT and *M. verrucaria* CAH will be constitutively expressed in the GM wheat plants under the Ubi-1 promoter, the total expression of each protein would still be expected to represent a small fraction of total plant protein production. The Ubi-1 promoter is likely to direct expression of both OAT and CAH in rapidly growing plant tissues and pollen, however neither protein is likely to be present on the surface.

178. Neither the *A. thaliana* OAT nor the *M. verrucaria* CAH proteins show any significant sequence homology to known allergens (data supplied by the applicant). This was determined by searching for stretches of identity of six or more contiguous amino acids with known allergens using the BLAST search tool (Altschul et al. 1997) and the approach described by Kleter and Peijenberg (2003). Identities of between six and eight contiguous amino acids with known allergens may reveal potential IgE binding epitopes (Hileman et al. 2002; Kleter & Peijnenburg 2002). Several three or four amino acid identities with known allergens were detected for OAT and CAH but no identities of six or more amino acids were detected. Identities of less than six amino acids are highly likely to be the result of random matches and are considered non-significant (Hileman et al. 2002).

179. Analysis of a Hopps-Wood hydropathy plot of the OAT protein sequence support the conclusion that these short four amino acid motifs would not be present on the surface of the OAT protein and therefore not available to be involved in IgE biding (data supplied by the applicant). Mapping the short identities detected on a hydropathy plot for CAH indicated that they are associated with minor hydrophilic peaks and potentially on the protein surface (data supplied by the applicant).

180. These data support the conclusion that neither the OAT nor CAH is likely to be allergenic.

Section 2.8 Toxicity and allergenicity assessment of the GM wheats

181. The UK Royal Society (2002) has concluded that there is at present no evidence that available GM foods cause allergic reactions, and that the risks posed by GM plants are in principle no greater than those posed by conventional breeding or by plants introduced from other areas of the world.

182. Neither the OAT nor CAH protein is predicted to be an allergen or toxin. Although the GM wheat plants are expected to contain increased levels of free proline, this is not considered to pose any risk of toxicity. Nevertheless, no material produced from the GM wheat in the field trial will be used in human food or animal feed. The scale of the trial is small (0.45 ha) and the possibility for exposure of humans or other organisms will be very limited. It is therefore concluded that the risk of the GM wheat resulting in toxic or allergic effects is very low

183. It is a licence requirement to report to the Regulator any adverse effects, such as allergenic responses of people as a result of working with the GM wheat.

SECTION 3 CONCLUSIONS REGARDING TOXICITY OR ALLERGENICITY

184. It is considered that the risk of the GM wheats being toxic or allergenic for humans or other organisms is very low because:

- the release is small in scale (0.45 hectares) and limited in duration (one wheat growing season);

- the introduced proteins occur naturally and are therefore already present in the environment;
- OAT enzymes that are very similar to the introduced A. thaliana OAT are present in many other organisms and widespread in the environment.
- the level of proline in the in the GM wheat plants is unlikely to have any toxic effects;
- none of the GM wheat materials from the release will be used in human food or animal feed;
- exposure to the introduced proteins through working with the GM wheat plants would be very low;
- processing of the GM wheat seed will only occur on a small scale (for preparation of seed for possible future trials); and
- the introduced proteins are not known to be allergenic, nor do they have significant homology with known allergenic proteins.

185. The licence holder is required to report to the Regulator any adverse effects on human health and safety (e.g. allergic reactions as a result of occupational exposure to the GM wheat) or to the environment.

SECTION 4 RESEARCH REQUIREMENTS

186. The release is a small and early-stage research trial to establish if the GM wheat plants have increased proline levels and salt tolerance under field conditions. However, before any application for larger scale or commercial releases of the GM wheat lines could be evaluated, further detailed information would be required on:

- expression levels of the OAT and CAH proteins in different plant tissues under Australian field conditions;
- the levels of free proline and metabolites in different plant tissues under Australian field conditions; and
- the potential toxicity and allergenicity of the GM wheat, particularly the introduced OAT and CAH proteins.

APPENDIX 3 WEEDINESS

187. Under Section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan (RARMP). In this Appendix, risks posed to the environment by the approved dealings are considered in relation to the potential for the GMOs to become problematic weeds.

SECTION 1 NATURE OF THE WEEDINESS HAZARD

188. There are numerous definitions of weeds including 'a plant growing where it should not be'. Weeds become a problem to the community when their presence or abundance interferes with the intended use of the land they occupy. Weeds may also represent a source of food to various organisms, hence the introduction of weeds to an environment may also bring about ecological change by altering the structure of food webs.

189. Weeds are thought to share a number of life history characters that enable them to rapidly colonise and persist in ecosystems, particularly those that are regularly disturbed (Roy 1990; Williamson & Fitter 1996). These characteristics include:

- ability to germinate, survive, and reproduce under a wide range of environmental conditions;
- long-lived seed with extended dormancy periods;
- rapid seedling growth;
- rapid growth to reproductive stage;
- long continuous seed production;
- ability to self-pollinate but not exclusively autogamous;
- use of unspecialised pollinators or wind when outcrossing;
- high seed output under favourable conditions;
- special adaptations for long distance and short distance dispersal; and
- being good competitors.

190. However, because environmental conditions have a substantial influence on these attributes, and other factors such as plant community composition and availability of key resources (e.g. space, water, light and nutrients) influence the potential of a plant species to invade, weedy characteristics alone are not enough to determine if a plant will become a problematic weed. Therefore, the most successful predictors of weediness remain taxonomic affinity to other weedy species and the history of a given species' weediness elsewhere in the world (Panetta 1993; Pheloung et al. 1999).

191. The two GM wheat lines differ from conventional non-GM wheat in the expression of two additional proteins. The enzymes ornithine aminotransferase (OAT) and the cyanamide hydratase (CAH) are present in both GM lines. Overexpression of the OAT enzyme results in increased levels of free proline in the GM wheat, which confers tolerance to saline conditions (see Appendix 1 for details).

192. The possibility was considered that the GM wheats might have the potential to be harmful to the environment because of inherent weediness or increased potential for

weediness, either as a result of the modified trait salt tolerance, expression of the introduced proteins, or as a result of unintended effects of the genetic modification.

193. This could occur if the GM wheats displayed altered characteristics such as increased fitness or increased seed shatter or increased dormancy. If the GM wheats were to spread in the environment as weeds, this could result in impacts such as loss of native biodiversity or adverse effects on agricultural systems.

SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

194. In assessing the likelihood of adverse impacts due to weediness of GM wheat, a number of factors were considered, including:

- the inherent weediness of conventionally bred non-GM wheat;
- the potential selective advantage conferred by the introduced proteins;
- the potential weediness of the GM wheat lines; and
- the potential for spread and persistence of the GM wheats beyond the release site.

Section 2.1 Inherent weediness of conventional non-GM wheat

195. Attributes of non-GM wheat associated with potential weediness are discussed in the document 'The Biology and Ecology of Bread Wheat (*Triticum aestivum* L. em Thell.) in Australia' (OGTR 2004) that was produced in order to inform the risk assessment processes for licence applications involving GM wheat. This document can be accessed at *www.ogtr.gov.au/.*

196. Wheat is not considered to be a problemtic weed in Australia. It does not possess the characteristics commonly associated with successful weeds, such as seed dormancy, long persistence in the soil, germination under a broad range of environmental conditions, rapid vegetative growth, short lifecycle, very high seed output, high seed dispersal and long-distance seed dispersal (Keeler 1989). Factors including soil moisture, nutrient limitation, temperature and roadside management practices limit the establishment and/ or persistence of wheat seedlings. Information on the weediness of non-GM wheat is included here to establish a baseline for comparison with the GM wheats being assessed.

197. An important element in predicting weediness is taxonomic relationship, considering weediness within a taxon, including its history of weediness in any part of the world (Bergelson et al. 1998; Panetta 1993; Pheloung 1995). Wheat has been grown for centuries throughout the world without any reports that it is a serious weed pest. Wheat is not considered to be a problematic weed in Australia (Groves et al. 2000; Groves et al. 2002). Bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* ssp*. durum* L.) are cultivated in Australia but other species of *Triticum* and the closely related genus *Aegilops* are classified as quarantine weeds in Australia and their import is not permitted. There are no established populations of these species at the release site.

Competitiveness and persistence

198. Wheat has a low competitive ability and does not have the potential to develop into a weed (Keeler 1989; Keeler et al. 1996).During the domestication of the modern wheat plant, characteristics that benefited farmers were modified and this also eliminated the ability of wheat germplasm to survive without human intervention (Eastham & Sweet 2002). Non-

shattering heads were favoured because of ease of harvest and this trait placed wheat plants at a competitive disadvantage to other species which could more efficiently distribute seed.

199. Wheat is not considered a problematic weed in Australia (Groves et al. 2003) or elsewhere. Nevertheless *T. aestivum* is considered to be naturalised and a minor weed of agricultural systems in all Australian states in which wheat is grown (Groves et al. 2003). Glover (2002) reported that wheat is a minor problem weed in some natural environments in Tasmania.

200. In the USA and Nepal *T. aestivum* is a common weed in some situations (Holm et al. 1979; Bridges 1992; Randall 2002). In Europe, wheat is often seen as a relic in fields, roadsides and waste ground, but these observations are likely the result of spillage (Eastham & Sweet 2002) and the wheat plants are rarely persistent.

201. A recent study by Anderson and Soper (2003) indicated that volunteer wheat in North America may persist for 16-24 months after harvest, but that emergence was extremely variable and that the causes were numerous and included genotypic, environmental, and production factors.

202. The persistence of wheat, and therefore its potential for weediness, is limited in Australia and elsewhere by climatic conditions including rainfall, temperature and frost (Nix 1987). In addition, many disease causing organisms infect wheat including bacteria, viruses, fungi and nematodes and can cause major crop production losses (Brennan & Murray 1988; Heyne 1987; OGTR 2005b).

203. In Australia, the establishment and persistence of wheat volunteers are further limited by high summer temperatures (resulting in severe moisture stress) and post-harvest practices such as stubble burning to reduce disease outbreaks.

Dormancy

204. Dormancy of seeds can contribute to the development of a seed bank in the soil and this represents an opportunity for GMOs to persist in the environment.

205. Red wheats (containing anthocyanin pigments in the seed coat) which are grown in North America and Europe exhibit some dormancy and sprouting tolerance and typically are dormant for extended periods after harvest (3-7 months, Pickett 1989). Red wheat seeds usually display primary dormancy at maturity and go through a period of 'after-ripening' during which time they will not germinate despite favourable soil moisture and temperature conditions.

206. In contrast, wheats grown in Australia are white wheats which are considered to have little seed dormancy (Nyachiro et al. 2002; OGTR 2005b). In fact, white wheats are subject to pre-harvest sprouting of grain in response to moisture. The parent cultivars, 'Carnamah' and 'Westonia' do not exhibit any dormancy and are very susceptible to pre-harvest sprouting (Littlewood 2004; 'Wheat varieties in Australia 1968-2001', Whiting 2002).

207. Expression of seed dormancy in wheat is also affected by temperature and declines if mean daily temperature increases over 12.5°C. Mean daily temperature in the Corrigin shire for the period September- December (possible seed maturing of the wheat crop) range from 12.5°C – 22.2°C (Bureau of Meteorology 2005), further reducing the possibility of dormancy occurring in the GM wheat. In addition, if wheat is buried (eg by ploughing) at a depth

where dormancy is enforced, if seeds do germinate they tend to be unable to reach the surface (Pickett 1993).

Dissemination

208. Wheat seed is not significantly disseminated by animals. Birds and animals, including feral pigs, marsupials, vermin and rabbits are likely to eat planted seed, wheat plants and mature grain or seed *in situ* rather than carry them elsewhere for storage or consumption. Dissemination into the environment is predominantly through human activity.

Section 2.2 Potential weediness of the GM wheat lines

209. The consideration here is whether or not there are any attributes of the GM wheat lines that would act to increase the potential weediness of the plants over that of non-GM wheat plants. Any potential selective advantage conferred by the introduced proteins that could result in weediness will be addressed in Section 2.3.

