



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 082/2007**

**Limited and controlled release of GM
perennial ryegrass and tall fescue with altered
fructan and lignin metabolism**

Applicant: DPI-Victoria

July 2008

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Executive Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of up to 500 perennial ryegrass and tall fescue lines genetically modified (GM) to improve forage qualities, into the environment in respect of application DIR 082/2007 from the Victorian Department of Primary Industries (DPI Victoria).

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

The application

DPI Victoria applied for a licence for dealings involving the intentional release of up to 2000 plants comprising 500 lines of genetically modified (GM) perennial ryegrass and tall fescue on a limited scale and under controlled conditions. The GM perennial ryegrass and tall fescue lines have been modified to improve forage qualities. The trial would take place at one site in the shire of Southern Grampians, Victoria, on an area of 800 m² per year between 2008 and 2010.

The GM perennial ryegrass and tall fescue lines contain one or more of 12 genes derived from perennial ryegrass and tall fescue. Expression of the introduced genes is expected to improve forage qualities by changing carbohydrate levels and improving digestibility of these pasture grasses.

The GM perennial ryegrass and tall fescue lines also contain an antibiotic resistance selectable marker gene that was used to identify transformed plants during initial development of GM plants in the laboratory.

The purpose of the trial is to conduct proof of concept research to assess the agronomic performance and forage qualities of the GM lines grown under field conditions. GM plants will be transferred from the trial site to a PC2 glasshouse prior to flowering for controlled breeding experiments. Seed and tissue samples will be collected and retained for analysis and possible future trials of lines that may be selected for further development, subject to further approval(s). The GM perennial ryegrass and tall fescue plants will not be used for human food or animal feed.

DPI Victoria proposed a number of controls to restrict the dissemination or persistence of the GM perennial ryegrass and tall fescue lines and the introduced genetic materials in the environment.

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

Confidential Commercial Information

Some details, including the names of the introduced genes, the names and origins of the promoters and terminators (regulatory sequences), and details of the cultivars used for transformation have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

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

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

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

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

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

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

Risk management

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

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

The licence conditions require DPI Victoria to **limit** the release to an area of 800 m² per year at one site between July 2008 and July 2010. The **control** measures to restrict the spread and persistence of the GMOs include preventing the use of GM plant materials in human food or

animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with OGTR transportation guidelines; and conducting post-harvest monitoring to ensure all GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 500 GM perennial ryegrass and tall fescue lines on an area of 800 m² per year over two years in the Victorian shire of Southern Grampians poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

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Abbreviations

The Act	<i>Gene Technology Act 2000</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
CCI	Confidential Commercial Information as declared under section 185 of the <i>Gene Technology Act 2000</i>
CaMV	Cauliflower mosaic virus
cv	cultivar
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
DPI Victoria	Victorian Department of Primary Industries
FAO	Food and Agricultural Organization of the United Nations
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
kDa	kilodalton
mRNA	Messenger Ribonucleic Acid
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OGTR	Office of the Gene Technology Regulator
RARMP	Risk Assessment and Management Plan
RNA	Ribonucleic Acid
TGA	Therapeutic Goods Administration
WHO	World Health Organisation

Technical Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence (DIR 082/2007) to the Victorian Department of Primary Industries (DPI Victoria) for dealings involving the intentional release of genetically modified (GM) perennial ryegrass and tall fescue lines into the Australian environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Acting Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public².

The application

DPI Victoria applied for a licence for dealings involving the intentional release of up to 2000 plants comprising 500 lines³ of perennial ryegrass and tall fescue (*Lolium perenne* L. and *Lolium arundinaceum* (Schreb) Darbysh.) on a limited scale and under controlled conditions. The GM perennial ryegrass and tall fescue lines have been genetically modified to improve forage qualities. The trial is authorised to take place at one site in the shire of Southern Grampians, Victoria, on an area of 800 m² per year over two years between July 2008 and July 2010.

The GM perennial ryegrass and tall fescue lines were produced by transforming plants of experimental cultivars, which are not grown commercially in Australia. The identity of the introduced genes has been declared commercial confidential information (see below).

Up to 250 of the GM perennial ryegrass lines contain one or more of three introduced perennial ryegrass genes encoding proteins involved in fructan biosynthesis in 15 different combinations. The expression of these genes is expected to alter the level of fructan carbohydrates in the GM plants to increase available energy and enhance animal productivity.

Up to 250 lines of GM perennial ryegrass and GM tall fescue contains one or more of nine introduced genes from perennial ryegrass or tall fescue encoding proteins involved in lignin metabolism in 15 different combinations. The expression of these genes is expected to alter lignin metabolism in the GM plants to increase cell wall digestibility and enhance animal productivity.

In addition, all of the GM perennial ryegrass and tall fescue lines contain an antibiotic resistance marker gene, *hph*, from the bacterium, *Escherichia coli*. The *hph* gene encodes *hygromycin phosphotransferase* and was used to select for modified plants in the laboratory. Hygromycin will not be applied to the plants during the proposed field trial.

² More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>>), and in the Regulator's Risk Analysis Framework (OGTR 2007) at

<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>

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

The purpose of the trial is to conduct proof of concept research to assess the agronomic performance and forage qualities of the GM lines grown under field conditions. GM plants will be transferred from the trial site to a PC2 glasshouse prior to flowering for controlled breeding experiments. Seed and tissue samples will be collected and retained for analysis and possible future trials of lines that may be selected for further development, subject to further approval(s). The GM perennial ryegrass and tall fescue plants will not be used for human food or animal feed.

DPI Victoria proposed a number of controls to restrict the dissemination or persistence of the GM perennial ryegrass and tall fescue lines and the introduced genetic materials in the environment. These controls were considered during the evaluation of the application.

Confidential Commercial Information

Some details, including the names of the introduced genes, the names and origins of the promoters and terminators (regulatory sequences), and details of the cultivars used for transformation have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application

Risk assessment

The risk assessment took into account information contained in the application, relevant previous approvals, current scientific knowledge and issues relating to risks to human health and safety and the environment raised in submissions received from consultation on the RARMP with a wide range of prescribed experts, agencies and authorities (summarised in Appendix B of the RARMP). No new risks to people or the environment were identified from the advice received on the consultation RARMP. However, feedback on the consideration of previously raised issues enabled their clarification in the final RARMP.

Advice received from the public on the consultation RARMP (one submission) and how it was considered, is summarised in Appendix C.

A reference document on the parent organism, *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum (Schreb.) Darbysh. (tall fescue)*, was produced to inform the risk assessment process for licence applications involving GM perennial ryegrass and tall fescue plants. The document is available from the OGTR or from the website <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir082-2007>.

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

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

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

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

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

Therefore, any risks of harm to the health and safety of people or the environment from the proposed limited and controlled release of the GM perennial ryegrass and tall fescue lines into the environment are considered to be **negligible**. Hence, the Acting Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment⁴

Risk management

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

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

Licence conditions to manage this limited and controlled release

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

- conduct the release on an area of up to 800 m² per year at one site in the shire of Southern Grampians, Victoria, between July 2008 and July 2010
- establish a 2 m monitoring zone around the trial site that is maintained in a manner that enables the identification of stoloniferous growth

⁴ As none of the proposed dealings were considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the *Gene Technology Act 2000* mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

- remove the GM plants from the field before flowering
- surround the 800 m² trial site with a 250 m border of *Triticale sp.*
- enclose the trial site with a 1 m high fence with a lockable gate
- locate the trial site at least 50 m away from natural waterways
- not permit any materials from the release to be used in human food or animal feed
- following harvest, clean the site, monitoring zone and equipment used on the site
- monitor the site for at least 12 months and destroy any perennial ryegrass and tall fescue plants that may grow until no volunteers are detected for a continuous 6 month period
- at the end of the trial, destroy all plant materials not required for further analysis.

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

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standard Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)⁵.

Identification of issues to be addressed for future releases

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

- characterisation of the introduced genetic material in the plants, including genotypic stability
- additional data on the potential toxicity of plant materials from the GM perennial ryegrass and tall fescue lines
- additional data on the potential allergenicity of pollen from the GM perennial ryegrass and tall fescue
- characteristics indicative of weediness including measurement of altered sexual and asexual reproductive capacity including seed persistence and plant establishment; altered growth rates, tolerance to drought, cold and other environmental stresses; and disease and pest susceptibility.

⁵ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

- information on characteristics indicative of weediness which may be conferred by the GM traits if gene transfer occurred to sexually compatible species.

Suitability of the applicant

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

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 500 GM perennial ryegrass and tall fescue lines on an area of 800 m² per year over two years in the Victorian shire of Southern Grampians poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

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Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people or the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation⁶, details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

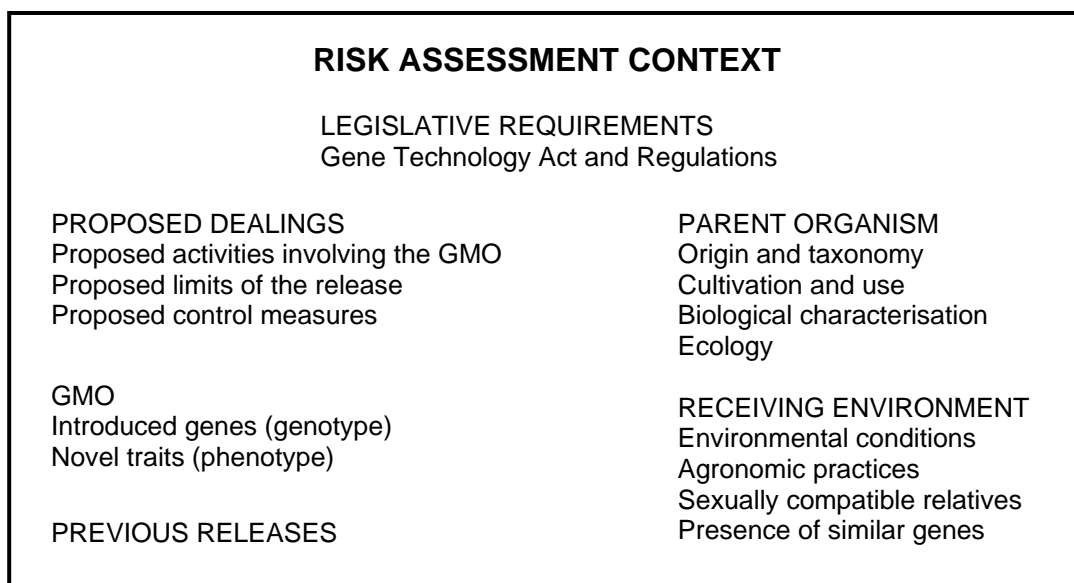


Figure 1 Components of the context considered during the preparation of the risk assessment

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

⁶ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1> and in the *Risk Analysis Framework* (OGTR 2005) <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

Section 2 The legislative requirements

3. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Regulator must take into account, and who she must consult with, in preparing the RARMPs that form the basis of her decisions on licence applications. In addition, the *Gene Technology Regulations 2001* (the Regulations) outlines matters the Regulator must consider when preparing a RARMP.