210. The applicant has advised that under non-saline conditions the GM wheat plants do not look different from the non-GM plants, but the GM plants are slightly smaller than the non-GM plants. Agronomic assessment of the GM salt tolerant wheat lines will be undertaken to determine the effects of the introduced genes on plant characteristics including grain yield.

Section 2.3 Potential selective advantage conferred by the genetic modification

2.3.1 OAT enzyme and increased proline content

211. The modified trait in the GM wheat lines is for tolerance to saline soil conditions. The salt tolerance is conferred by over-expression of the OAT enzyme resulting in increased levels of the amino acid proline. Increased levels of free proline could confer a selective advantage to GM wheat plants in saline conditions.

212. The degree of salt tolerance of the GM wheat under field conditions is not known and would be tested in the release. The GM wheat lines display increased tolerance to saline conditions, relative to non-GM wheat, under glasshouse conditions. The GM salt tolerant wheat had a greater than 2-fold increase in tiller number, seed number and seed weight in comparison to the non-GM wheat under saline hydroponic conditions (150 mM salt, information provided by the applicant).

213. Under non-saline conditions the GM plants are slightly smaller than the non-GM plants otherwise the plants do not look different. The applicant has suggested that this may be due to higher metabolic turnover of proline in the GM plants. This may result in reduced competitiveness. Growth retardation has been observed in other plants genetically modified to have elevated levels of osmolytes, in the absence of stress (Maggio et al. 2002). Therefore it is possible that there is a metabolic cost incurred through the overproduction of proline which may impact on the overall fitness of the GM wheat.

214. Increases in free proline are implicated in responses of many plants to a range of abiotic and biotic stresses other than salt stress, including: frost and cold acclimation (Chu et al. 1978; Dorffling et al. 1997; Tanatau et al. 2004); drought or water stress (Nabizadeh et al. 2004; Stewart et al. 1977; Vajrabhaya et al. 2001; Ramachandra et al. 2004); ultraviolet light (Demir 2000); and pathogens (Fabro et al. 2004; 1995; Reddy 2000).

215. There are a number of studies linking increased proline content in wheat with either the response or tolerance to a variety of stresses, including: salt (1996; Chauhan et al. 1983; Keles & Öncel 2004); drought or water stress (Keles & Öncel 2004; Gusta & Chen 1987); frost (1996); and pathogens (1995; Reddy 2000).

216. Increased levels of free proline have been achieved by genetic modification in a number of plants. The modifications have either overexpressed or decreased key enzymes involved in proline metabolism and the resultant GM plants have exhibited increased tolerance to a range of abiotic stresses as summarised in Table 5 (for details of the enzymes modified refer to Figure 2, Appendix 1). The elevation of other osmolytes through genetic modification has also increased plant tolerance of abiotic stresses (recently reviewed by, Blumwald et al. 2004).

Table 5 Elevated proline and stress tolerance in GM plants

Genetic modification	Plant	Stress tolerance	Reference
increased OAT	*Arabidopsis thaliana*	salt	Roosens et al. (2002)
increased P5CS	*Nicotiana tabacum*	salt	Kishor et al. (1995), Hong et al. (2000)
	Rice (*Oryza sativa* L.)	salt water stress	Zhu et al. (1998)
increased P5CR	Soybean (*Glycine max* L.)	salt water stress	de Ronde et al. (2004)
reduced ProDH	*Arabidopsis thaliana*	salt freezing	Nanjo et al. (1999a)

217. While there is also a growing body of evidence that proline may be involved in a range of plant stress responses, the level of free proline is under tight control (Delauney & Verma 1993; Verbruggen et al. 1996; Nanjo et al. 1999b; Yoshiba et al. 1997) and it is only one element in a complex regulatory system involving the expression of many genes, modulation of biochemical pathways and physiological responses (Peng et al. 1996; Nakashima et al. 1998; Nakashima et al. 1998; Verbruggen et al. 1996; Rizhsky et al. 2004).

218. It is important to note that the increases in proline achieved to date by single genetic modifications have generally been low and the increases in stress tolerance relatively small (Rontein et al. 2002). Even in GM plants modified to achieve elevated proline, the effects of feedback regulation on the proline synthesis and catabolism still effect control over the pool of free proline (Blumwald et al. 2004).

219. Whether any of these increased proline modifications provide the various GM plants with significant advantages under field conditions has not been established, and there is often little correlation between laboratory and field results (Blumwald et al. 2004). Some authors therefore suggest that to achieve significant increases in stress tolerance of crop plants multiple genes or pathways may need to be manipulated (Blumwald et al. 2004; Rontein et al. 2002).

220. If the GM wheat exhibits increased proline levels under field conditions this may confer an advantage, relative to non-GM wheat, in response to abiotic and/or biotic stresses. However, the survival of the GM wheat plants would still be limited by water availability, temperature, low intrinsic competitive ability and the other environmental factors which normally limit the persistence of wheat plants in Australia.

221. In conclusion, while the genetic modification may provide the GM wheat with an advantage in some environmental conditions relative to non-GM wheat, it is unlikely to increase other characteristics normally associated with intrinsic weediness (which non-GM wheat does not possess).

2.3.2 CAH enzyme

222. The CAH enzyme confers tolerance to cyanamide which is used elsewhere as a herbicide, nitrogenous fertiliser and to promote budburst in fruit trees.

223. In Australia, cyanamide is only registered for budburst regulation on grapes and kiwifruit (APVMA 2005a; APVMA 2005b) and it is not used as fertiliser or herbicide.

224. Cyanamide was only used during laboratory selection of the GM wheat plants. The *cah* gene will not provide any selective advantage to the GM wheat plants because they will not be exposed, either intentionally or unintentionally, to this chemical during the release.

Section 2.4 Persistence of the GM wheats at the release site

225. The GM wheat might persist at the release site as a result of planted seed that has not germinated or seed from the GM wheat plants that may fall to the ground at maturity (seed shatter) and during harvest which may also enter the soil. Some non-viable plant material might also be incorporated into the soil after harvest. As explained in Section 2.1 above, wheat is not prone to shattering and white wheats have very little seed dormancy. The parental cultivars are very susceptible to pre-harvest sprouting and do not exhibit seed dormancy (Littlewood 2004).

226. The applicant proposed a number of measures to minimise the entry of GM wheat seeds into the soil (seedbank) and the persistence of GM wheat plants at the release site, including: hand harvesting the GM wheat; following harvest, removal of residual plant material and destruction by incineration; promoting the germination of residual seed by three post-harvest irrigations; monitoring the release site for two years after harvest and destruction of any emerging volunteers by herbicide application. As noted in section 2.1, there are reports of volunteer wheat persisting for up to two years in commercial production systems (Anderson & Soper 2003).

227. Post-harvest tillage of the release site is not required as the parent cultivars do not exhibit dormancy. Three irrigations of the release over three months is likely to encourage germination of volunteers and treatment with herbicides will eliminate any volunteers.

228. Licence conditions have been imposed that require two years of post-harvest monitoring of the release site at conclusion of the trial and destruction of volunteers before flowering so that the GM wheat is unable to persist in the environment at the release site.

Section 2.5 Spread of GM wheats beyond the release site

229. If GM wheat seed is spread from the release site it could result in establishment and persistence of the GM wheat in the environment. The release is one site, one season, small scale (0.45 ha) trial limiting the possibility of dissemination of the GM wheat from the site. Licence conditions impose a 500 m isolation zone (from any other wheat) around the release site. This will include a 10 m monitoring zone in which the presence of wheat and sexually compatible species will be monitored and any found, destroyed.

230. Possible causes of dissemination could be spillage during transport, sowing and harvesting (ie through human activity). Workers moving within the GM wheat would need to ensure that GM wheat seeds are not transported to other wheat growing sites on their clothing. If seed were disseminated from the site, it would require favourable conditions (moisture, temperature) to germinate.

231. Movement by animals, particularly vermin and birds is also possible. The applicant has advised that birds are not considered a problem for crops at this site. It is far more likely that birds and vermin would eat wheat seed on site rather than carry the seed to another location. These GM wheat lines are in a white wheat parental background, which have a thin seed coat and are therefore easily broken down in the digestive system of mammals and birds. Wheat is widely used as a feed for birds because it is nutritious and easily digested (Yasar 2003). To further minimise the potential for spread of the GM whet, a fence around the release site would limit the movement of plant materials from the site through the exclusion of rabbits and large animals, while covering the GM wheat from early seed set (also known as milky-dough stage of grain development) until harvesting would discourage birds.

232. Mouse numbers fluctuate every three to four years on average in Australia and after a mouse plague can remain very low for up to two years (Brown & Singleton 2002). Periods of between four and seven years are typical between mouse plagues in a particular region (Brown & Singleton 2002). Rodents are opportunistic feeders and their diet can include seeds, the pith of stems and other plant materials (Caughley et al. 1998). If any grain is removed from the trial sites, it is likely to be consumed by the rodents.

233. The average territory size of mice varies between breeding and non-breeding seasons, from 0.015 to 0.2 hectares respectively (Caughley et al. 1998). A circle with a radius of 25 m has an area of just over 0.2 hectares, suggesting that the 500 m isolation zone would prevent rodents moving GM wheat seeds to unmonitored growing environments.

234. A variety of insects are likely to feed on the crop, however it is unlikely that most of these would contribute to the dispersal of material from the GM wheat plants beyond the trial sites. It is possible that ants may remove seeds for underground storage but to depths where germination is highly unlikely. No data is available on the species of ants present at the trial sites, so typical territory size and seed storage behaviour is unknown. Although there are differences in ant behaviour and territory size across species, seed dispersal occurs at a local scale, such that seeds are usually only moved a few metres (Cain et al. 1998; Peters et al. 2003). Maximum seed dispersal distances by ants in Australia and the rest of the world are typically less than 40 m, with a mean dispersal distance of 0.96 m (Berg 1975; Beattie 1982; Gómez & Espadaler 1998). Therefore GM wheat seed is unlikely to be removed beyond the trial site or monitoring zone.

235. Seeds can be carried by water away from the release site. However, the release site is situated on slightly sloping ground (the applicant has advised that the land slopes 1 m in 60 m south to north) and slopes into the salt scalded soil. Very heavy rainfall would be required to carry wheat seed down the slope into the highly saline or scalded area. Records show the average rainfall (mm) for this area for the months November – February is only 16.5, 12.6, 14.3, and 16.7, respectively. Over the same period, average daily maximum temperatures (°C) are 26.8, 30.3, 32.5, and 31.4, respectively. These high temperatures would cause rapid desiccation of any exposed wheat seed and germinating seedlings. Therefore it is considered highly unlikely that volunteer wheat seeds would germinate in this area. The risk of seed escape is further minimised because the trial is small in scale and of limited duration. The licence requires that the release site be 50 m from the nearest natural waterway to minimise the risk of seed dispersal by water.

236. Gene flow via cross-pollination with non-GM wheat plants would contribute to the potential spread and persistence of the GMOs in the environment outside the release sites. This issue is discussed in detail in Appendix 4.

237. The approved dealing includes cultivation of the GM wheat lines and retention of all wheat seed for storage, laboratory research or future plantings (subject to separate application). The applicant proposes that wheat seeds would be harvested by hand at the end of the growing season, double-bagged and transported in containers to secure storage facilities, thus limiting the potential for accidental dispersal of the GM wheat seed beyond the trial sites. Wheat seed produced in the field trials would not be used for human food, nor would wheat seed, straw or other wheat products be used for stockfeed.

238. Licence conditions have been imposed to limit dispersal of seed and plant material from the trial sites. These include procedures for preventing accidental transfer on workers' clothing in the trial sites and containing the trail with bird proof netting from early seed set to harvest and a fence. Monitoring conditions are also imposed on the area surrounding the trial sites to ensure the identification, removal and destruction of any volunteers resulting from the trial.