4. In accordance with section 50A of the Act, the Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits on the size, location and duration of the release and controls have been proposed by the applicant to restrict the dissemination or persistence of the GMOs or their genetic material in the environment. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application qualifies as a limited and controlled release and the Regulator has prepared a RARMP for this application.

5. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public.

6. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a 'significant risk' to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d).

Section 3 The proposed dealings

7. The Victorian Department of Primary Industries (DPI Victoria) proposes to release up to 500 genetically modified (GM) perennial ryegrass and tall fescue lines⁷ into the environment under limited and controlled conditions. The GM lines have been modified to improve forage qualities by altering carbohydrate levels and improving digestibility.

8. Some details of the application, including the names of the introduced genes, the names and origins of the promoters and terminators, and the names of the cultivars that were transformed, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

3.1 The proposed activities

9. The applicant has stated that the principal purpose of the proposed release is to conduct proof of concept experiments to assess the agronomic performance and forage qualities of the GM lines grown under field conditions. GM plants will be transferred from the trial site to a PC2 glasshouse prior to flowering for controlled breeding experiments. Some seed will be saved for possible future trials (subject to further approvals). The GM perennial ryegrass and tall fescue plants will not be used for human food or animal feed.

⁷ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

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

10. The release is proposed to take place at one site in the Department of Primary Industries research station at Hamilton in the shire of Southern Grampians, Victoria (VIC) on an area of 800 m² per year between June 2008 and July 2010.

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

11. The trial site is located 11 km from the closest population centre (Hamilton, population 11,000). The trial will be planted in the centre of 28.6 ha paddock that is surrounded by a 1 m high fence with a locked gate to which only trained staff will have access.

12. The applicant has proposed a number of controls to restrict the dissemination or persistence of the GM perennial ryegrass and tall fescue lines into the environment including:

- surrounding the 800 m² trial site with a 250 m border of *Triticale sp*⁸.
- restricting access to the trial site with a 1 m high fence with a locked gate
- planting at a time when other grasses in the release area are not flowering
- establishing a 2 m monitoring zone around the trial site that is maintained in a manner that enables the identification of volunteers
- ensuring that the trial site and *Triticale sp.* border are free of pasture grasses
- transferring GM plants to a PC2 glasshouse prior to flowering
- analysing GM plant materials in a certified PC2 facility and then destroying the unwanted materials
- destroying all (GM and non-GM) plant materials remaining at the field site by spraying with herbicide
- post harvest monitoring of the trial site for 12 months and destroying any volunteers
- transporting GM plant materials to and from the proposed trial site in accordance with OGTR transportation guidelines
- not using the GMO in human food or animal feed.

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

Section 4 The parent organisms

13. The parent organisms are perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh.) which are both exotic to Australia. Perennial ryegrass and tall fescue are used in both pasture and turf in Australia. They are used, generally in combination with other grass species, for dairying and sheep grazing predominantly in the temperate areas of Australia (New South Wales, Victoria and Tasmania) (Blair 1997; Lazenby 1997; Callow et al. 2003). In addition, both species are also used for turf, often in

⁸ The applicant originally proposed a 300 m border of *Triticale sp.* but changed this to 250 m during the consultation process

combination with other grass species and primarily in temperate regions (Lamp et al. 2001; Canturf 2006; Gardenet 2006; Yates 2006).

14. The GM perennial ryegrass and tall fescue were derived from endophyte-free callus lines. Endophytes are fungi that live between the plant cells of many forage grasses (Kemp et al. 2007). These fungi do not cause any disease in the grasses, and under most circumstances they are beneficial to the growth and survival of infected plants (Clay 1990; Joost 1995; Schardl & Phillips 1997; Schardl et al. 1997; Clay & Schardl 2002; Grewal & Richmond 2003; Schardl et al. 2004).

15. Further detailed information on perennial ryegrass and tall fescue can be found in the reference document *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum (Schreb.) Darbysh. (tall fescue)*, that was produced to inform the risk assessment process for licence applications involving GM perennial ryegrass, Italian ryegrass and tall fescue plants (OGTR 2008). This document is available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

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

5.1 Introduction to the GMOs

16. The GM perennial ryegrass and tall fescue lines contain one or more of twelve introduced genes, that are subject to a CCI declaration, encoding proteins involved in fructan or lignin metabolism. The genes introduced into the GM plants are derived from either perennial ryegrass or tall fescue, with the exception of the selectable marker genes (Table 1).

Table 1 The genes used to genetically modify perennial ryegrass and tall fescue

Gene	Protein produced	Protein involved in	Source
GOI 1A	CCI	Fructan biosynthesis	Perennial ryegrass
GOI 2A	CCI	Fructan biosynthesis	Perennial ryegrass
GOI 3A	CCI	Fructan biosynthesis	Perennial ryegrass
GOI 1B	CCI	Lignin biosynthesis	Tall fescue
GOI 2B	CCI	Lignin biosynthesis	Tall fescue
GOI 3B	CCI	Lignin biosynthesis	Perennial ryegrass
GOI 4B	CCI	Lignin biosynthesis	Perennial ryegrass
GOI 5B	CCI	Lignin biosynthesis	Perennial ryegrass
GOI 6B	CCI	Lignin biosynthesis	Perennial ryegrass
GOI 7B	CCI	Lignin biosynthesis	Perennial ryegrass
GOI 8B	CCI	Lignin biosynthesis	Perennial ryegrass
GOI 9B	CCI	Lignin biosynthesis	Perennial ryegrass
<i>hph</i>	Hygromycin phosphotransferase	Hygromycin resistance (selectable marker)	<i>Escherichia coli</i>

17. Up to 500 GM perennial ryegrass and tall fescue lines are proposed for release, with each line generated using 1 of 32 constructs (see Table 2). All GM plants were generated using the biolistics transformation method (see Chapter 1, Section 5.4) and each transformation was used to generate up to 150 GM perennial ryegrass and tall fescue plants, with a total of 2000 GM plants. Up to 400 non-GM plants would be used as controls.

Table 2 Gene constructs used to generate the GM perennial ryegrass and tall fescue lines proposed for release

Identity of construct	Target organism	Promoter	Gene of interest	Terminator	DNA type
1A	<i>L. perenne</i>	endogenous	GOI 1A	endogenous	Cassette
2A	<i>L. perenne</i>	endogenous	GOI 1A	endogenous	Vector
3A	<i>L. perenne</i>	constitutive	GOI 1A	35S	Vector
4A	<i>L. perenne</i>	constitutive	GOI 2A	35S	Vector
5A	<i>L. perenne</i>	constitutive	GOI 3A	35S	Vector
6A	<i>L. perenne</i>	Constitutive constitutive	GOI 1A GOI 2A	35S 35S	Vector Vector
7A	<i>L. perenne</i>	Constitutive endogenous	GOI 2A GOI 1A	35S endogenous	Vectors
8A	<i>L. perenne</i>	constitutive	GOI 1A (hairpin)	<i>nos</i>	Vector
9A	<i>L. perenne</i>	Inducible 1	GOI 1A	terminator to inducible 1	Vector
10A	<i>L. perenne</i>	Inducible 1	GOI 2A	terminator to inducible 1	Vector
11A	<i>L. perenne</i>	Inducible 1	GOI 1A - GOI 2A (fusion)	terminator to inducible 1	Vector
12A	<i>L. perenne</i>	Inducible 2	GOI 1A	endogenous	Vector
13A	<i>L. perenne</i>	Inducible 3	GOI 2A	endogenous	
14A	<i>L. perenne</i>	Inducible 2 Inducible 3	GOI 1A GOA 2A	endogenous	Cassettes
15A	<i>L. perenne</i>	Inducible 2	GOI 1A – GOI 2A	endogenous	Cassette
16A/16B	<i>L. perenne</i> <i>L. arundinaceum</i>	<i>Actin 1</i>	<i>Hph</i>	35S	Cassette
17A/17B	<i>L. perenne</i>	<i>Actin1</i>	<i>Hph1</i>	35S	Vector
1B	<i>L. arundinaceum</i>	endogenous 1	GOI 1B	endogenous 1	Cassette
2B	<i>L. arundinaceum</i>	endogenous 2	GOI 1B	endogenous 1	Cassette
3B	<i>L. arundinaceum</i>	endogenous 3	GOI 1B	endogenous 1	Cassette
4B	<i>L. perenne</i>	endogenous 2	GOI 2B	endogenous 1	Vector
5B	<i>L. perenne</i>	constitutive	GOI 3B (hairpin)	<i>nos</i>	Vector
6B	<i>L. perenne</i>	constitutive	GOI 4B (hairpin)	35S	Vector
7B	<i>L. perenne</i>	constitutive	GOI 5B (hairpin)	<i>nos</i>	Vector
8B	<i>L. perenne</i>	constitutive	GOI 4B – GOI 5B (hairpin)	<i>nos</i>	Vector
9B	<i>L. perenne</i>	constitutive	GOI 6B (hairpin)	<i>nos</i>	Vector
10B	<i>L. perenne</i>	constitutive	GOI 7B (hairpin)	<i>nos</i>	Vector
11B	<i>L. perenne</i>	constitutive	GOI 8B (hairpin)	<i>nos</i>	Vector
12B	<i>L. perenne</i>	constitutive	GOI 9B	<i>nos</i>	Vector
13B	<i>L. perenne</i>	endogenous 3	GOI 8B	endogenous 2	Vector
14B	<i>L. perenne</i>	endogenous 4	GOI 4B	endogenous 3	Vector
15B	<i>L. perenne</i>	<i>LpCCR1</i>	GOI 4B (fs)	endogenous 4	Vector