SECTION 3 CONCLUSIONS REGARDING WEEDINESS

239. It is concluded that the risk of the GM wheat establishing as a problematic weeds as a result of the limited and controlled release is very low because:

- the release is small in scale (0.45 hectares) and limited in duration (April 2005 – January 2006);
- wheat has a low potential for dispersal by natural means;
- the introduced gene , oat, may confer a selective advantage under saline and other stress conditions;
- the increased proline trait may incur a metabolic cost that might reduce the fitness of the GM wheat;
- the introduced cah gene would only confer a selective advantage where cyanamide is used. Cyanamide is not used as a herbicide in Australia;
- major constraints on weediness of both GM and non-GM wheats are water availability, nutrient availability, disease, temperature, plant competition, herbivory, frost and fire;

- wheat does not possess characteristics commonly associated with weediness, and is not known to be a problematic weed in any environment; and
- the genetic modifications in the GM wheat lines are not likely to affect these characteristics of weediness (or to confer a selective environmental advantage).

240. It is considered that the risk of the GM wheats establishing as a weed is very low. The elevated proline trait may confer a selective advantage under saline conditions and potentially to some other stresses, relative to non-GM wheat. However, the GM wheat plants would still be limited by water availability and the range of other environmental factors which normally limit the persistence of wheat plants in Australia. It is considered unlikely that the modification would improve other characteristics normally associated with intrinsic weediness which wheat does not possess. It is also possible that there will be a metabolic cost incurred through the overproduction of proline which may impact on the overall fitness of the GM wheat.

241. The consequences of any increase in weediness will be managed by various strategies to limit the spread and persistence of the GM wheats from the release site. Licence conditions have been imposed to manage this risk, including a requirement to maintain a monitoring and an isolation zone around the trial site and to conduct post-harvest inspections of the release site to ensure volunteer plants are destroyed before flowering to minimise the risk of GM wheat spreading and persisting in the environment (Appendix 5 for details).

SECTION 4 RESEARCH REQUIREMENTS

242. The release is a small and early-stage research trial to establish if the GM wheat plants have increased proline levels and salt tolerance under field conditions. If the applicant makes any application for future, particularly larger releases of the GM wheat more information would be required on:

- the magnitude of the tolerance of the GM wheat to salt and other abiotic stresses; and
- the agronomic characteristics of the GM wheat lines that relate to fitness and potential weediness.

APPENDIX 4 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

243. Under Section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan (RARMP). This Appendix considers potential hazards that may be posed through the transfer of the introduced genetic materials from the GM wheats to other organisms.

244. Gene transfer is the movement of genetic material between individuals. Within a species genetic material is routinely exchanged between individuals of successive generations through sexual reproduction. Hybrids can sometimes be produced between closely related species through sexual reproduction although this may require significant assistance. For example, in plants, cross-pollination of wheat and rye in the laboratory produces primary triticale (2n =21, ABR); the application of a mutagenic chemical (colchicine) to F_1 hybrids is then required to double chromosome number and generate fertile triticales (2n=42, AABBRR). In animals, fertilisation of a mare by a donkey produces a mule. Hybrid progeny may be fertile or sterile, meaning hybridisation may or may not lead to the introgression of new genetic material into a population.

245. Without the application of gene technology, gene transfer is not readily observed between distantly related species, except between bacteria and between viruses. However transfer of genetic material between sexually incompatible organisms can occur. Detailed examination of DNA sequence similarities reveals that ancestral plants have occasionally exchanged small DNA fragments with distantly related organisms. However, there seems to have been only very limited transfer of genetic materials from plants to other types of organisms.

246. The likelihood of hazards arising from gene transfer is dependent on a number of factors that must form a necessary chain for a hazard to be realised, including:

- **opportunity** for gene transfer to occur such that the recipient organism is exposed to the genetic material of the donor in the form of pollen, plant cells or DNA;
- occurrence of the genetic material of the donor being incorporated into the genetic material of the recipient organism at a site and in a configuration that allows the genetic material to be functional;
- persistence of the transferred genetic material such that the recipient organism is able to survive, reproduce and maintain the genetic modification; and
- significance of the transferred genetic material such that its presence and/or expression in the recipient organism will result in an adverse impact on human health and safety or the environment.

247. For ease of reference, the assessment of gene transfer to other organisms is presented in four sections:

- **Section 1** details the nature and likelihood of a hazard arising through transfer of the introduced genetic materials from the GM wheats to other plants, including other wheat plants;
- Section 2 details the nature and likelihood of a hazard arising through transfer of the introduced genetic materials from the GM wheats to microorganisms;

- Section 3 details the nature and likelihood of a hazard arising through transfer of the introduced genetic materials from the GM wheats to animals, including humans; and
- Section 4 draws together the conclusions from these sections.

SECTION 1 GENE TRANSFER FROM THE GM WHEATS TO OTHER PLANTS

Section 1.1 Nature of the gene transfer hazard

248. Transfer of the introduced genes (*oat* and *cah*) or regulatory sequences to other cultivated (including volunteer) or naturalised (feral) wheat plants would present the same hazards and have the same potential impacts as their presence in the GM wheats (see Appendices 2-3 for details). However, if such a transfer occurred, it would increase the possibility that these genetic materials would further spread and persist where environmental conditions are suitable.

249. If gene transfer to other plant species were to occur, any resulting hazards to the environment could be highly varied, broadly depending upon the nature of the genetic materials and of the species to which transfer occurred. Transfer of the introduced genes or regulatory sequences into other plant species may have adverse effects on biodiversity, particularly native flora, if the recipient plants and their progeny gained a selective advantage, such as enhanced survival or reproductive capacity.

Section 1.2 Potential hazards from the introduced genetic materials

1.2.1 The *oat* gene

285. Over-expression of the OAT protein as a result of the genetic modification results in elevated levels of the amino acid proline. Elevated proline is expected to confer tolerance to saline conditions, and possibly to other stress conditions such as frost and water stress (see Appendices 1 and 3 for details). Transfer of the *oat* gene to other plants might confer tolerance to these stress conditions to those plants. A possible adverse outcome from transfer of the *oat* gene to other plants might be increased weediness.

1.2.2 The *cah* gene

250. Plants expressing the *cah* gene produce the CAH protein which confers tolerance to cyanamide. CAH expression is unlikely to be toxic or allergenic to humans and other organisms (see Appendix 2 for details) and is unlikely to affect weediness (see Appendix 3 for details). Transfer of the *cah* gene to other plants from the GM wheat would enable growth in the presence of cyanamide.

1.2.3 Promoters and regulatory sequences

286. If these sequences were to be transferred to other plants without the associated introduced genes of the GM wheats, the expression of endogenous plant genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change.

287. The introduced regulatory sequences are derived from maize (*Zea mays* L.). All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory sequences. The transfer of endogenous regulatory sequences to a new genetic context occurs naturally in all plant genomes and could also result in unpredictable effects.

Thus the potential hazard from the introduced sequences is no different to that posed by sequence transfer from non-GM plants or sequence transfer occurring within the genome of a plant species.

Section 1.3 Likelihood of a hazard arising through transfer of the introduced genetic materials to other plants

251. For a detailed consideration of the likelihood of gene transfer occurring between wheat plants, and between wheat and related species, including an overview of the pollination biology of wheat, see the document "The Biology and Ecology of Bread Wheat (*Triticum aestivum* L. em Thell.) in Australia" (OGTR 2004). This document is available at *www.ogtr.gov.au/* and was produced in order to inform the risk assessment processes for licence applications involving GM wheat.

252. Bread wheat belongs to the tribe *Triticeae* (Subfamily Pooideae, Family Poaceae). Gene transfer from bread wheat to other members of the *Triticeae* is dependent upon pollen movement and compatibility of the parent genomes (Table 6). Bread wheat is predominantly self pollinating.

1.3.1 Transfer to other wheat (*Triticum aestivum*) plants

253. Gene transfer through cross pollination can only occur to other wheat plants that are in very close proximity and that flower synchronously and even then at low frequencies.

254. Pollen dispersal in wheat plants is wind mediated. Outcrossing can occur at frequencies of up to ~5 % between adjacent rows (Waines & Hedge 2003). However, wheat pollen is heavy in comparison to other grass pollen and falls rapidly, 60 cm/second from a plant height of 1 m (Lelley 1966). Field conditions including temperature, relative humidity and wind intensity have a great influence upon pollen viability. Under field conditions, wheat pollen is viable for only a limited period of time (up to 30 minutes) (OECD 1999).

255. The NSW certified seed specifications require no separation distance between wheat crops grown for certified seed, but simply that a physical barrier (eg. fence) be in place to prevent seed mixing during harvest (Glover 2002). Similarly the international body that issues seed certification guidelines, AOSCA, requires only that foundation crops of wheat be isolated from other wheat crops so as to prevent mechanical mixing during harvest (Association of Official Seed Certifying Agencies 2001). The USDA has similar guidelines for various pedigree seed classes (summarised in Waines & Hedge 2003; Hucl & Matus-Cadiz 2001).

256. Hucl and Matus-Cadiz (2001) studied outcrossing in Canadian spring wheats and noted that the single sowing time reduced synchrony of flowering in different cultivars. In this study, the highest outcrossing rate was 3.8 % at 0.5 m from the pollen source. Outcrossing decreased with distance from the pollen source and in some Canadian wheat cultivars outcrossing was restricted to a distance of 3 m from the pollen source. In two cultivars, 'Robin' and 'Oslo', outcrossing was observed at 27 m. The authors suggested that 30 m separation is required during the production of certified seed and registered seed of these 2 cultivars, to avoid contamination through outcrossing. The study indicated that a 30 m separation zone would be expected to eliminate outcrossing problems in wheat.

257. Gene flow studies on wheat under Australian conditions indicated out-crossing frequency was very low, 0.012% at the outer edge of a 2 m wide buffer. These studies were

conducted by CSIRO during releases under the former voluntary Genetic Manipulation Advisory Committee (GMAC) (final report on PR65 and PR66). Wheat generally was rated as having low potential for outcrossing and consequently, low potential for causing impacts upon farming and other environments (Glover 2002).

Table 6. Chromosome number and genome(s) of the species of the tribe *Triticeae* (Dewey, 1984; Kimber and Sears, 1987)

Species	Synonyms	Chromosome Number	Genome code
I. Diploid species			
Triticum boeoticum L.		14	A
Triticum speltoides	*Aegilops speltoides*	14	S
Triticum tauschii (Coss.) Schmal.	*Aegilops squarrosa*	14	D
Triticum bicorne Forsk.	*Aegilops bicornis*	14	S^b
T. longissimum (Schweinf. & Muschli in Muschli) Bowden	*Aeg. longissima*		S^l
T. searsii (Feldman & Kislev) Feldman, comb. nov.		14	S^s
T. tripsacoides (Jaub & Spach) Bowden	*Aeg. mutica*	14	Mt
T. comosum (Sibth. & Sm.) Richter	*Ae. comosa*	14	M
T. uniaristatum (Vis.) Richter	*Ae. uniaristata*	14	Un
T. dichasians (Zhuk.) Bowden	*Ae. caudata*	14	C
T. umbellulatum (Zhuk.) Bowden	*Ae. umbellulata*	14	U
II. Polyploid species			
T. zhukovskyi Men & Er.		42	A.A.G
Triticum timopheevii (Zhuk.) Zhuk.	*T. araraticum*	28	A.G
T. crassum (4x) (Boiss.) Aitch. & Hensl.	*Ae. crassa*	28	D.M
T. ventricosum Ces.	*Ae. ventricosa*	28	D.Un
T. crassum (6x) (Boiss.) Aitch. & Hensl.	*Ae. crassa*	42	D.D.M
T. syriacum Bowden	*Ae. crassa* ssp. *vavilovii*	42	D.M.S
T. juvenile Thell.	*Ae. juvenalis*	42	D.M.U
T. kotschyi (Boiss.) Bowden	*Ae. kotschyi*	28	U.S
T. ovatum (L.) Raspail	*Ae. ovata*	28	U.M
T. triaristatum (4x) (Willd.) Godr. &Gren.	*Ae. triaristata*	28	U.M
T. triaristatum (6x) (Willd.) Godr. &Gren.	*Ae. triaristata*	42	U.M.Un
T. machrochaetum (Schuttl. &Huet. Ex Duval-Jouve) Richter	*Ae. biuncialis*	28	U.M

Species	Synonyms	Chromosome Number	Genome code
T. columnare (Zhuk.) Morris & Sears	*Ae. columnaris*	28	U.M
T. triuciale (L.) Raspail		28	U.C
T. cylindricum Ces.	*Ae. cylindrica*	28	C.D
Thinopyrum ponticum		70	J-E
Thinopyrum intermedium		42	E1.E2.S
III. Domesticated species			
Triticum turgidum (durum wheat)		28	A.B
Triticum aestivum (bread wheat)		42	A.B.D
Secale spp. (ryes)		14	R
Hordeum spp. (barleys)		14	H

258. The applicant proposed measures to limit gene transfer including a 1 m pollen trap of non-GM wheat and, where salt scalding would prevent the growth of non-GM wheat, a 1.5 m high interceptor wall to prevent the dispersal of seed and pollen.

259. Because wheat is primarily self-pollinating and pollen movement is mediated by wind, a pollen trap is considered unlikely to be an effective measure to prevent pollen escape from the release site. Pollen traps are most suitable for insect pollinated crops (eg canola) where insects that leave the trial site are attracted to forage on the adjacent non-GM buffer. Overseas reports have measured out-crossing frequency decreasing to 0.005% at 300 m from wheat fields usually in the prevailing wind direction (Matus-Cadiz et al. 2004). Advice received suggests that a physical barrier such as a wall might not be effective in reducing wind-mediated pollen movement.

260. The applicant has advised that the nearest commercial wheat crop will be 600 m away. To limit the likelihood of gene transfer occurring, licence conditions have been imposed requiring separation of the GM wheat from other wheat crops by a distance of at least 500 metres; removing sexually compatible plants from the monitoring zone and *Triticum*, *Secale* and *Triticale* populations from the isolation zone, followed by 2 years of post-harvest monitoring of the release site to remove volunteers before they flower (see Appendix 5 for details).

261. The likelihood of a hazard arising through transfer of the introduced genetic materials to other cultivated wheat will be further minimised by the small scale (0.45 hectares) and limited duration (April 2005 – January 2006) of the release.

262. Non-GM bread wheat will also be planted as part of the trial and outcrossing from the GM wheat plants to these plants is possible. Licence conditions have been imposed that require these plants and resultant seed to be treated in the same manner as the GM wheat.

1.3.2 Transfer to other sexually compatible (*Triticum* and *Aegilops*) species

263. Wheat is a hexaploid with a complex genome (AABBDD, see Table 6). It is sexually compatible with various species which share similar, compatible genomes, however even where pollination does occur hybrids are frequently male sterile (Heyne 1987). Wheat is not sexually compatible with plants outside the *Triticeae* tribe within the family Poaceae.

264. Wheat is sexually compatible with many species both within the genus *Triticum* and in other related genera such as *Aegilops* and *Elytrigia* (OGTR 2005b). Wheat also hybridises readily with rye (*Secale cereale*). Successful hybridisation would be most likely between the GM wheat plants and sexually compatible relatives if they are in close proximity and flower in synchrony.

265. In Australia, the major compatible relatives that wheat can hybridise with are durum wheat (*T. turgidum* ssp. *durum* L.), triticale and rye. Bread wheat is incompatible with all other grass species in Australia. Other species of *Triticum* and the closely related genus *Aegilops* are recognised as quarantine weeds in Australia (see AQIS ICON database at: www.aqis.gov.au/icon32/asp/ex_querycontent.asp).

266. Triticale is derived from artificial breeding of rye and wheat. Most commercial triticale grown in Australia is hexaploid triticale (2n = 42, AABBRR) (Larter & Gustafson 1980). Wheat/triticale or wheat/rye hybrids occurring naturally will most likely be sterile or

unsuccessful because of cytological instability caused by the interaction of the non-homoeologous R and D genomes (CFIA 1999; OECD 1999; Larter & Gustafson 1980).

267. In field experiments with mixed plantings, hybrids were obtained between bread wheat and *T. durum*, *Aegilops biuncialis*, *A. cylindrica*, *A. ovata*. Embryo rescue was required to produce plants from the cross between bread wheat and *A. cylindrica* (Jacot et al. 2004).

268. To limit the likelihood of gene transfer occurring, licence conditions have been imposed requiring separation of the GM wheat from other wheat crops by a distance of at least 500 metres; removing sexually compatible plants from the monitoring zone and *Triticum*, *Secale* and *Triticale* populations from the isolation zone, followed by 2 years of post-harvest monitoring of the release site to remove volunteers before they flower (see Appendix 5 for details).

1.3.3 Transfer to other members of the Triticeae tribe

269. Where interspecific and intergeneric hybrids between wheat and its wild relatives have been developed, embryo rescue and tissue culture procedures have been required to produce hybrid plants. Hybridisation between wheat and *Hordeum* spp., *Elytrigia* spp. and *Leymus* spp. has been achieved, but embryo rescue was required to produce plants (Eastham & Sweet 2002). There are unpublished reports of introgression of wheat traits into sea barley (*Hordeum marinum*) but at extremely low frequency (OGTR 2005a).

270. Ellstrand et al. (1999) observed that natural hybrids between wheat and its wild relatives are highly sterile 'although seeds may occasionally be found'. Hybrid sterility may explain why hybridisation appears to be restricted to F_1s with little evidence of subsequent introgression.

271. The applicant has advised that a commercial crop of barley (*Hordeum vulgare*) will be planted approximately 70 m from the GM trial site. The genomes of wheat and barley are considered incompatible. Hybrid plants derived from crossing wheat and barley have been achieved, but this have required extensive human intervention such as manual pollination, chemical treatment and embryo rescue and resultant plants are self-sterile (Islam et al. 1978; Koba et al. 1991; Molnar-Lang et al. 2005). The only successful artificial crosses have involved barley as the male parent (ie pollen donor) (Koba et al. 1991). This is the reverse direction from the consideration of hybridisation from the GM wheat, which would be the male parent.

272. Hybridisation between the GM wheat and barley would also require synchronous flowering. The applicant has advised that the barley would be sown at least two weeks in advance of the GM wheat. In addition, barley flowers earlier than wheat. It is likely that flowering in the barley will be close to completion before commencement of flowering in the GM wheat crop.

273. Wheat pollen is relatively heavy and generally travels only a few metres before falling to the ground (see section 1.3.1 for details). Given that successful hybridisation between completely sexually compatible wheat plants is generally only observed over short distances of a few metres, and then at very low frequency, the likelihood of hybridisation between the GM wheat and sexually incompatible barley over a distance of 70 metres is concluded to be negligible.

274. To limit the likelihood of gene transfer occurring, licence conditions have been imposed requiring the removal of all plants of *Hordeum* spp. and *Elytrigia* spp. from the trial site and a surrounding 10 metre monitoring zone (see Chapter 2 and Appendix 5 for details).

SECTION 2 GENE TRANSFER FROM THE GM WHEATS TO MICROORGANISMS

Section 2.1 Nature of the gene transfer hazard

275. Gene transfer from plants to microorganisms cannot occur through cross-pollination. Horizontal gene transfer is defined as the transfer of genetic material from one organism (the donor) to another organism (the recipient) which is not sexually compatible with the donor (Conner et al. 2003). There is growing evidence that horizontal gene transfer has been a principal force in the evolution of bacteria (Ochman et al. 2000; Nielsen 1998; Smalla et al. 2000; Stanhope et al. 2001).

276. The potential hazards associated with the introduced genetic materials of the GM wheats transferring to microorganisms could be highly varied, broadly depending upon the phenotype of the recipient and any changes to its survival, reproductive capacity and/or pathogenicity. The impact of any hazard arising through gene transfer would also depend on other sources of the introduced genetic materials in the environment.

Section 2.2 Potential hazards from the introduced genetic materials

2.2.1 The *oat* gene

277. The OAT enzyme is present in plants, animals and bacteria as part of the proline biosynthetic pathway. Transfer of the *oat* gene to microorganisms could result in increased synthesis of proline. Increased proline has been implicated in the response to osmotic stress of a number of bacteria and yeast (Cayley et al. 1992; Takagi et al. 2000; Siripornadulsil et al. 2002). Christian (1955) cited by Csonka (1989) reported that in *Salmonella oranienburg*, exogenous proline could alleviate growth inhibition imposed by osmotic stress.

278. Delauney and Verma (1993) noted that proline is a potent osmoprotectant in bacteria. Csonka (1989) had earlier reported that osmotic stress resulted in large increases in intracellular proline in a large variety of bacteria. Many species of gram positive bacteria are able to increase their proline pool size upon exposure to osmotic stress in the absence of exogenous proline.

279. An increase in proline levels might provide bacteria and other microorganisms with a selective advantage under conditions of osmotic stress.

2.2.2 The *cah* gene

280. The *cah* gene present in the GM wheats was isolated from the soil fungus *Myrothecium verrucaria*. Transfer to and expression of the *cah* gene in microorganisms would confer the ability to convert cyanamide to urea. This might provide a selective advantage to microbes exposed to cyanamide. Cyanamide will not be used during the release, so no selective pressure would be applied.

2.2.3 Promoters and other regulatory sequences

281. The introduced regulatory sequences, the *Ubi* promoter and intron, and the *zein* terminator are derived from *Zea mays* L.

282. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory sequences. The transfer of endogenous regulatory sequences to a new genetic context could also result in unpredictable effects. Thus the likelihood of a hazard arising due to transfer of the introduced sequences is no different to that of transfer from non-GM plants.

Section 2.3 Other sources of the introduced genetic materials in the environment and their potential for horizontal transfer

283. Information on other sources of the introduced genetic materials in the environment is discussed here (see Appendices 1 and 2 for details) to provide baseline information on the prevalence and transfer of these genetic materials that would happen naturally, irrespective of the GM wheats. Gene transfer between bacteria is a well established natural process that is central to their survival and evolution (Nielsen 1998; Ochman et al. 2000; Smalla et al. 2000; Stanhope et al. 2001; EFSA 2004). Thus, where the introduced genes already exist in bacterial populations, the likelihood of transfer between bacterial species would greatly exceed that from GM plants to bacteria.

2.3.1 The *oat* gene

284. The *oat* gene introduced to the GM wheat was derived from the common plant *Arabidopsis thaliana*, which is widespread in the environment.

285. The OAT enzyme is part of the biosynthetic pathway for proline in plants, animals and microorganisms. The *oat* gene is therefore very widely dispersed in the environment.

2.3.2 The *cah* gene

286. The *cah* gene introduced to the GM wheat was isolated from the soil fungus *Myrothecium verrucaria*.

287. It is well established the breakdown of cyanamide to urea in soil involves microbial activity (Estermaier et al. 1992). While the enzyme from the *M. verrucaria* is the only CAH described in the literature to date, and biochemical confirmation of the production of CAH enzyme activity has not been demonstrated in other species, it is likely that CAH is present in other soil microorganisms.

288. The applicant has advised that putative *cah* genes (identified from gene sequencing projects) have been identified through genome sequencing projects in a number of fungal and bacterial species (refer to Appendix 1 section 4.2). The hypothetical protein sequences predicted from these putative genes share significant homology with CAH from *M. verrucaria* and on this basis are predicted to encode CAH proteins.

289. It is therefore likely that other microbes do possess the *cah* gene and that genes conferring the ability to utilise cyanamide are widespread in the environment.

2.3.3 Promoters and other regulatory sequences

290. The introduced regulatory sequences, the *Ubi* promoter and intron, and the *zein* terminator are derived from *Zea mays* L.