5.2 The introduced genes, encoded proteins and end products

5.2.1 Genes expected to alter fructan metabolism, and their encoded proteins

18. Up to 250 of the GM perennial ryegrass lines contain one or two of three different genes isolated from perennial ryegrass that encode proteins involved in fructan biosynthesis (Figure 2). The expression or suppression of these genes either singly or in combination is expected to alter the level of fructan carbohydrates present in perennial ryegrass tissues. Non-GM perennial ryegrass plants already contain the biosynthetic pathway for fructan biosynthesis and the genes that are inserted into perennial ryegrass are all endogenous genes. Some of the constructs are expected to either reduce or increase the level of some of the enzymes, and some are intended to alter the timing of their production.

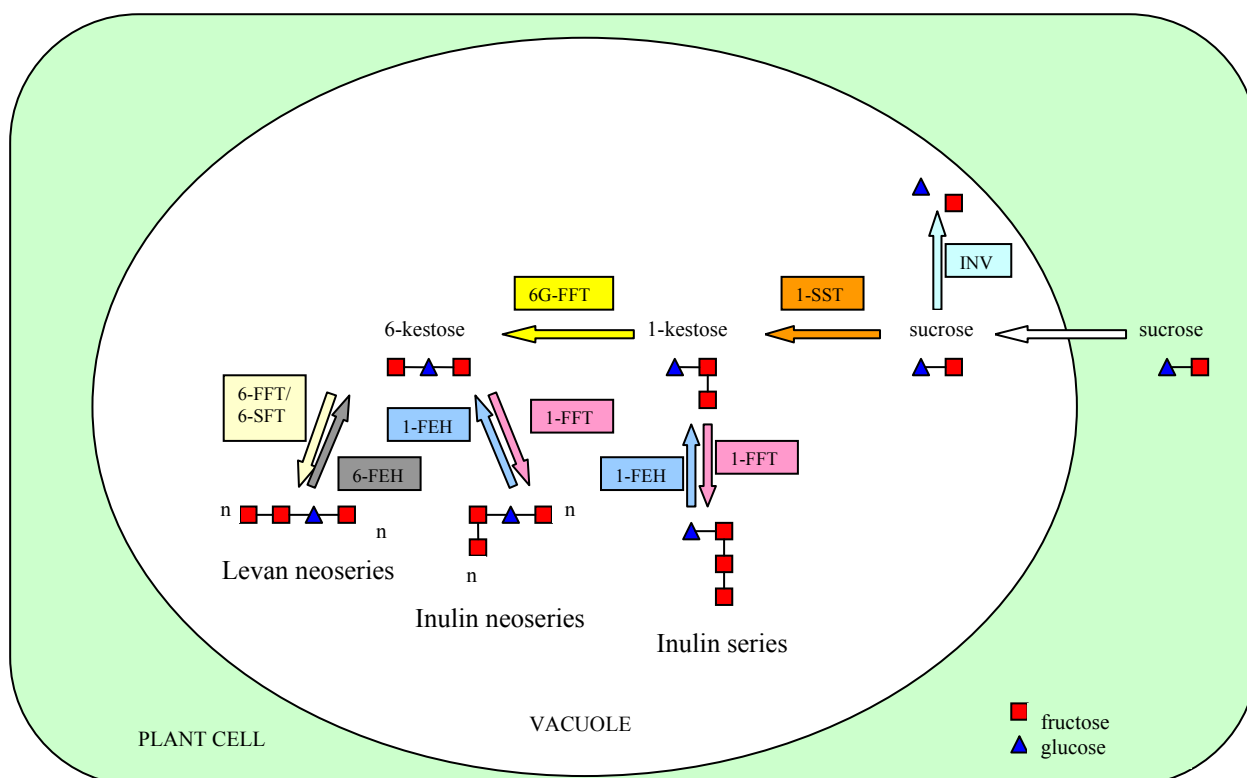


Figure 2 Schematic representation of the fructan biosynthetic pathway in grasses

Abbreviations used: 1-SST (sucrose:sucrose 1-fructosyltransferase); 6G-FFT (6-glucose fructosyltransferase); INV invertase; 1-FEH fructan exohydrolase; 6-FEH fructan exohydrolase; 1-FFT (fructan:fructan 1-fructosyltransferase); 6-FFT (fructan:fructan 6-fructosyltransferase); 6-SFT (sucrose:fructan 6-fructosyltransferase).

5.2.2 Genes expected to alter lignin metabolism, and their encoded proteins

19. Up to 250 of the GM perennial ryegrass and tall fescue lines contain one or two of nine different genes that encode proteins involved in lignin metabolism (Figure 3). The expression or suppression of these genes, either singly or in a combination, is expected to alter the levels or type of lignin present in perennial ryegrass and tall fescue tissues. Non-GM perennial ryegrass and tall fescue plants already contain the biosynthetic pathway for lignin biosynthesis and the genes that are inserted into perennial ryegrass and tall fescue are all endogenous genes, with one exception where a tall fescue gene is inserted into perennial ryegrass. Some of the constructs are expected to either reduce or increase the level of some of the enzymes, and some are intended to alter the timing of their production.

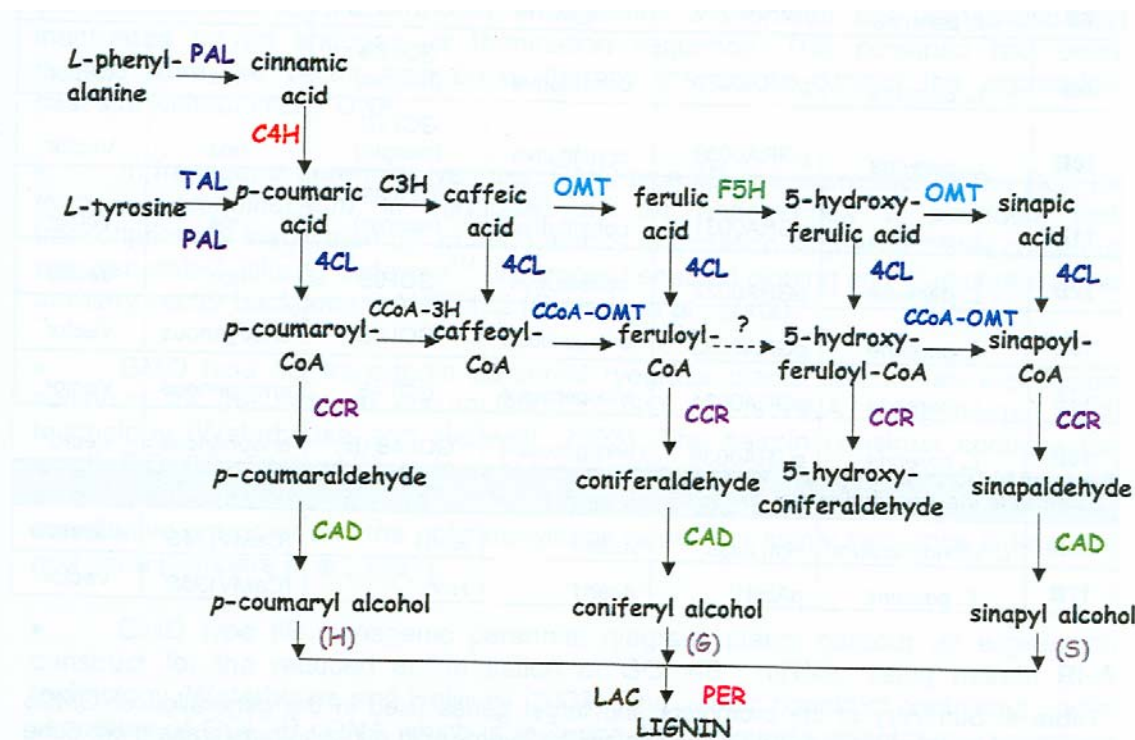


Figure 3 Schematic representation of the lignin biosynthetic pathway

Abbreviations used: PAL phenylalanine ammonia lyase; C4H coumarate 4 hydroxylase, TAL tyrosine ammonium lyase; C3H coumarate 3 hydroxylase; OMT *O*-methyltransferase; F5H ferulate 5 hydroxylase; 4CL 4-coumarate CoA ligase; CCoA-3H caffeoyl CoA 3-hydroxylase; CCoA-OMT caffeoyl CoA-methyltransferase; CCR cinnamoyl CoA reductase; CAD cinnamyl alcohol dehydrogenase; LAC laccase, PER peroxidase.

5.2.3 Toxicity/allergenicity of the proteins encoded by the introduced genes for fructan and lignin metabolism

20. The genes introduced into the GM plants were isolated from perennial ryegrass and tall fescue plants. Homologues of all of the encoded proteins occur naturally in a range of organisms, including plants widely consumed by people and animals (see discussion in Section 6.5). On this basis, people and other organisms have a long history of exposure to the proteins involved in fructan and lignin metabolism.

21. No toxicity/allergenicity tests have been performed on any of the purified encoded proteins as the proposed trial is still at proof of concept stage.

22. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The predicted amino acid sequences of the proteins encoded by each of the introduced genes for lignin and fructan metabolism were compared to a database of known allergens. The results of this analysis showed that all three of the fructan metabolism genes showed up to 72% sequence homology over 80 amino acids and up to 54% homology with the full length protein with two known minor food allergens from tomato. This figure is above the 35% threshold often used to highlight an allergenicity concern (Fiers et al. 2004). However, the proteins encoded by the introduced genes are naturally present in the non-GM perennial ryegrass plants and similar proteins are present in many other plants, including plants that are widely consumed by people and animals and they are not reported as allergens.

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

5.2.4 The selectable marker gene (*hph*) and encoded protein

24. The GM perennial ryegrass and tall fescue lines contain the antibiotic resistance selectable marker gene hygromycin phosphotransferase (*hph*). The *hph* gene (also known as *hpt* or *aph(4)Ia*), encodes the enzyme hygromycin phosphotransferase (HPT). It is derived from *Escherichia coli* and confers hygromycin resistance on the GM plant by catalysing the phosphorylation of the 4-hydroxy group on the hyosamine moiety, thereby inactivating hygromycin (Rao et al. 1983) and preventing it from killing cells producing HPT. The *hph* gene was used as selective marker to identify transformed plant tissue during initial development of GM plants in the laboratory.

25. The *hph* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004) and, as discussed in the RARMP for DIR 073/2007 (available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir073-2007>>, or by contacting the OGTR) its use has been assessed as not posing a risk to human health and safety or the environment. HPT is easily digested by simulated gastric juices and the amino acid sequence contains no similarities to known allergens (Lu et al. 2007). An assessment of antibiotic resistance genes used in GM plants by the European Food Safety Authority concluded that inclusion of the *hph* gene in GM plants would not significantly affect the health of humans or animals (EFSA 2004).

5.2.5 The end products associated with the introduced genes for fructan and lignin metabolism

26. Perennial ryegrass is one of the most important forage crops in temperate regions of the world and, along with tall fescue, is useful for dairy and sheep forage. The nutritional value of perennial ryegrass is limited by inefficient utilisation of amino acids by microorganisms in the rumen due to low carbohydrate levels (Gadegaard et al. 2008). Increasing carbohydrate content in grass forage has been shown to increase the nutritional value and hence milk production (Miller et al. 2001) and also decrease the production of ammonia released into the environment (Miller et al. 2001; Lee et al. 2003). The nutritional value of grasses is also limited by lignification, which renders much of the cellulose and hemicellulose inaccessible to rumen microorganisms (He et al. 2003). This is especially important during plant maturation as the wall composition changes from ~ 10% protein with very little lignin in young plants to very low concentrations of protein and 20-30% lignin in mature plants (Vogel & Jung 2001). Feeding and grazing studies have shown that small changes in forage digestibility can have a significant impact on animal performance (Casler & Vogel 1999).

Fructan

27. Fructans are polymers of fructose (Pavis et al. 2001a) which occur in 25% of flowering plant species (Crafts-Brandner 2005) as reserve carbohydrates (Ritsema & Smeekens 2003). Fructan may account for more than 30% of the dry weight of grass leaves, stems and ears depending on their state of development and on environmental conditions (Pollock & Jones 1979). Fructans are synthesised in the vacuoles (Darwen & John 1989) of many economically important orders of Asterales (chicory, Jerusalem artichoke), Liliales (onion, tulip) and Poales (barley, wheat) (Weyens et al. 2004). The Poaceae contain fructans of a complex structure with $\beta(2-6)$ and $\beta(2-1)$ fructose linkages (Pavis et al. 2001b).

28. Fructans from *Lolium* belong to the:

- inulin series with a terminal glucose residue and $\beta(2-1)$ linked fructose residues

- inulin neoseries with an internal glucose residue and $\beta(2-1)$ linked fructose residues
- levan neoseries with an internal glucose residue and $\beta(2-6)$ linked fructose residues (Pavis et al. 2001b)(see Figure 2).

29. The global distribution of fructan-accumulating plants shows that they are especially abundant in temperate climate zones with seasonal drought or frost (Hendry & Wallace 2008) and fructans have been implicated in drought and cold tolerance (Valliyodan & Nguyen 2006). Fructans accumulate in wheat (Bancal & Gaudillère 1989; Jeong & Housley 1990) and *Lolium temulentum* (Pollock 1984) following cold stress and in perennial ryegrass following drought stress (Amiard et al. 2003). Under drought stress, levels of sucrose:sucrose 1-fructosyltransferase 1 (SST-1) in wheat stems were reduced and in both rice and wheat 1-fructan exohydrolase (1-FEH) increased, allowing depolymerisation of fructans (Yang et al. 2004; Ji et al. 2007), yet in chicory plants increases in SST-1 levels and fructan accumulation have been observed (De Roover et al. 2000).

30. GM plants with altered levels of enzymes involved in the fructan biosynthetic pathway have various phenotypes. GM tobacco plants expressing bacterial levansucrase produced fructan and were more resistant to drought stress (Pilon-Smits et al. 1995) and freezing (Konstantinova et al. 2002). GM Italian ryegrass (*Lolium multiflorum*) expressing bacterial levansucrase had altered fructan metabolism, were stunted and had slowed growth in the reproductive phase (Ye et al. 2001). Yet, GM rice plants expressing wheat 1-SST showed fructan accumulation and enhanced chilling tolerance (Kawakami et al. 2008), as did GM GM tobacco plants expressing lettuce 1-SST (Li et al. 2007). GM tobacco plants expressing barley 6-SFT also accumulated fructans (Sprenger et al. 1997), but did not show altered resistance to drought stress (Schellenbaum et al. 1999).

31. Although expression of fructan synthesis genes from bacteria has led to aberrant growth phenotypes (Cairns 2003), expression of plant fructosyltransferase in plants does not seem to affect growth or total carbohydrate content (Ritsema & Smeekens 2003). For example, GM sugarbeet plants expressing onion SST and fructan:fructan 6-glucose fructosyltransferase (6G-FFT) produced inulin neoseries and showed no abnormal phenotype (Weyens et al. 2004). Similarly, perennial ryegrass plants expressing the same genes produced 3-fold higher fructan levels with a higher degree of polymerisation than non-GM plants but no abnormal phenotype (Gadegaard et al. 2008).

32. Fructans have also been implicated in disease resistance. More fructans were seen to accumulate in wheat cultivars which were resistant to snow mold, than in susceptible cultivars, and levels of 6-SFT and 1-SST were higher in the resistant cultivars (Kawakami & Yoshida 2002). Fructans are also thought to contribute to resistance to severe defoliation in tall fescue (Damiani et al. 2004).

Lignin

33. Lignin is a complex phenolic polymer found in plant cell walls and is essential for mechanical support, water and mineral transport, and defence against pathogens in vascular terrestrial plants (He et al. 2003). Lignin often comprises over 20% of the dry weight of plant cell walls and is, after cellulose, the second most abundant polymer in nature (Lewis & Yamamoto 1990). It is comprised of three monomers –*p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol which differ in their degree of methylation and are polymerised together to form *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) phenylpropanoid units (Boerjan et al. 2003). Changes in the relative proportion of these monomers differs between plants and even within a plant between cell types or in response to environmental stresses (Boudet

2000). The lignin biosynthesis pathway has been extensively reviewed by (Rastogi & Dwivedi 2008; Lewis & Yamamoto 1990; Boudet 2000; Boerjan et al. 2003; Halpin 2004).

34. A review of the impact of reduced lignin on plant fitness concluded that, although in general crop yields are depressed by significant reductions in lignin content, there are also examples which show either no changes in fitness (Pedersen et al. 2005) or improved fitness. For example, GM poplar plants with anti-sense 4-coumarate CoA ligase (4CL1) show a reduction in lignin, an increase in cellulose content and an increased growth rate (Hu et al. 1999). Lignin reduction in *bml* maize mutants leads to earlier flowering (Vermerris & McIntyre 1999; Vermerris et al. 2002a). However, sorghum *bmr* mutants with altered lignin composition generally flower later than their wildtype counterparts (Vermerris et al. 2002b). In *Arabidopsis*, a *fah1* mutant which has a structural mutation in a ferulic acid hydroxylase gene has reduced protection against UV light (Landry et al. 1995).

35. Stress lignin has been reported under pathogen attack (Vance et al. 1980) and the induction of various genes involved in lignin biosynthesis has been reported (see Table 3). This is quite specific as in some plants differential induction of lignin biosynthesis genes, or members of gene families has been observed following different stresses. For example, different sequences of the Caffeic acid O-methyltransferase (COMT) II promoter have been identified which were responsible for gene induction by methyl jasmonic acid, UV light, TMV or wounding (Toquin et al. 2003; Vincent et al. 2005). Members of the 4CL gene family in parsley are differentially induced by treatment with fungal elicitors or infection with compatible or incompatible races of *Phytophthora megasperma* f.sp. *glycinea* (Uhlmann & Ebel 1993). During the hypersensitive response of wheat to stem rust a syringyl-rich lignin is deposited (Menden et al. 2007), consistent with the induction of a sinapyl alcohol specific form of cinnamyl alcohol dehydrogenase (CAD) in elicitor treated wheat leaves (Mitchell et al. 1994; Mitchell et al. 1999).

Table 3 Lignin genes involved in disease resistance

Plant spp	Disease/inducer	Effects	Reference
<i>Medicago truncatula</i>	Infection Elicited cell cultures	Phenylalanine ammonia lyase (PAL) upregulated	(Dixon et al. 2002)
Pearl millet (<i>Pennisetum glaucum</i> (L.) R.Br.)	<i>Sclerospora graminicola</i> infection	PAL activity correlated with disease resistance	(Nagarathna et al. 1993)
Tobacco	TMV	Caffeic acid O-methyltransferase (COMT) induced	(Pellegrini et al. 1993; Maury et al. 1999)
Tobacco	TMV	Caffeoyl CoA O-methyltransferase (CCoAOMT) induced	(Maury et al. 1999)
Parsley	Disease response	CCoAOMT induced	(Pakusch et al. 1989; Schmitt et al. 1991)
	Elicited cell cultures	Caffeoyl CoA 3 hydroxylase (CCoA-3H) induced	(Cornish et al. 1979; Kneusel et al. 1989)
Carrot	Disease response	CCoAOMT induced	(Schmitt et al. 1991)
Barley	Fungal pathogens UV light	O-methyl transferase (OMT) induced	(Gregersen et al. 1994)
Bean (<i>Phaseolus vulgaris</i>)	Fungal elicitor	Cinnamyl alcohol dehydrogenase (CAD) induced	(Grand et al. 1987; Walter et al. 1988)
Flax (<i>Linum usitatissimum</i>)	Fungal elicitor	CAD induced	(Hano et al. 2006).
Wheat	Fungal elicitor	Sinapyl alcohol dehydrogenase (SAD) induced	(Mitchell et al. 1994; Mitchell et al. 1999).

36. Increases in lignin formation itself has also been observed following pathogen attack in a number of plant species. Banana plants tolerant to Panama disease (*Fusarium oxysporum* f. sp. *cubense*) showed increased levels of lignin biosynthesis enzymes and lignin deposition following application of a fungal elicitor compared to a susceptible cultivar (De Ascensao & Dubery 2000). Chemical inhibition of lignification in wheat results in a decreased resistance to stem rust (*Puccinia graminis*) (Moerschbacher et al. 1990) and higher lignin accumulation was seen in wheat resistant to take-all fungus (*Gaeumannomyces graminis* var *tritici*) than in a susceptible genotype (Rengel et al. 1994). However, pathogen infection does not induce lignin formation in all plants, and not all plants with altered lignin show altered disease resistance. For example, no increased susceptibility to *Fusarium* or *Alternaria* spp. was seen for sorghum mutants with altered CAD and COMT levels and reduced lignin (Funnell & Pedersen 2006). Similarly, field grown GM poplar trees with reduced CAD or COMT showed no altered responses to pests or diseases (Pilate et al. 2002).

37. Activation of enzymes of lignin metabolism has also been observed under stress conditions (Dixon & Paiva 1995). During drought, the level of lignin decreases, this may be to prevent the accumulation of lignin in the absence of growth which would lead to lignification in the elongation zone and thus compromise growth recovery upon rehydration (Vincent et al. 2005). Survival of shattercane (*Sorghum bicolor*) seed was positively correlated with caryopsis lignin content, with the higher lignin content thought to protect the seed from microbial invasion (Fellows & Roeth 1992). In *Arabidopsis*, microarray analysis of genes induced by cabbage white butterfly (*Pieris rapae*) feeding showed increases in cinnamyl CoA reductase (CCR) and COMT (Reymond et al. 2004), although COMT was also induced by wounding (Reymond et al. 2004). Caffeoyl CoA O-methyltransferase (CCoAOMTs) were also induced in response to drought in maritime pine needles (*Pinus pinaster*) (Costa et al. 1998) and in *Arabidopsis* roots (Bianchi et al. 2002).

38. Changes in lignin content and composition can also have impacts on decomposition rates. GM tobacco plants with reduced lignin content or altered composition were shown to decompose faster in soil (Hopkins et al. 2001; Webster et al. 2005), as were roots from field grown GM poplar trees with reduced CAD and COMT levels (Pilate et al. 2002). However, stem sections from the same trees showed similar decomposition rates to non-GM trees (Tilston et al. 2004).

5.2.6 Toxicity of the end products associated with the introduced genes for fructan and lignin metabolism

Fructan

39. Up to 250 of the GM perennial ryegrass lines proposed for release are expected to produce increased or decreased fructan accumulation compared to the parent cultivars. Data has been provided on the fructan content of some lines, with some lines showing doubled levels of fructans in stems (information supplied by applicant). Fructans are not known to be toxic to humans and are found in many common food plants (see Section 5.2.5). Fructans have been claimed to have favourable effects in the prevention of cardiovascular diseases, colon cancer and osteoporosis and can be used as a low calorie food ingredient to replace sugar or fat as they are not digested by humans (Weyens et al. 2004).

40. In horses, excess fructan consumption has been implicated in illnesses such as laminitis, equine polysaccharide storage myopathy (EPSM) and equine metabolic syndrome (also called insulin resistance or peripheral Cushing's) (Watts & Chatterton 2004). Fructan is not digested in the small intestine of horse, but is fermented by microflora in the colon which causes a change in pH of the gut. This encourages the growth of gram-positive bacteria some of which release substances that activate matrix metalloproteins. These enzymes act in the hoof wall

and break down the material which anchors the hoof to the pedal bone, leading to pain and distortion of the hoof and laminitis (Jones 2004; King & Mansmann 2004). There have been no reports identified of adverse effects of fructan on other organisms.

Lignin

41. Up to 250 of the GM perennial ryegrass and tall fescue lines proposed for release are expected to produce altered levels or composition of lignin compared to the parent cultivars. Data has been provided on the lignin content of some lines, with some lines showing reduced lignin content in stems (information supplied by applicant). Lignin is not known to be toxic to living organisms and is found in different quantities and compositions in all land plants.

5.3 The regulatory sequences

5.3.1 Regulatory sequences for the introduced genes for fructan and lignin metabolism

42. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Ten promoters are being used in the proposed release. Part of the purpose of the proposed trial is to determine the efficacy of these promoters in the perennial ryegrass and tall fescue plants. The detail of these promoters has been declared CCI.

43. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. Eight terminators are being used in the GMOs proposed release. The detail of these terminators has been declared CCI.

44. An intron from the perennial ryegrass has been used in GMOs 8A 5B, 6B, 7B, 8B, 9B, 10B, 11B, 12B to separate sense and antisense orientations of the gene sequences to create a hairpin construct which is expected to lead to gene silencing (Waterhouse & Helliwell 2003).

45. While some of the regulatory sequences are derived from plant pathogens (*Agrobacterium tumefaciens*, CaMV) the sequences are not pathogenic in themselves nor do they cause any disease symptoms in the GM plants. Those regulatory sequences derived from plants that are associated with allergic or toxic responses in humans (ie. *Lolium perenne*, *Triticum aestivum*, *Lolium arundinaceum*, and *Zea mays*) are not in themselves allergenic or toxic.

5.3.2 Regulatory sequences for the expression of the hph gene

46. The expression of the *hph* gene in the GM perennial ryegrass and tall fescue lines is controlled by the *Actin1* promoter from rice (*Oryza sativa*) (McElroy et al. 1990).

47. The mRNA termination region for the *hph* gene in the GM perennial ryegrass and tall fescue lines is derived from the CaMV 35S terminator (Gardner et al. 1981). Although CaMV is a plant pathogen, the regulatory sequence comprises only a small part of its total genome, and has not been reported to cause any adverse effects on human health or the environment.

5.4 Method of genetic modification

48. The genes were introduced into perennial ryegrass and tall fescue by biolistic transformation, also known as particle bombardment. This technique involves coating very small gold particles with the transformation vector or cassette containing the introduced genes. The particles were then 'shot' into callus derived from meristematic tissue of either tall fescue or one of four cultivars of perennial ryegrass. GM plant tissues were recovered by survival on tissue media containing the antibiotic hygromycin. Particle bombardment has been widely used in Australia and overseas for introducing new genes into plants and is not known to cause any adverse effects on human health or the environment.

49. The genes were introduced into perennial ryegrass and tall fescue as either cassettes or whole plasmids. The constructs for GMOs 1A, 12A-15A and 1B-3B are cassettes, so contain no vector DNA. The remaining candidate genes were introduced using whole plasmids with vector backbone sequences present so the GM plants may contain vector DNA. All of the GM perennial ryegrass and tall fescue lines were generated from independent transformation events, and therefore the introduced genes are expected to be located at different sites in the perennial ryegrass and tall fescue genomes for each line.

5.5 Characterisation of the GMOs

5.5.1 Stability and molecular characterisation

50. All inserts within the transformation vectors have been fully sequenced to confirm the nucleotide sequence of each segment that was used for vector and cassette construction.

51. The GM perennial ryegrass and tall fescue lines are at an early development stage. To eliminate genetic variation for the trial, vegetatively propagated primary transformants (T₀) will be employed. The T₀ plants have shown mitotically stable integration of the transgenes (information supplied by applicant).

52. The exact location of the inserted genes within the perennial ryegrass and tall fescue genome of the lines is not known.

53. The copy number of inserted genes for all of the GM perennial ryegrass and tall fescue lines is not known. Initial results suggest that between one and eight copies of the transgene have been inserted, with approximately 10% of the plants containing a single copy insertion. RT-PCR and/or RNA and DNA blot hybridisation has been performed on all plants to confirm the presence and expression of the introduced gene(s) (information supplied by applicant).

5.5.2 Characterisation of the phenotype of the GMOs

54. The main aim of the proposed trial is to characterise the growth of the GMOs and assess their fructan or lignin content/composition under normal field conditions. Traits that will be measured include: growth rate, tiller number, water soluble carbohydrates, forage quality and other growth characteristics including yield traits.

55. When grown under glass house conditions, no gross morphological abnormalities were seen, although some plants which accumulated less lignin flowered slightly earlier (information supplied by applicant).

56. While there may be changes in the levels of products produced as a result of the activity of the encoded proteins, no new products should be produced by expression of the introduced genes, except for the selectable marker gene. This will produce the HPT protein, which will give resistance to hygromycin (as discussed in Chapter 1, Section 5.2.4). However, there may be unintended effects due to random insertion of the introduced genes (see Chapter 2, Event 6).

Section 6 The receiving environment

57. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the size, location and duration of the dealings, any relevant biotic/abiotic properties of the geographic regions where the release would occur; intended agronomic practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2007).