291. The *Ubi1* promoter and the *zein* terminator are present in maize plants and will thus be common in agricultural environments where maize is present. Maize forms a part of the diet of humans and livestock and thus gut bacteria would commonly be exposed to maize DNA, including the *Ubi1* promoter and *zein* terminator sequences. Very similar promoters exist in all plant species.

Section 2.4 Likelihood of a hazard arising through gene transfer from the GM wheats to microorganisms

292. The likelihood of gene transfer to microorganisms creating a hazard for human health and safety or the environment depends on the characteristics of the introduced genetic materials, as well as on the likelihood of the transfer itself.

293. Most gene transfers have been identified through analyses of gene sequences (Ochman et al. 2000; Worobey & Holmes 1999). In general, gene transfers are detected over evolutionary time scales of millions of years (Lawrence 1999). Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman et al. 2000). In contrast, transfers of plant genetic materials to other microorganisms such as bacteria, viruses or fungi have been exceedingly rare (see Sections 2.4.1, 2.4.2 and 2.4.3 of this Appendix).

2.4.1 Bacteria

294. Mechanisms of conjugation (gene transfer between bacteria) and transduction (gene transfer from bacterial viruses to bacteria) will not be considered here as both these mechanisms are one step removed from the only possible route of plant to bacteria DNA transfer – natural transformation in the environment.

295. Natural transformation is a mechanism by which transfer of DNA from plants to microorganisms could have occurred during evolution (Bertolla & Simonet 1999) and is the mechanism that is most likely to contribute to a horizontal gene transfer from GM plants to bacteria (Smalla et al. 2000). Natural transformation enables competent bacteria to generate genetic variability by taking up and integrating free DNA that is present in their surroundings. This uptake of DNA does not necessarily depend on DNA sequence, thus indicating the potential of gene transfer from divergent donor organisms (Nielsen 1998).

296. A number of steps and conditions would need to be fulfilled for functional natural transformation to occur (Bertolla & Simonet 1999), many of which are highly unlikely, making the overall likelihood of gene transfer, and of resulting hazard, extremely low. The steps are:

- **release of the** DNA molecules from plant cells into the environment;
- persistence of the free DNA in the environment;
- presence of bacterial genotypes capable of developing competence for natural transformation;
- appropriate biotic and abiotic conditions for the development of the competent state;
- uptake of DNA fragments;

- **chromosomal** integration via recombination or autonomous replication of the transforming DNA;
- expression of the genes by the recipient bacterium; and
- selective advantage to fix (maintain) the transferred DNA in the gene pool of the recipient species.

297. Horizontal gene transfer from plants to bacteria has not been demonstrated under natural conditions (Syvanen 1999) and deliberate attempts to induce such transfers have so far failed (e.g. (Schlüter et al. 1995; Coghlan 2000). Transfer of plant DNA to bacteria has been demonstrated under highly artificial laboratory and glasshouse conditions, between homologous sequences and under conditions of selective pressure (Nielsen et al. 1998; (De Vries & Wackernagel 1998; De Vries et al. 2001) and even then only at a very low frequency.

298. A recent study reported evidence of low frequency transfer of a small fragment (180 bp) of an introduced gene derived from GM soybean to microorganisms within the small intestine of human ileostomists (i.e. individuals in which the terminal ileum is resected and digested material is diverted from the body to a colostomy bag) (Netherwood et al. 2004). However, only very low concentrations (1-3 copies per 10^6 bacteria) of the small fragment were detected in samples of microorganisms taken from the small bowel of three of seven ileostomists. Furthermore, the small fragment was only detected after two levels of amplification; (i) extensive culturing of the microorganism samples, and (ii) Polymerase Chain Reaction (PCR) analysis. The introduced gene could not be detected in faeces from human volunteers with intact digestive tracts following the consumption of a meal containing GM soy, indicating that the introduced gene is normally completely degraded in the large intestine.

299. Introduced genetic materials acquired by bacteria are unlikely to be of significance unless they are expressed or alter the expression of host genes. There are barriers to the expression of extraneous by genes in bacteria. For example:

- many plant promoters will not be active in bacteria;
- processing of the intermediate RNA may be required for protein expression (e.g. removal of introns to generate functional mRNA for translation) which will not occur in bacteria;
- coding sequences of plant genes may not be efficiently translated in bacteria due to differences in codon usage; and
- processing of an encoded 'pro-protein' may be required for production of a functional product.

300. Prokaryotes have efficient genomes and generally do not contain extraneous DNA sequences. If the genes are not useful to the organism then there will be no selective advantage in maintaining them in the genome, and they are not likely to persist. Thus the risk of gene transfer leading to harmful consequences is extremely low, and greatly exceeded by the likelihood of transfer of these genes and regulatory sequences from sources other than the GM wheats (see Section 2.3 of this Appendix).

301. The Scientific Panel on genetically modified organisms of the European Food Safety Authority (EFSA 2004) in its recent evaluation of the risks associated with the use of antibiotic resistance marker genes in GM plants, concluded that the frequency of horizontal

gene transfer from GM plants to microorganisms is very low (www.efsa.eu.int/science/gmo/gmo_opinions/384_en.html)..

2.4.2 Viruses

302. Homologous recombination between introduced viral genes and infecting viruses has been observed in GM plants, although at low frequencies and under conditions of selective pressure (Borja et al. 1999; Frischmuth & Stanley 1998; Gal et al. 2002; Greene & Allison 1994; Greene & Allison 1996; Schoelz & Wintermantel 1993). These cases involved restoration of an infective virus through complementation of a defective virus by viral sequences introduced into a GM plant genome. Interactions between introduced viral sequences in GM plants and infecting viruses, and an assessment of the likelihood of hazards to human health and the environment occurring from these interactions, are discussed in detail in the Consultation RARMP for licence application DIR 047/2003.

2.4.3 Fungi

303. Fungi are known to be transformable, and horizontal gene transfer from plants to plant-associated fungi has been claimed. Uptake of DNA from the host plant by *Plasmodiophora brassicae* (Bryngelsson et al. 1988; Buhariwalla & Mithen 1995) and uptake of the hygromycin B resistance gene from a GM plant by *Aspergillus niger* (Hoffman et al. 1994) have been reported. However, stable integration and inheritance of the plant DNA in the genome of these fungi has not been substantiated by experimental evidence (Nielsen 1998).

304. Thus the risk of gene transfer occurring and leading to harmful consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.3 of this Appendix).

SECTION 3 GENE TRANSFER FROM THE GM WHEATS TO ANIMALS, INCLUDING HUMANS

Section 3.1 Nature of the gene transfer hazard

305. The potential hazards associated with the introduced genetic materials in the GM wheats transferring to animals, including humans, could be highly varied, broadly depending upon the phenotype of the recipient and any changes to the survival or reproductive capacity of it or its progeny.

Section 3.2 Potential hazards from the introduced genetic materials

3.2.1 The *oat* gene

306. Animals cells and their gut microflora are likely to express the OAT enzyme, this is not likely to lead to any harmful effects.

307. Animals will naturally express native OAT enzymes, so the expression of the *Arabidopsis thaliana* OAT should not present any particular hazard *per se*. However, if the *oat* gene were transferred to animal cells, and expressed, this could result in elevating the free proline concentration. If the level of proline was elevated sufficiently the balance of amino acid metabolism might be perturbed, however it is more likely that any excess proline would be metabolised.

3.2.2 The *cah* gene

308. If the *cah* gene were transferred to animal cells, and expressed, the CAH protein might be produced. CAH would be a novel protein for animal cells. However, it would not have any effects on cell metabolism in the absence of cyanamide. Exposure of animal cells to cyanamide is highly unlikely.

3.2.3 Promoters and other regulatory sequences

309. If any of these sequences were to be transferred to animals without the associated introduced genes of the GM wheat lines, it would be highly unlikely that they would work.

310. The introduced regulatory sequences are derived from *Zea mays*. The same or similar genetic elements are common in animal feed sources including human food. Thus no new hazard will arise due to the presence of the introduced regulatory sequences in the GM wheat plants.

Section 3.3 Likelihood of a hazard arising through gene transfer from the GM wheats to animals (including humans)

311. The likelihood of gene transfer creating a hazard for human health and safety or the environment depends on the likelihood of transfer itself, as well as on the characteristics of introduced sequences, as discussed in previous sub-sections.

312. The most significant route for entry of foreign DNA into animals, including humans, would be through food as it passes through the gastrointestinal tract. The epithelial lining of the gastrointestinal tract is exposed to foreign DNA released from food. Microorganisms colonise the whole length of the gastrointestinal tract, aiding the digestive process. However, the proportion of DNA derived from the introduced genetic materials of GM plants in the animal diet is extremely low. For example, it has been estimated that in a diet comprising 40% GM maize, the introduced genes would represent 0.00042% of total dietary DNA intake (Beever & Kemp 2000). The UK Royal Society have concluded that consumption of introduced DNA in GM foods, viewed in the context of the normal diet, poses no new or additional risk. Animals, including humans, consume large amounts of DNA derived not only from food organisms but also from contaminating microorganisms and viruses (The Royal Society 2002)

313. The fate of DNA in the digestive tract of various animals has been studied and is discussed in detail in the risk assessments for licence applications DIR 020/2002, DIR 021/2002 and DIR 022/2002. These risk assessments concluded that the likelihood of transfer via food is extremely low, and not greater than the likelihood of transfer from other sources of the introduced genetic materials in the environment (see Section 2.3 of this Appendix).

314. Even in the rare event of plant DNA uptake by animal cells, a further step of chromosomal integration has not been demonstrated. Furthermore, any uptake of plant DNA is likely to occur in non-reproductive (somatic) cells such as immune system or gut epithelium cells, and the introduced gene would not be transmitted to the cells of any progeny.

315. A recent study reported evidence of low frequency transfer of a small fragment of an introduced gene derived from GM soybean to microorganisms within the small intestine of human ileostomists. In this study, the potential for gene transfer from intestinal

microorganisms to mammalian intestinal epithelial cells was also investigated in the laboratory but could not be detected, despite the use of very large numbers of GM bacteria carrying the introduced gene (Netherwood et al. 2004).

316. The release is small in scale and limited in duration. No products from the GM wheat from the field trial will be used for human food or animal feed. Overall the likelihood of gene transfer from the GM wheats to animals, including humans is effectively zero and the consequences of any such transfer are negligible.

SECTION 4 CONCLUSIONS REGARDING GENE TRANSFER TO OTHER ORGANISMS

Section 4.1 Conclusions regarding gene transfer to non-GM wheat and other plants

317. It is considered that risks through gene transfer from the GM wheats to non-GM wheat and other plants are very low because:

- out-crossing frequencies in wheat are low;
- gene transfer to sexually compatible species outside the trial (including cultivated and naturalised wheat and related species) is unlikely to occur during the trial due to isolation from other wheat plants (e.g. at least 500 m from any other wheat plants);
- gene transfer to sexually compatible non-GM bread wheat proposed to be planted as part of the trial is possible;
- gene transfer to less closely related grass species is unlikely to occur due to genetic incompatibility;
- well established genetic incompatibilities prevent gene transfer to non-grass plant species;
- gene transfer to other wheat would not pose any risks additional to the low risks posed by the GM wheat lines themselves; and
- the field trial is small and of limited duration.

318. Gene transfer to other wheat plants will be limited by the licence conditions imposed. The licence conditions require separation of the GM wheat from other wheat crops by a distance of at least 500 metres; removing sexually compatible plants from the monitor zone and *Triticum, Secale* and *Triticale* populations from the Isolation Zone followed by 2 years of post-harvest monitoring of the release site to remove volunteers before they flower (Appendix 5 for details). Non-GM wheat planted as part of the trial will be treated as the GM wheat.

Section 4.2 Conclusions regarding gene transfer to microorganisms

319. It is considered that risks through transfer of the introduced genetic materials from the GM wheats to microorganisms are negligible because:

- all of the introduced genetic materials in the GM wheats are already present in the environment;
- the likelihood of gene transfer from plants to microorganisms is extremely low and greatly exceeded by the likelihood of transfer from other sources of the introduced genes;
- the introduced genes in the GM wheat lines, and other genes with similar functions, are widespread in the environment and are available for transfer; and

- the field trial will be small and of limited duration limiting the potential for the introduced genetic material to persist in the environment.