6.1 Relevant abiotic factors

58. The abiotic factors relevant to the growth and distribution of perennial ryegrass and tall fescue in Australia are discussed in *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum Schreb. (tall fescue)* document (OGTR 2008).

59. The release is proposed to take place in the Victorian local government area of Southern Grampians. This region is in the temperate climatic type (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology). The rainfall and temperature statistics for Hamilton Research Station, in which the proposed site is located, are given in Table 4.

Table 4 Monthly temperature and rainfall statistics for Hamilton Research Station –Victoria (Temperate)*

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Mean max temp (°C)	25.2	26.1	23.2	19.0	15.3	12.7	12.0	13.0	14.7	17.1	19.5	22.5	18.4
Mean min temp (°C)	10.2	10.9	9.6	7.7	6.3	4.4	4.1	4.6	5.4	6.1	7.2	8.9	7.1
Mean rainfall (mm)	33.8	25.4	38.6	50.9	64.0	65.4	85.6	83.0	75.7	63.3	53.6	47.0	685.5

* data taken from the Australian Bureau of Meteorology website (<http://www.bom.gov.au/index.shtml>). Temperature data are an average of 35 years of records and rainfall data are an average of 38 years of records, ending in 2000.

60. The proposed field location is located within an 1100 ha research farm. There are no natural streams, creeks or springs or artificial drains or irrigation channels running through or near the trial site.

6.2 Relevant biotic factors

61. The biotic factors pertaining to the growth and distribution of perennial ryegrass and tall fescue in Australia are discussed in *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum Schreb. (tall fescue)* document (OGTR 2008). Of relevance to this proposed release are the following points:

- the Research Station, in which the proposed site is located, occurs within a major grazing region
- on the Research Station *L. perenne*, *L. arundinaceum* and *L. multiflorum* are sown and *L. rigidum* is naturalised
- the nearest commercial site for perennial ryegrass and tall fescue seed production is at Colac, 160 km away from the site
- invertebrates, vertebrates and microorganisms would all be exposed to the introduced genes, their encoded proteins and end products. In particular, native birds and mammals (either introduced or native) may visit the proposed release site. This is discussed in Chapter 2. There are beef cattle and sheep present at the Research Station on which the proposed site is located.

6.3 Relevant agricultural practices

62. The size, location and duration of the proposed limited and controlled release of the GM perennial ryegrass and tall fescue lines are outlined in Section 3.2 of this Chapter.

63. The applicant proposes to transport the planting material as vegetative tillers to the site from a PC2 facility.

64. The GM perennial ryegrass and tall fescue will initially be planted into the proposed field site in July-August 2008 and then removed to a PC2 glasshouse between November and March prior to flowering. Some of these plants will be used for crossing and the resulting seedlings or the original plants will be re-planted into the proposed field site in 2009 and removed to the PC2 glasshouse prior to flowering.

65. A 250 m border crop of *Triticale sp.* will be planted to surround the GM perennial ryegrass and tall fescue, as is commonly used for commercial seed production of grasses. This is expected to act as a physical barrier to limit pollen dispersal and the dense planting of *Triticale sp.* is expected to suppress the growth of weeds, including pasture grasses.

66. At the end of the season all plants will be removed from the soil and transported to a PC2 glasshouse (as detailed above). Any material remaining at the field site will be treated with a non-selective herbicide and allowed to decompose.

67. The applicant has stated that the field site will not be irrigated during the trial, as it will be planted in Spring, a time of regular and reliable rainfall in Hamilton. Prior to planting the trial the area will be fertilised, and broadleaf weeds will be controlled with manual weeding or herbicide application. Monocot weeds will be controlled manually. No pest control is anticipated during the trial.

6.4 Presence of related plants in the receiving environment

68. The GM perennial ryegrass and tall fescue lines proposed for release will be grown together at the field trial site. Perennial ryegrass and tall fescue are able to hybridise with each other under natural conditions (OGTR 2008).

69. Perennial ryegrass and tall fescue are also able to hybridise with other grass species present in Victoria including Italian ryegrass (*Lolium multiflorum* Lam.), annual ryegrass (*L. rigidum* Gaud.), rigid ryegrass (*L. loliaceae* Bory & Chaub.), hardy ryegrass (*L. remotum* Schrank), meadow fescue (*Lolium pratensis* (Huds.) Darbysh.), red fescue (*Festuca rubra* L.) and darnel (*L. temulentum* L.), although some of the hybrids are sterile (OGTR 2008). However, these grass species have not been recorded in the vicinity of the trial site (information supplied by applicant).

70. The applicant has indicated that most of the DPI research station will be sown to perennial ryegrass (*L. perenne*), tall fescue (*L. arundinaceum*) and Italian ryegrass (*L. multiflorum*). There may be some naturalised annual ryegrass (*L. rigidum*), although it is not common in the area.

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

71. All of the introduced genes for forage quality were isolated from perennial ryegrass or tall fescue. The isolated genes have not been modified by the applicant and have been reintroduced into the perennial ryegrass and tall fescue plants in their native form with one exception where a single base pair deletion has been introduced into a gene. Many of the genes are being introduced in a construct designed to prevent expression of the plants endogenous genes, so these will result in the absence of a gene product. Therefore, it is expected that humans, herbivores/omnivores and microorganisms routinely encounter the introduced genes and their gene products, or their homologues, through contact with plants and food derived from plants. This information forms the baseline data for assessing the risks from exposure to these proteins as a result of the trial of the GM perennial ryegrass and tall fescue lines.

72. One gene (GOI 4B) has been introduced in the native form and also with a single base pair deletion at the start (5' end) of the gene which will cause it to read as a frameshift so it does not produce a functional protein.

73. Fructans and lignins are both present in a wide variety of plants. Fructans are present in a number of foods and feedstuff eaten by people and animals with generally no ill-effects, although harmful effects have been observed in horses due to excess consumption (Chapter 1, Section 5.2.6). People and animals come into contact with lignin through consumption of various foods which, while not toxic, can have negative effects on feed quality (Chapter 1, Section 5.2.5).

74. The *hph* gene is derived from *E. coli*, which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997). As such, it is expected that humans, animals and microorganisms routinely encounter the encoded protein (see Chapter 1, Section 5.2.4).

Section 7 Australian and international approvals

7.1 Australian approvals of the GM perennial ryegrass and tall fescue lines

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

75. There has been no release of these GM perennial ryegrass and tall fescue lines or any other GM perennial ryegrass and tall fescue lines in Australia.

7.1.2 Approvals by other Australian government agencies

76. The Regulator is responsible for assessing risks to the health and safety of people or the environment associated with the use of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the Australian Quarantine and Inspection Service (AQIS). This is discussed further in Chapter 3.

7.2 International approvals

77. There has been no release of these GM perennial ryegrass and tall fescue lines internationally. However, there have been releases of other GM perennial ryegrass and tall fescue plants in both France and the USA. The traits which have been modified include altered lignin levels, fungal resistance, drought tolerance, increased salt tolerance and reduced pollen allergens⁹.

⁹ <http://www.isb.vt.edu/2002menu/regulatory_information.cfm>, <http://gmoinfo.jrc.it/gmp_browse.aspx> accessed 10 April 2008

Chapter 2 Risk assessment

Section 1 Introduction

78. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 4) considers risks from the proposed dealings with the GMOs that could result in harm to the health and safety of people or the environment posed by, or as a result of, gene technology. It takes into account information in the application, relevant previous approvals and current scientific knowledge.

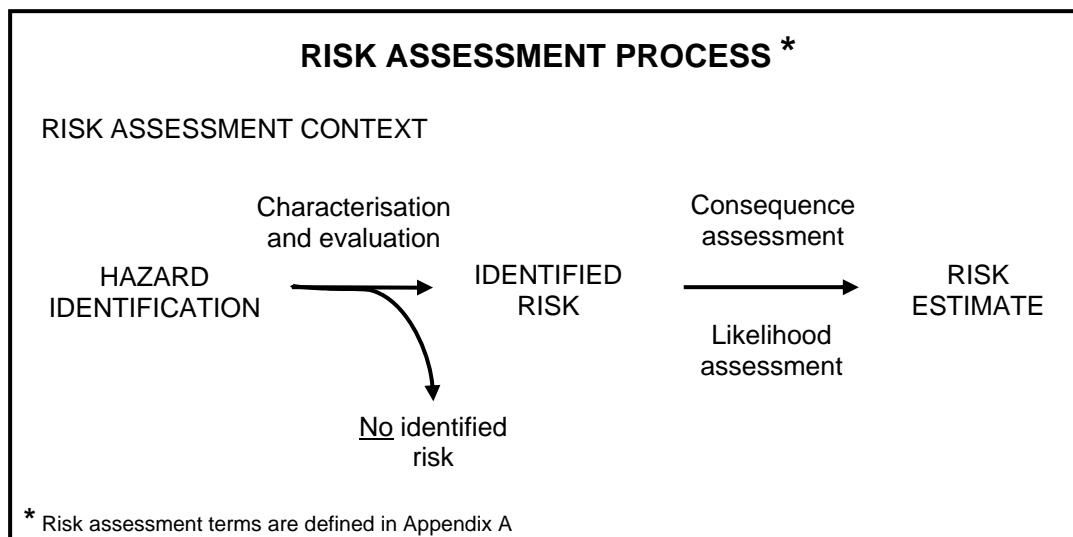


Figure 4 The risk assessment process.

79. Once the risk assessment context has been established (see Chapter 1) the next step is hazard identification to examine what harm could arise and how it could happen during a release of these GMOs into the environment.

80. It is important to note that the word 'hazard' is used in a technical rather than a colloquial sense in this document. The hazard is a source of *potential* harm. There is no implication that the hazard will *necessarily* lead to harm. A hazard can be an event, a substance or an organism (OGTR 2005).

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

82. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2005). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

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

Section 2 Hazard characterisation

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

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

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

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

87. The seven events that were characterised are discussed in detail later in this Section. They are summarised in Table 5 where events that share a number of common features are grouped together in broader hazard categories. None were considered to lead to an identified risk that required further assessment.

88. The GM perennial ryegrass and tall fescue lines contain a selectable marker gene; the *hph* gene encoding the HPT protein which confers tolerance to the antibiotic hygromycin.

89. The prevalence of the *hph* gene in the environment and the lack of evidence for toxicity or allergenicity of the HPT protein, to humans and animals are discussed in Chapter 1, Section 5.2.4. Therefore, the potential effects of the *hph* gene will not be further assessed for this application.

Table 5 Summary of events that may give rise to an adverse outcome through the expression of the introduced genes for altered fructan and lignin metabolism.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Exposure to GM plant material containing proteins encoded by the introduced genes or their end products.	Allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The encoded proteins and their end products are widespread in the environment and are unlikely to be toxic/allergenic to people or toxic to other organisms. The limited scale, short duration and other proposed limits and controls, minimise exposure of people and other organisms to products of the introduced genes.
Section 2.2 Spread and persistence of the GM perennial ryegrass and/or tall fescue lines in the environment	2. Expression of the introduced genes improving the survival of GM perennial ryegrass and/or tall fescue plants.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The limits and controls proposed for the release would minimise spread and persistence. Survival of GM perennial ryegrass and tall fescue would still be limited by environmental factors that also limit non-GM plants.
	3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including, animals and extreme weather conditions.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Perennial ryegrass and tall fescue will not be permitted to flower or set seed. Rooting of cuttings has only been seen under experimental conditions. The other proposed limits and controls would also minimise dispersal.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced genes or regulatory sequences in other perennial ryegrass and tall fescue plants or in other sexually compatible plants	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Tall fescue and perennial ryegrass pollen does not travel long distances. Natural barriers exist for pollination between species. The applicant proposed a number of controls, including not allowing the GM plants to flower in the field and ensuring that the trial site and border is free of sexually compatible species which would limit the potential for vertical gene flow. Events 1 – 3 did not identify any risks to people or the environment associated with expression of the introduced genes.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence and expression of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The introduced genes or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms. Events 1 – 3 did not identify any risks to people or the environment associated with expression of the introduced genes.
Section 2.5 Unintended changes in biochemistry, physiology or ecology	6. Changes to biochemistry, physiology or ecology of the GM perennial ryegrass and tall fescue lines resulting from expression, or random insertion, of the introduced genes.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Unintended, adverse effects, if any, would be minimised by the proposed limits and controls. Unexpected alterations are likely to be detected and eliminated during early growth in the glasshouse.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.6 Unauthorised activities	7. Use of the GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	<ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

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

90. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000).

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

92. A range of organisms may be exposed directly or indirectly to the proteins encoded by the introduced genes for altered fructan and lignin metabolism. Workers cultivating the perennial ryegrass and tall fescue would be exposed to all plant parts. Other organisms may be exposed directly to the proteins through biotic interactions with GM perennial ryegrass and tall fescue plants (vertebrates, insects, symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include organisms that feed on organisms that feed on GM perennial ryegrass and tall fescue plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

Event 1: Exposure to GM plant materials containing proteins encoded by the introduced genes, or their end products

93. Expression of the introduced genes for altered fructan and lignin metabolism could potentially result in the production of novel toxic or allergenic compounds in the GM perennial ryegrass and tall fescue lines, or alter the expression of endogenous perennial ryegrass and tall fescue proteins. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these humans or other organisms.

94. Non-GM perennial ryegrass and tall fescue is not known to be toxic to humans or other organisms. Inhalation of grass pollen is a major cause of hayfever and non-GM perennial ryegrass and tall fescue pollen can produce allergic responses in susceptible individuals on inhalation (reviewed in OGTR 2008). It is not known if any of the introduced genes for altered fructan and lignin metabolism are involved in the expression of proteins in the pollen allergen production pathway.

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

96. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. The short duration and small size of the trial will limit the potential for exposure of humans and animals to the GM plant tissues. Contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff associated with the field trial. Although preliminary glasshouse studies suggest that some plants with reduced lignin levels may show early flowering, the applicant proposes to remove all GM plants from the field prior to flowering, thereby limiting the potential of exposure to pollen in the field that could result from flowering. Only people authorised to work with the GMOs would be exposed to pollen in the PC2 glasshouse.

97. The proposed trial site will be surrounded by a 1 m high fence with access to the trial site being via a locked gate and access to the research farm is also restricted. This will reduce inadvertent access by humans and prevent grazing livestock entering the site, which limits exposure of the public and grazing livestock to the GM plant material. Livestock would not be intentionally exposed as the GM plant material will not be used as feed. There is limited potential for exposure of the public to plant materials via ingestion, skin contact or inhalation as no plant material will be used as animal feed or plant products. The short duration (2008-2010) and small size (800 m² per year) of the proposed trial would also limit the potential for exposure to the GM plant material.

98. **Conclusion:** The potential for allergic reactions in people, or toxicity in people and other organisms as a result of exposure to GM plant materials containing proteins encoded by the introduced genes, or their end products, is **not an identified risk** and will not be assessed further.

2.2 Spread and persistence of the GM perennial ryegrass and tall fescue in the environment

99. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM perennial ryegrass and tall fescue plants in particular, is given in *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum Schreb. (tall fescue)* (OGTR 2008). In summary, perennial ryegrass and tall fescue share some characteristics with known weeds, such as wind-pollination, sexually and asexual reproduction, rapid adaptation to their environment, production of large amounts of seed, and spread through wind, water, animals and humans. In addition, both species have been identified as weeds of natural and agricultural systems although they are not classed as weeds of national significance (Department of the Environment and Heritage 2004). However, both species lack other characteristics that are common to many weeds, such as the ability to produce a persisting seed bank, dormant seeds, rapid growth to flowering, continuous seed production, and long-distance seed dispersal (OGTR 2008).

100. Scenarios that could lead to increased spread and persistence of the GM perennial ryegrass and tall fescue lines include expression of the introduced genes conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These events could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins.

Event 2: Expression of the introduced genes improving the survival of the GM perennial ryegrass and/or tall fescue plants

101. If the GM perennial ryegrass and tall fescue lines were to establish or persist in the environment they could increase the exposure of humans and other organisms to the GM plant

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

102. If the expression of the introduced genes for altered fructan and lignin metabolism were to provide the GM perennial ryegrass and tall fescue plants with a significant selective advantage over non-GM perennial ryegrass and tall fescue plants and they were able to establish and persist in favourable non-agricultural environments this may give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. Similarly, the GM perennial ryegrass and tall fescue plants could adversely affect agricultural environments if they exhibited a greater ability to establish and persist than non-GM perennial ryegrass and tall fescue.

103. The impact of the genetic modifications on survival of the GM perennial ryegrass and tall fescue lines is uncharacterised under field conditions. However, a number of predictions can be made based on knowledge of the gene functions and their predicted effect when expressed in the modified plants. Predictions can also be made based on the observed phenotypes of the GM perennial ryegrass and tall fescue lines grown under glasshouse conditions and comparing them to the phenotype of the non-GM perennial ryegrass and tall fescue plants. These predictions are summarised in Chapter 1, Section 5.5.2.

104. The GM plants with altered lignin levels may have altered susceptibility to pests and diseases; altered fertility, flower morphology or flowering times; altered growth rates; or altered seed survival. Altered fructan content has been seen to affect resistance to disease, drought survival and cold tolerance (see Chapter 1, Section 5.2.5). These may impact on the spread and persistence of the GM perennial ryegrass and tall fescue plants.

105. Although earlier flowering has been observed in several of the GM lines proposed for release which have reduced lignin content, the applicant has proposed a number of conditions to limit and control the release, including weekly monitoring for flowering and the removal of plants from the trial site prior to flowering.

106. The survival of the GM perennial ryegrass and tall fescue plants would still be limited by high temperatures, low intrinsic competitive ability, nutrient availability and other environmental factors that normally limit the spread and persistence of perennial ryegrass and tall fescue plants in Australia (OGTR 2008). Furthermore, in the unlikely instance that an advantage in one ability is conferred, the GM plants will most likely be less fit as compared to other commercially available perennial ryegrass and tall fescue varieties because of the potential metabolic/physiological burdens (eg as discussed in Pretty 2001). For example, the perennial ryegrass and tall fescue may have stunted growth, produce less seeds, and have a decreased ability to tolerate competition from other plants. Therefore, the expression of the introduced genes for altered fructan and lignin metabolism is not expected to provide the GM perennial ryegrass and tall fescue plants with a significant selective advantage over non-GM perennial ryegrass and tall fescue plants.

107. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM perennial ryegrass and tall fescue lines proposed for release. The release would be of limited size and short duration and the applicant proposes a number of control measures, including removal of plants prior to flowering, which would prevent the occurrence of seed in the field; destruction of all plant materials not required for further analysis and post harvest monitoring of the proposed site with destruction of any volunteers for twelve months.

108. The purpose of the proposed release is to conduct proof of concept experiments with the GM perennial ryegrass and tall fescue lines to assess growth and yield characteristics. Thus, any characteristics that may impact on the survivability of the GM plants including tolerance to biotic or abiotic stresses will be closely monitored during the proposed trial.

109. **Conclusion:** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced genes for altered fructan and lignin metabolism improving the survival of the GM perennial ryegrass and tall fescue lines is **not an identified risk** and will not be assessed further.

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

110. If the GM perennial ryegrass and tall fescue lines were to be dispersed from the release site they could increase the exposure of humans and other organisms to the GM plant material and/or establish and persist in the environment. The effects of contact, inhalation or ingestion of the GM perennial ryegrass and tall fescue lines have been assessed in Event 1 and were not an identified risk. The introduced genes improving survival of the GM perennial ryegrass and tall fescue lines in the environment was assessed in Event 2 and was also found not to be an identified risk. Therefore the dispersal of reproductive GM plant material is not expected adversely affect the health of humans or other animals; or to increase the survival of the GM perennial ryegrass and tall fescue lines compared to non-GM perennial ryegrass and tall fescue.