Section 4.3 Conclusions regarding gene transfer to animals, including humans

320. It is considered that risks through transfer of the introduced genetic materials from the GM wheats to animals, including humans, are negligible because:

- the likelihood of gene transfer from plants to animals is extremely low and greatly exceeded by the likelihood of transfer from other sources of the introduced genetic materials;
- all of the introduced genetic materials in the GM wheats are already present in the environment;
- products from the GM wheats will not be used for animal feed or human food;
- the probability of interaction, uptake and integration of intact plant genes by other organisms occurring is extremely low, especially if it involves unrelated sequences (non-homologous recombination);
- natural events of horizontal gene flow from plants to distantly related organisms are extremely rare; and
- in the extremely unlikely event of such a transfer occurring, human health and safety and the environment are unlikely to be adversely affected.

SECTION 5 RESEARCH REQUIREMENTS

321. The release of the GM wheat lines is a small and early-stage research trial to characterise wheat lines with a genetic modification to overproduce proline. If the applicant makes any application for future, particularly larger releases of these GM wheat lines more information would be required on the occurrence of gene flow from GM wheat to non-GM wheat under Australian field conditions.

APPENDIX 5 LICENCE CONDITIONS

Gene Technology Regulation in Australia

The *Gene Technology Act 2000* (Cth) and corresponding State and Territory legislation form a substantial part of a range of integrated regulatory measures relevant to controlling genetically modified organisms (GMOs) and their use.

The Gene Technology Regulator is required to consult with, and take into account advice from a range of key stakeholders, including regulatory authorities on risks to human health and safety and the environment in assessing applications for dealings involving the intentional release of GMOs into the Australian environment.

Note in relation to approval of genetically modified foods for human consumption

Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment. FSANZ approval would need to be obtained before the GM wheat could be used as human food. This licence contains a condition that prohibits this use.

Note about where the GMOs are being planted pursuant to this licence

Information about where the GMOs are being planted pursuant to this licence can be found in a separate document entitled 'DIR053/2004 Site Details'. This document can be viewed by accessing the document directly at http://www.ogtr.gov.au/ir/dir053.htm or clicking here.

SECTION 1 INTERPRETATION AND DEFINITIONS

This licence does not authorise dealings with GMOs that are otherwise prohibited as a result of the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

In this licence:

(a) Words and phrases used in this licence have the same meaning as they do in the Act and the Regulations;

(b) Words importing a gender include any other gender;

(c) Words in the singular include the plural and words in the plural include the singular;

(d) Words importing persons include a partnership and a body whether corporate or otherwise;

(e) References to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

(f) Where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning;

(g) Specific conditions prevail over standard conditions to the extent of any inconsistency.

In this licence:

'Act' means the *Gene Technology Act 2000* (Cth) and equivalent provisions in corresponding State law;

'Annual Report' means a written report provided to the Regulator within 90 days of each anniversary of this licence containing all the information required by this licence to be provided in the Annual Report.

'Clean' (or **'Cleaned'**), as the case requires, means:

(a) in relation to a Location or other area, the Destruction of the GMOs and Plant Material in that Location or area, to the reasonable satisfaction of the Regulator; or

(b) in relation to Equipment, the removal and Destruction of the GMOs and Plant Material from the Equipment, to the reasonable satisfaction of the Regulator;

'Destroy' (or **'Destroyed'** or **'Destruction'**) means, as the case requires, killed by one or more of the following methods:

(a) stalk pulling; or

(b) uprooting by ploughing; or

(c) burning; or

(d) treatment with herbicide; or

(e) autoclaving or incineration; or

(f) hand weeding.

Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate. For example, in the case of killing the remains of harvest of the GMOs, treatment of post harvest remains by herbicide would not be a sufficient mechanism.

'Equipment' includes harvesters, seeders, storage equipment, transport equipment (e.g. bags, containers, trucks), clothing and tools**;**

'GM' means genetically modified;

'GMOs' means the genetically modified organism or organisms authorised for release by this licence;

'Isolation Zone' means the area of land, extending outwards 500 metres in all directions from the outer edge of the area of land where the GMOs are planted and grown**;**

'Location' means the area of land where the GMOs are planted and grown;

'Monitoring Zone' means the area of land, extending outwards 10 metres in all directions from the outer edge of the area of land where the GMOs are planted and grown.

'Natural Waterways' means waterways other than irrigation channels, holding dams or storage ponds used to collect water runoff from irrigated areas;

'OGTR' means the Office of the Gene Technology Regulator;

'Plant Material' means viable parts of GMOs, including seed, stubble, pollen, whether from the plant itself or derived from or produced by the plant;

'Reference Cereal' means non-genetically modified *Triticum aestivum* planted in a Location for the purpose of agronomic comparisons.

'Regulator' means the Gene Technology Regulator;

'Related Species' means plants in the genera *Triticum, Hordeum, Secale, Aegilops, Elytrigia;*

'Sign-off' means a notice in writing from the Regulator, in respect of a place, that post harvest inspection conditions no longer apply in respect of that place;

'Triticum population' means groups of 5 or more plants per square metre of land belonging to the genera *Triticum, Secale, Triticale*

'Volunteer plants' means progeny of the GMOs or regrowth of previous GM or non-GM wheat.

SECTION 2 GENERAL CONDITIONS

Duration of Licence

1. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with GMOs are authorised during any period of suspension.

Holder of Licence

2. The holder of this licence ('the licence holder') is Grain Biotech Australia Pty Ltd.

Project Supervisor

3. The Project Supervisor in respect of this Licence is identified at Attachment A*.

4. The licence holder must immediately notify the Regulator in writing if any of the contact details of the Project Supervisor change.

No dealings with GMOs except as authorised by this licence

5. Persons covered by this licence must not deal with the GMOs except as expressly permitted by this licence.

GMOs covered by this licence

6. The GMOs covered by this licence are described at Attachment B*.

Permitted dealings

7. The permitted dealings with the GMOs are to plant, grow and conduct experiments with the GMOs, and the possession, supply, use, transport and disposal of the GMOs for the purpose of any of the permitted dealings with the GMOs, or in the course of any of these dealings.

Persons covered by this GMO licence

8. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged to undertake any activity in connection with GMOs grown in a Location pursuant to this Licence.

Informing people of their obligations

9. The licence holder must inform any person covered by this licence, to whom a particular condition of this licence applies, of the following:

(a) the particular condition (including any variations of it);

(b) the cancellation or suspension of the licence;

(c) the surrender of the licence.

10. The licence holder must provide the Regulator, on the Regulator's written request, signed statements from persons covered by this licence that the licence holder has informed those people of the conditions of this licence that apply to them.

Licence holder to notify of circumstances that might affect suitability

11. The licence holder must immediately, by notice in writing, inform the Regulator of:

* Attachments are included in the licence.

(a) any relevant conviction of the licence holder occurring after the commencement of this licence;

(b) any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment;

(c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of this licence to meet the conditions in it.

Licence holder must provide information on matters related to suitability

12. The licence holder must provide information related to the licence holder's ongoing suitability to hold a licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Additional information to be given to the Regulator

13. It is a condition of a licence that the licence holder inform the Regulator if the licence holder:

(a) becomes aware of additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

(b) becomes aware of any contraventions of the licence by a person covered by the licence; or

(c) becomes aware of any unintended effects of the dealings authorised by the licence.

14. The licence holder must provide the information required by paragraphs (a) (b) and (c) of the immediately preceding condition to the Regulator as soon as practically and reasonably possible and must also include the information in the Annual Report.

People dealing with GMOs must allow auditing and monitoring of the dealing

15. If a person is authorised by this licence to deal with GMOs and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Remaining an accredited organisation

16. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and comply with its instrument of accreditation.

SECTION 3 SPECIFIC CONDITIONS

Locations and size of trial

1. The permitted dealings with the GMOs may only be undertaken during the winter wheat growing seasons between April 2005 and January 2006 although disposal of the GMOs for purposes of the dealings may take place after January 2006.

2. The GMOs may only be grown at a single Location in the Corrigin Shire of Western Australia at GPS coordinates advised to the Regulator.

3. Reference Cereals may be grown at the Location while the GMOs are growing.

4. Reference Cereals must be handled and controlled as if they are the GMO.

Note: The above condition has the effect that all ensuing conditions which apply to the GMO will also apply to Reference Cereals.

5. The maximum permitted size of the Location is 0.45 hectares.

6. The licence holder must be able to access and control the Location where the GMOs are grown to the extent necessary to comply with this licence, for the duration of the life of the licence.

7. No GMOs may be planted at the Location after 31 July 2005.

Notice of planting

8. The licence holder must provide a notice in writing to the Regulator each time the GMOs are planted at the Location. The notice must set out:

   (a) the cultivars and lines of the GMOs that have been planted;

   (b) the date on which planting of the GMOs commenced;

   (c) details of the Location where the GMOs are planted, including GPS coordinates for the Location;

   (d) the period during which the licence holder considers the GMOs are likely to flower; and

   (e) the period during which the licence holder considers the GMOs are likely to be harvested (or Destroyed in lieu of harvest).

Note: Information contained in notices given to the Regulator pursuant to this condition can be viewed in the Site details that accompany this licence.

9. The notice must be provided to the Regulator within 14 days of the date on which planting of the GMOs commenced.

Conditions relating to the Location

10. The Location must be surrounded by:

    (a) a fence at least 1.8 metres high with lockable gates that will exclude rabbits and large animals including macropods; and

    (b) an Isolation Zone which includes the Monitoring Zone.

11. The outer edge of the Location must not be within 50 metres of a Natural Waterway.

12. Access to the Location must be restricted to persons covered by this licence.

13. Appropriate control measures must be implemented to minimise rodent numbers at the Location. These must include, but are not limited to, mowing the Monitoring Zone and keeping the Monitoring Zone free of weeds and other material capable of attracting and/or harbouring rodents while the GMOs are being grown at the Location.

14. Any steps taken to control rodents and any evidence of rodent activity must be recorded in a log book and be available for inspection by the Regulator on request.

15. The Location must be covered by a bird-proof net from 'milky dough' stage of the GMO until the GMO is harvested.

Conditions about the Isolation Zone

16. No *Triticum* population may be grown in the Isolation Zone while the GMOs are being grown at the Location within it.

17. If any *Triticum* population occurs in the Isolation Zone while the GMOs are being grown at the Location within it, either the *Triticum* population or the GMOs in the Location must be destroyed prior to flowering of the GMO. If GMOs are destroyed pursuant to this condition, they are taken to have been harvested for the purposes of this licence and conditions relevant to harvested GMOs apply.

18. An Isolation Zone must be able to be accessed and controlled by the licence holder to an extent that is commensurate with the licence holder's rights to access and control the Location within it.

Inspections to be conducted in the Location while the GMOs are being grown

19. Fourteen days before the expected commencement of flowering of the GMOs at a Location, as notified to the Regulator pursuant to this licence, the Location must be inspected for the presence of Related Species that are not GMOs which must be destroyed before flowering.

20. Inspections must be conducted at least once every 14 days thereafter until the GMOs at the Location have finished flowering.

Inspections to be conducted in the Monitoring Zone while the GMOs are being grown

21. Fourteen days before the expected commencement of flowering of the GMOs at a Location, as notified to the Regulator pursuant to this licence, the Monitoring Zone must be inspected for the presence of Related Species and GMOs which must be destroyed before flowering.

22. Inspections must be conducted at least once every 14 days thereafter until the GMOs at the Location have been harvested.

Inspections to be conducted in the Isolation Zone while GMOs are being grown

23. Fourteen days before the expected commencement of flowering of the GMOs at a Location, the Isolation Zone must be inspected for the presence of *Triticum* populations.

24. Inspections must be conducted at least once every 14 days thereafter until the GMOs at the Location have finished flowering.

Note: Other conditions in this licence, above, in relation to Isolation Zones, require the Destruction of the Triticum populations or the Cleaning of the Location if a Triticum population is found in an Isolation Zone.

Notice of Harvest

25. The licence holder must not harvest the GMOs without providing the Regulator with at least 7 days and not more than 20 days notice of an intention to harvest at the Location. Any change of intention prior to the intended harvest date must be notified to the Regulator as soon as is reasonably and practically possible.

26. The licence holder must provide the actual date or dates of commencement of harvesting of the GMOs at the Location. This notice must be provided within 7 days of commencement of harvesting of the GMOs at the Location.

Note: There are 2 relevant notices with respect to a forecast of harvest. One is a long-term forecast provided under conditions 8-9 and the other is a short term forecast under condition 25.

GMOs must be either harvested or Destroyed

27. Within 9 months of being planted, the GMOs must be either harvested or Destroyed.

28. If the GMOs are harvested, they must be harvested and stored separately from any other bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum*), rye (*Secale cereale*), barley (*Hordeum vulgare*) or triticale and must be harvested either by hand or with a mechanical harvester.

29. If a mechanical harvester is used, the mechanical harvester must be fitted with a dust extractor and modified so as to capture Plant Material.

Seed and other Plant Material may be collected and stored

30. Parts of GMOs (including leaf tissue, flower buds, seed, roots and stems) may be collected from the GMOs at the Location for the purpose of conducting experiments.

31. Parts of GMOs (including leaf tissue, flower buds, seed, roots and stems) that are collected may only be transported off the Location to:

(a) storage within a secure enclosed area that is signed so as to indicate GM Plant Material is stored within that area. GMOs must be stored in a sealed primary container capable of preventing dispersal of the GMO and/or Plant Material and which is enclosed by a locked outer container that is signed so as to indicate that it contains GM Wheat; or

(b) a facility certified by the Regulator to physical containment level 2 (PC2).

32. After any experiments with the GMOs or Parts of GMOs (including leaf tissue, flower buds, roots and stems) are completed, the GMOs, or Parts of GMOs, must be Destroyed.

Conditions in relation to the Cleaning of Location after GMOs are grown

33. After the GMOs are harvested or Destroyed at the Location, the Location must be Cleaned.

34. The Location must be Cleaned within 14 days of harvest or Destruction of the GMOs in it, whichever occurs first.

Notice of Cleaning

35. The licence holder must provide a notice in writing to the Regulator when a Location is Cleaned pursuant to this licence.

36. The notice must be provided to the Regulator within 14 days of the date on which Cleaning the Location concluded.

General conditions in relation to the Cleaning of all other places and Equipment used in connection with this licence

37. If,

(a) an area or place other than the Location is used in connection with this licence, or

(b) Equipment is used in connection with the GMOs or Plant Material,

then that area, place or Equipment must also be Cleaned.

38. Cleaning must occur immediately or as soon as practicable after the use and before it is used for any other purpose.

39. If Equipment is Cleaned, the area in which the Equipment is Cleaned must also be Cleaned immediately or within 14 days of Cleaning of Equipment. (It is not necessary for Equipment to be Cleaned only at a Location).

40. On the request of the Regulator, the Regulator must, within 14 days of the request, be provided with written documentation of the procedures in place to ensure continuing compliance with these Cleaning conditions.

General conditions that apply wherever inspections must be undertaken for the existence of Volunteer plants and Related Species

41. After harvest or destruction, the Location must be irrigated 3 times at intervals of at least 28 days so as to promote the growth of Volunteers.

42. After a Location is Cleaned, the following places must be inspected for the existence of Volunteer plants and Related Species:

(a) the Location;

(b) the Monitoring Zone; and

(c) any areas used to Clean Equipment.

43. Inspection must be performed by a person who is able to recognise Volunteer plants and Related Species.

44. The results of inspection activities must be recorded in a logbook. The logbook must be available on request for examination or photocopying by the OGTR. The findings of the inspections as recorded in the logbook must be provided to the Regulator every month and included in the licence holder's Annual Report to the Regulator. The logbook must contain at least the following:

(a) details of the areas inspected;

(b) details of the date of inspection;

(c) the names of the person or persons who undertook the inspection and details of the experience, training or qualification that enabled them to recognise Volunteer plants and Related Species;

(d) the number of Volunteer plants and Related Species observed, if any;

(e) details of the development stages reached by the Volunteer plants and Related Species, if any; and

(f) details of methods used to Destroy Volunteer plants and Related Species, if any.

45. Any Volunteer plants and Related Species identified must be Destroyed prior to the plants flowering.

46. Unless this licence provides otherwise, places subject to condition 42 must be inspected at least once every 30 days until the Regulator has issued a Sign-off.

47. If,

(a) inspections have been routinely completed in a place for a period of 2 years, and

(b) inspection records for that place show that no Volunteer plants and Related Species have been observed in the most recent 6 month inspection period,

the licence holder may make written application to the Regulator that these inspection conditions no longer apply in respect of that place.

48. Inspection conditions do not apply in respect of a place if the Regulator has issued a Sign-off in respect of that place.

Restrictions during and after the GMOs are grown

49. If the GMOs are grown at the Location, and Sign-off has not occurred, no Related Species may be grown at the Location.

50. After Cleaning of the site the licence holder may plant species that are approved by a notice in writing by the Regulator.

Note: The Regulator will not approve the growing of Related Species prior to Sign off.

Transportation of the GMOs and Plant Material

51. Subject to the conditions immediately below in respect of transportation, the GMOs and Plant Material must be transported in accordance with the OGTR Guidelines for the Transport of GMOs (June 2001) issued by the Regulator.

52. Every container used to transport the GMOs and Plant Material must be labelled:

(a) to indicate that it contains GM wheat; and

(b) with telephone contact numbers for the licence holder and instructions to contact the licence holder in the event that the container is broken or misdirected.

53. Harvested seed from the GMOs may only be transported to the extent necessary to store it, export it, Destroy it or relocate it to a facility certified by the Regulator to physical containment level 2 (PC2).

54. The licence holder must have in place accounting procedures to verify whether the same quantity of GMOs and Plant Material that is sent is delivered. Routes, methods and procedures used for transportation in accordance with this licence must be documented.

Contingency Plans

55. Within 30 days of the date of the commencement of this licence, a written Contingency Plan must be submitted to the Regulator detailing measures to be taken in the event of the unintended presence of the GMOs or Plant Material, outside an area that must be inspected.

56. The Contingency Plan must include details of procedures to:

(a) ensure the Regulator is notified immediately if the licence holder becomes aware of the event;

(b) destroy any of the GMOs and Plant Material; and

(c) inspect and Destroy any Volunteer plants and Related Species that may exist as a result of the event.

57. The Contingency Plan must be implemented in the event that the unintended presence of the GMOs or Plant Material is discovered outside an area that must be inspected, and the Regulator notified immediately or as soon as is practicable.

Compliance Management Plan

58. Prior to growing the GMOs, a written Compliance Management Plan must be provided to the Regulator. The Compliance Management Plan must describe in detail how the licence holder intends to ensure compliance with these conditions and document that compliance.

Reporting

59. The licence holder must provide an Annual Report to the Regulator.

Testing methodology

60. The licence holder must provide a written instrument to the Regulator describing an experimental method that is capable of reliably detecting the presence of the GMOs and the presence of the genetic modifications described in this licence (at Attachment B) in a recipient organism. The instrument must be provided within 30 days of planting the GMOs.

GMOs and Plant Material must not be consumed

61. The licence holder must not allow the GMOs or any products from the GMOs to be used as food for humans or as stockfeed.

APPENDIX 6 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES

Section 1 The regulation of gene technology in Australia

322. The *Gene Technology Act 2000* (the Act) took effect on 21 June 2001. The Act, supported by the *Gene Technology Regulations 2001,* an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, underpins Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs). The regulatory system replaces the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC).

323. The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation.

324. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), an Australian Government regulatory agency located within the Health and Ageing portfolio.

325. The Act prohibits persons from dealing with GMOs unless the dealing is exempt, a Notifiable Low Risk Dealing, on the Register of GMOs, or licenced by the Regulator (see Section 31 of the Act).

326. The requirements under the legislation for consultation and for considering and assessing licence applications and preparing risk assessment and risk management plans (RARMPs) are discussed in detail in Division 4, Part 5 of the Act and summarised below.

327. Detailed information about the national regulatory system and the gene technology legislation is also available from the OGTR website (*www.ogtr.gov.au*).

Section 2 The licence application

328. Licence applications for dealings involving the intentional release (DIR) of a genetically modified organism into the environment must be submitted in accordance with the requirements of Section 40 of the Act. As required by Schedule 4, Part 2 of the Regulations, the application must include information about:

- the parent organism;
- the GMOs;
- the proposed dealing with the GMOs;
- interaction between the GMOs and the environment;
- risks the GMOs may pose to the health and safety of people;
- risk management;
- previous assessments of approvals; and
- the suitability of the applicant.

329. The application must also contain:

- additional information required for a GMO that is:

- a plant;
- a microorganism (not living in or on animals and not a live vaccine);
- a microorganism that lives in or on animals;
- a live vaccine for use in animals;
- a vertebrate animal;
- an aquatic organism;
- an invertebrate animal;
- to be used for biological control;
- to be used for bioremediation; and
- intended to be used as food for human or vertebrate animal consumption;

- supporting information from the Institutional Biosafety Committee.

330. A preliminary screening of an application is undertaken by OGTR staff to determine whether it complies with the Act and the Regulations, by containing the required information. If this information is provided in the application, the Regulator may then accept the application for formal consideration. Section 43 of the Act provides that the Regulator is not required to consider an application if the application does not contain the required information.

331. After accepting an application for consideration, the Regulator must decide to issue, or refuse to issue, a licence. The decision must be taken following an extensive consultation and evaluation process, as detailed in Sections 3-6 of this Appendix. Regulation 8 of the Regulations prescribes a period of 170 working days within which this decision must be taken. This period does not include weekends or public holidays in the Australian Capital Territory. Also, this period does not include any days in which the Regulator is unable to progress the application because information sought from the applicant in relation to the application has not been received.

Section 3 The initial consultation processes

332. In accordance with Section 50 of the Act, the Regulator must seek advice in preparing a RARMP from prescribed agencies:

- State and Territory Governments;
- the Gene Technology Technical Advisory Committee (GTTAC);
- prescribed Australian Government agencies (Regulation 9 of the *Gene Technology Regulations 2001* refers);
- the Australian Government Minister for Environment and Heritage; and
- relevant local council(s) where the release is proposed.

333. Section 49 of the Act requires that if the Regulator is satisfied that at least one of the dealings proposed to be authorised by the licence may pose significant risks to the health and safety of people or to the environment, the Regulator must publish a notice (in national and regional newspapers, in the *Gazette* and on the OGTR website) in respect of the application, inviting written submissions on whether the licence should be issued.

334. As a measure over and above those required under the Act, in order to promote the openness and transparency of the regulatory system, the Regulator may take other steps. For example, receipt of applications is notified to the public by posting a notice of each application's receipt on the OGTR website and directly advising those on the OGTR mailing list. Copies of applications are available on request from the OGTR.

Section 4 The evaluation processes

335. The risk assessment process is carried out in accordance with the *Act* and *Regulations*, using the Risk Analysis Framework (the Framework) developed by the Regulator (available on the OGTR website). It also takes into account the guidelines and risk assessment strategies used by related agencies both in Australia and overseas. The Framework was developed in consultation with the States and Territories, Australian Government agencies, GTTAC and the public. Its purpose is to provide general guidance to applicants and evaluators and other stakeholders in identifying and assessing the risks posed by GMOs and in determining the measures necessary to manage any such risks.

336. In undertaking a risk assessment, the following are considered and analysed:

- the data presented in the proponent's application;
- data provided previously to GMAC, the interim OGTR or the OGTR in respect of previous releases of relevant GMOs;
- submissions or advice from States and Territories, Australian Government agencies and the Australian Government Minister for Environment and Heritage and the public;
- advice from GTTAC;
- information from other national regulatory agencies; and
- current scientific knowledge and the scientific literature.

337. In considering this information and preparing the RARMP, the following specific matters are taken into account, as set out in Section 49 and required by Section 51 of the Act:

- the risks posed to human health and safety or risks to the environment;
- the properties of the organism to which the dealings relate before it became a GMO;
- the effect, or the expected effect, of the genetic modification that has occurred on the properties of the organism;
- provisions for limiting the dissemination or persistence of the GMO or its genetic material in the environment;
- the potential for spread or persistence of the GMO or its genetic material in the environment;
- the extent or scale of the proposed dealings; and
- any likely impacts of the proposed dealings on the health and safety of people.

338. In accordance with Regulation 10 of the Regulations, the following are also taken into account:

- any previous assessment, in Australia or overseas, in relation to allowing or approving dealings with the GMO;
- the potential of the GMO concerned to:
    - be harmful to other organisms;
    - adversely affect any ecosystems;
    - transfer genetic material to another organism;
    - spread, or persist, in the environment;
    - have, in comparison to related organisms, a selective advantage in the environment; and
    - be toxic, allergenic or pathogenic to other organisms.
- the short and long term when taking these factors into account.

SECTION 5 FURTHER CONSULTATION

339. Having prepared a risk assessment and a risk management plan, the Regulator must, under Section 52 of the Act, seek comment from stakeholders, including those outlined in Section 3 and the public.

340. All issues relating to the protection of human health and safety and the environment raised in written submissions on an application or a risk assessment and a risk management plan are considered carefully, and weighed against the body of current scientific information, in reaching the conclusions set out in a final RARMP. Section 56 of the Act requires that these be taken into account in making a decision on whether or not to issue a licence for the release.

341. Comments received in written submissions on this RARMP are very important in shaping the final RARMP and in informing the Regulator's decision on an application. A summary of public submissions and an indication of where such issues have been taken into account are provided in an Appendix to the final RARMP.

342. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings are able to be managed so as to **protect human health and safety and the environment**. Matters in submissions that do not address these issues and/or concern broader issues outside the objective of the legislation will not be considered in the assessment process. In most instances, as determined in the extensive consultation process that led to the development of the legislation, they fall within the responsibilities of other authorities.

SECTION 6 DECISION ON LICENCE

343. Having taken the required steps for assessment of a licence application, the Regulator must decide whether to issue or refuse a licence (Section 55 of the Act). The Regulator must not issue the licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in such a way as to protect the health and safety of people and the environment.

344. The Regulator must also be satisfied, under Section 57 of the Act, that the applicant is a suitable person to hold the licence. Section 58 outlines matters the Regulator must consider in deciding whether a person or company is suitable to hold a licence e.g.:

- any relevant convictions;
- any relevant revocations or suspensions of a licence or permit; and
- the capacity of the person or company to meet the conditions of the licence.

345. The Regulator carefully considers all of this information which is supplied in a declaration signed by licence applicants.

346. The Monitoring and Compliance Section of the OGTR compiles compliance histories of applicants, considering all previous approvals to deal with GMOs under the Act and the previous voluntary system. These histories as well as other information such as follow-up actions from audits may be taken into account. The ability of an organisation to provide resources to adequately meet monitoring and compliance requirements may also be taken into account.

347. If a licence is issued, the Regulator may impose licence conditions (Section 62 of the Act). For example, conditions may be imposed to:

- limit the scope of the dealings;
- require documentation and record-keeping;
- require a level of containment;
- specify waste disposal methods;
- manage risks posed to the health and safety of people, or to the environment;
- require data collection, including studies to be conducted;
- limit the geographic area in which the dealings may occur;
- require contingency planning in respect of unintended effects of the dealings; and
- limit the dissemination or persistence of the GMO or its genetic material in the environment.

348. It is also required as a condition of a licence that the licence holder inform any person covered by the licence of any condition of the licence which applies to them (Section 63 of the Act). Access to the site of a dealing must also be provided to persons authorised by the Regulator for the purpose of auditing and monitoring the dealing and compliance with other licence conditions (Section 64 of the Act). It is a condition of any licence that the licence holder inform the Regulator of:

- any new information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence;
- any contraventions of the licence by a person covered by the licence; and
- any unintended effects of the dealings authorised by the licence.

349. It should be noted that, as well as imposing licence conditions, the Regulator has additional options for risk management. The Regulator has the legislative capacity to enforce compliance with licence conditions, and indeed, to direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment.

The OGTR also independently monitors trial sites to determine whether the licence holder is complying with the licence conditions, or whether there are any unforseen problems.

APPENDIX 7 SUMMARY OF PUBLIC SUBMISSIONS

350. The Regulator received three public submissions on this application. A summary of the submissions is provided below. The key issues raised in the public submissions that relate to risks to human health and safety or the environment are:

- stability of the genetic modifications (Appendix 1 refers)
- adverse effects on human and animal health due to the genetic modifications (Appendix 2 refers);
- potential for adverse environmental effects (Appendix 2 refers);
- potential for seed dispersal by water (Appendix 3 refers);
- potential for gene transfer (Appendix 4 refers) and
- adequacy of containment and cleaning measures (Appendix 2, 3 and 4 refer).

351. The public submissions also raised projected impacts on international markets, and segregation issues. However, the focus of the gene technology legislation is the protection of human health and safety and the environment and these matters are outside the scope of assessments the Regulator is required to conduct under the Act.

352. In accordance with Section 56 of the Act, the Regulator has taken into account all issues raised in written submissions that related to risks to human health and safety and to the environment in finalising the RARMP. These issues were considered carefully and weighed against the body of current scientific information in reaching the conclusions set out in this document.

Abbreviations:

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

Issues raised: AL: allergenicity; D: insufficient data; E: environment; EC: ethical concerns; FSANZ: issues for Food Standards Australia New Zealand GT: gene transfer; H: human health and safety; LC: licence conditions M: marketing; R: research; RA: risk assessment; RM: risk management; S: segregation; T: toxicity; W: weediness

App: Appendix; Ch: Chapter; FSANZ: Food Standards Australia New Zealand; NA: not applicable; OSA: outside scope of assessment

[a] Submission from: A: agricultural/industry organisation; I: individual; NGO: non-government organisation

No.	Type[a]	Summary of issues raised	Issue	Consideration of issue
1	NGO	Opposes the proposed GM salt tolerant wheat trial as an unnecessary risk:		
		• International markets will not tolerate any level of contamination of non-GE wheat;	S	OSA
		• The industry has demonstrated on a regular basis its inability to prevent genetic contamination;	S	OSA
		• There is no market for GE wheat;	M	OSA

		• The notion of GE wheat plants to become salt tolerant will only reinforce the appalling practices that led to salinity issues in the first place and will create a climate of agricultural expansion in marginal lands, frequently with high conservation and environmental values, and prevent farmer cooperation in measures intended to reduce incipient and existing salinity problems;	E, EC	OSA, this is a small scale (0.45ha) proof of concept, limited and controlled field trial.
		• Analysis of expression of the GE construct in animals highlights the deficiencies in the RARMP;	RA, GT, D	App 1, 2 and 4, this is a proof of concept trial. Not permitted for use as human food or animal feed.
		• What is the potential impact of raised proline concentrations in any native animals that might be exposed to this wheat;	T	App 2 (Sections 2.6, 2.7) Proline is an amino acid present in all organisms and ubiquitous in the environment. Proline is not toxic in normal dietary exposure or at high doses.
		• What is the basis for the claim that raised proline concentrations are likely to be metabolised?	D	App 1 (Section 3) App 2 (Section 2.7)
		• The conditions proposed will not prevent access to the site by a variety of animals;	RM	Ch 2, App 2 and App 5 (LC). This small scale (0.45ha) field trial will be contained by a 1.8 m high fence and bird proof netting will be placed over the trial from milk dough stage of grain development to harvest.
		• …flawed commonality argument made regularly by the OGTR, ie that the safe presence of natural form of a genetically engineered construct within ecosystems necessarily means that the genetically engineered construct in a plant will necessarily be safe (with respect to horizontal gene transfer);	GT	Prevalence of naturally occurring form of introduced genes in the environment is an important part of baseline comparison used to assess this hazard. Abundance in the environment increases probability that natural form would be the source, if such transfer occurred, rather than the GMO. Both the likelihood and consequences of horizontal gene transfer from plants to other organisms have been assessed and found to be negligible (see App 4)
		• The RARMP identifies two contradictory purposes, 'to characterise wheat lines with genetic modification to overproduce proline' (para 318) and 'purpose is to evaluate salt tolerance and agronomic performance' (Executive Summary);	D	Ch 1 (Section 1.1) Not contradictory, Chapters & Appendices always provide greater detail than Exec Summary (overproduction of proline is expected to confer salt tolerance and is the central aspect of the characterisation)
		• Any such trial must be fully contained;	RM	Ch 2, App 2, 3, 4 and 5 (LC). This is a small scale (0.45ha) proof of concept, limited and controlled field trial.
		• There are no measures intended or able to prevent the spread of wheat in a storm or severe wind;	W, RM	Ch 2, App 3. Corrigin shire in Western Australia has marginal rainfall and elevated temperatures from November to February, limiting the likelihood of seed and seedling survival.
		• No discussion of exposure of wheat or seed to water and viability should seeds be carried to and by water;	W, RM	Discussed in Ch 2, App 3 (Section 2.5) and App 5 (LC)
		• The proposed 50 m distance to a watercourse is inadequate;	W, RM	Risk Assessments disagree: low rainfall and low likelihood of survival
		• Cleaning conditions are not specific and could amount to no more than a hosedown;	W, RM	Cleaning conditions to be read in conjunction with monitoring conditions (to minimise regrowth)
		• Contingency plans are not assessed as part of the application, but developed post approval.	RM	Contingency plans cannot be developed until licence conditions are finalised.

2	I	Licence conditions that should be imposed: • Stringent tests on allergenicity of this GMO • Long term feeding studies on stock, poultry to ascertain the food safety of this GMO and its products/by-products to test for possible health impacts	A F, T	Limited & controlled proof of concept trial only. Not permitted for human/animal use. Any adverse effects to be reported, App5 (LC)
		• Alert FSANZ if allergenicity is shown to be a concern	A	Noted (FSANZ approval would be required before this GMO could be used in food)
		• RARMP should include: - potential human health ramifications arising from using products from animals directly or indirectly fed on GM wheat	H, A, T	App 2 (NB not permitted for animal feed)
		- potential ramifications for animal health from consumption of GM wheat	T	App 2(NB not permitted for animal feed)
		• Typographical error (cyanide hydratase)	Typing error	Noted/changed
		• Stability of inserted materials and potential impact	D	App1, 2
		• CAH protein not found in plants, therefore cannot demonstrate lack of allergenicity	D	App2. Similar proteins are produced by bacteria and fungi that are widespread in the environment and are present in food. Neither the *A. thaliana* OAT nor the *M. verrucaria* CAH proteins show any significant sequence homology to known allergens
		• Unknown impact of genes if location of insertion not known	D	Early stage limited & controlled trial for research purposes. Future applications would require such information, Ch 2, App1
		• Possible adverse impact of additional proline expression on microorganisms	T	Discussed in detail in App2
		• Persistence data not available	E	Early stage limited & controlled field trial. Future applications would require such information Ch 2, App2
		• Gene transfer from divergent donor organism	GT	Discussed in Appendix 4
		• CAH protein is a novel protein for animal cells	T	Similar proteins are widely present in environment (bacteria & fungi). See App 2
3	A	• Consequences of unintended presence of wheat in wheat supply	M, S	OSA
		• Support research into development of GM crops	R	Noted
		• Urge OGTR to ensure that the trial is carefully managed to prevent contamination of other crops	RM	App 5 (LC)

APPENDIX 8 REFERENCES

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