111. Grass seeds may be dispersed by grazing animals since they are capable of germination after passing through the digestive systems of cattle and sheep (Yamada & Kawaguchi 1972; Yamada et al. 1972; Johns & Greenup 1976; Janzen 1984; Chambers & MacMahon 1994; Hulme 1994; Fischer et al. 1996)} or horses (Campbell & Gibson 2001), and may be transported in the wool of grazing sheep (Fischer et al. 1996). Seed dispersal in irrigation water has been observed for *Lolium spp.* in Chile, with germinable seeds recovered from the irrigation water (Tosso et al. 1986). Human activity is also a likely source of seed dispersal with perennial ryegrass seed transported on cars (Hodkinson & Thompson 1997).

112. Seed production, dispersal and digestibility characteristics could be altered in the GM perennial ryegrass and tall fescue lines compared to non-GM parental perennial ryegrass and tall fescue lines. Seed persistence may be increased due to altered lignin levels (Fellows & Roeth 1992) and the palatability of the GM perennial ryegrass and tall fescue plants to animals may be increased due to changed fructan and lignin content.

113. However, the applicant intends to remove the GM perennial ryegrass and tall fescue plants from the field site prior to flowering and therefore seed set is unlikely to occur in the field. Both grass species lack physiological dormancy (Hill & Pearson 1985; Lodge 2004) and grasses form transient seed banks, with only approximately 10% of the seed remaining after 14 months and none after two years (Lodge 2004).

114. Perennial ryegrass and tall fescue are both capable of asexual reproduction. Perennial ryegrass is classed as a bunchgrass (Oregon State University 2000; Thorogood 2003), although it can produce stolons and short rhizomes. The propensity for stoloniferous development is linked to cultivar genotype as well as to environmental factors such as soil type, degree of shading and grazing pressure (Donaghy 2001). Aerial tillers have been found to spread up to 15.5 cm and to occur at a density of up to 57.8 tillers/m² (Sawada 1991). Tall fescue is classed either as a sod-forming grass with short rhizomes (Oregon State University 2000) or as a tufted bunchgrass that may or may not have rhizomes (Meyer & Watkins 2003). Generally in pasture cultivars, most rhizome growth occurs after eighteen months, but their

activity can be high in flood irrigated environments (Milne 2005) and cultivars exist with an extensive rhizomatous habit. Both species can form roots from stem cuttings under experimental conditions (Uchida & Arasea 2005). However, there is no evidence that broken pieces of plant can establish in the field.

115. Altering lignin or fructan may alter the growth habit of the GM perennial ryegrass and tall fescue and could potentially alter their propensity to form rhizomes or stolons. However, the applicant has proposed a 2 m wide zone around the field site which will be kept free of vegetation that will inhibit the identification of volunteers. Monitoring and removal of volunteers is also proposed to minimise persistence.

116. Humans or animals and extremes of weather could disperse stolons (and seed if present). However, control measures have been proposed by the applicant to minimise dispersal. The GMOs will be transported to and from the field as plants and they will be removed from the field before flowering so no seed will be present. The proposed release site will be located 50 m away from natural water ways to minimise dispersal in the event of flooding. Beef cattle and sheep are present on the DPI Victoria farm. However, the proposed release site will be surrounded by a 1 m fence with access through a locked gate limiting the possibility of seed or stolon dispersal by any grazing livestock or inadvertent dispersal by unauthorised people. Dispersal by authorised people entering the proposed trial site would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing. Finally all GM plant material will be transported in accordance with the OGTR transport guidelines which will minimise the opportunity to disperse the GM material.

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

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

118. Vertical gene flow is the transfer of genetic information from an individual organism to its progeny by conventional heredity mechanisms, both asexual and sexual. In flowering plants, pollen dispersal is the main mode of gene flow (Waines & Hedge 2003). For GM crops, vertical gene flow could therefore occur via successful crosspollination between the crop and neighbouring crops, related weeds or native plants (Glover 2002).

119. Baseline information on vertical gene transfer associated with non-GM perennial ryegrass and tall fescue plants can be found in *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum Schreb. (tall fescue)* (OGTR 2008). In summary, perennial ryegrass and tall fescue plants are wind-pollinated, primarily outcrossing with self-incompatibility mechanisms and can form natural hybrids with a number of other species.

Event 4. Expression of the introduced genes and regulatory sequences in other perennial ryegrass and tall fescue plants or in other sexually compatible plants

120. Transfer and expression of the introduced genes for altered fructan and lignin metabolism in other perennial ryegrass and tall fescue or sexually compatible plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

121. However, as discussed in Event 2, the survival of the GM perennial ryegrass and tall fescue plants proposed for release would be limited by factors such as high temperatures, low intrinsic competitive ability, nutrient availability and other domestication and environmental factors that normally limit the spread and persistence of perennial ryegrass and tall fescue plants in Australia. Therefore, similar to the GM perennial ryegrass and tall fescue plants, expression of the introduced genes in other perennial ryegrass and tall fescue plants would also result in plants limited by these factors. The expression of the introduced genes in other sexually compatible species is also unlikely to give these plants a significant selective advantage. The conditions that limit the spread and persistence of any hybrids between non-GM perennial ryegrass and tall fescue and other sexually compatible plants would be expected to limit the spread and persistence of any hybrids between the GM perennial ryegrass and tall fescue and other sexually compatible species.

122. As discussed in Event 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM perennial ryegrass and tall fescue plants by the introduced genes or regulatory sequences. This will be the same if the introduced genes are expressed in other perennial ryegrass and tall fescue plants. Similarly, if the introduced genes are expressed in other sexually compatible species, allergenicity and toxicity are not expected to be altered.

123. All of the introduced regulatory sequences are expected to operate in the same manner as regulatory elements endogenous to the perennial ryegrass and tall fescue plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. However, it is highly unlikely that a regulatory element would be transferred since the GM plants will not be permitted to flower and, even if it did occur, the chance of an adverse effect to people or the environment is highly unlikely.

124. Both perennial ryegrass and tall fescue are wind-pollinated, predominately outcrossing species, with self-incompatibility mechanisms in place which limit self-pollination (Cornish et al. 1979; Fearon et al. 1983). Pollen viability varies from 30 mins to 2 ½ hours, depending on weather conditions (Wang et al. 2004). Gene flow rates in both perennial ryegrass and tall fescue have been studied on an experimental scale and for commercial seed standards (reviewed in OGTR 2008). Low levels of gene flow from perennial ryegrass have been detected as far as 144 m away (<2%) (Cunliffe et al. 2004) and at 150 m for tall fescue (0.96%). No tall fescue pollen flow occurred at 200 m (Wang et al. 2004). Commercial seed production standards require an isolation distance of 274 m (Wang & Ge 2006) in the USA, and 200 m (if the area is less than 2 ha) in South Australia (Smith & Baxter 2002). (For further discussion see Chapter 3).

125. Therefore, if the GM plants were to flower, the introduced genes and regulatory sequences could be transferred to sexually compatible plants close to the trial site; however, long distance gene transfer is unlikely.

126. Stacking of the introduced genes could occur, either to produce individual plants with multiple introduced lignin or fructan genes or an individual plant containing introduced genes for both lignin and fructan metabolism. This could give rise to synergistic, additive or antagonistic effects as a result of the expression of multiple gene constructs or the two pathways in the same plant. This could lead to the redirecting of metabolites, possibly reducing the overall fitness of the plant.

127. As discussed in Chapter 1, Section 6.2, the applicant has indicated the presence of commercial plantings of perennial ryegrass and tall fescue at or near the proposed release site. Also, as identified in *The Biology of Lolium multiflorum Lam. (Italian ryegrass)*, *Lolium perenne L. (perennial ryegrass)* and *Lolium arundinaceum Schreb. (tall fescue)*, (OGTR

2008), there are species outside the *Lolium* genus (for example, *Festuca* spp.) that are sexually compatible with perennial ryegrass and tall fescue and known to form hybrids under natural conditions although some of these hybrids are sterile.

128. A number of natural barriers exist that preclude hybrid formation between perennial ryegrass and tall fescue and their relatives. These natural barriers may include: asynchronous flowering, gametic, zygotic or endosperm incompatibility, and reduced hybrid fitness or hybrid sterility. The barriers arise mainly from the fact that the chromosomes of the different genomes may not pair during gamete formation in the F1 hybrids or may result in developmental instability, which reduces the hybrids ability to survive.

129. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for pollen flow and gene transfer to sexually compatible plants. In particular, the applicant proposes to monitor weekly and remove the GM perennial ryegrass and tall fescue plants from the field site prior to flowering. The applicant also proposes to surround the site with a border of *Triticale* sp. which, they suggest, would act as a physical barrier to pollen flow in the event of flowering, ensure that the trial site and border rows are free from pasture grasses and plant at a time when other grasses in the area are not flowering. Furthermore, the applicant proposes to perform post harvest monitoring of the site for twelve months and destroy any volunteer plants found.

130. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genes and regulatory sequences in other perennial ryegrass and tall fescue plants or other sexually compatible plant species as a result of gene transfer is **not an identified risk** and will not be assessed further.

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

131. Horizontal gene transfer is the movement of genetic information (DNA) between sexually unrelated organisms (Thomson 2000). In the context of genetic modification, a major concern has been whether DNA introduced into crops could transfer into bacteria in the soil or into the cells of organisms that may eat the crops. Horizontal gene transfer has been considered in previous RARMPs (including in detail in DIR 057/2004, available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir057-2004>) or by contacting the OGTR). These assessments have concluded that horizontal gene transfer from plants to sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. There are no more recent reviews that alter this conclusion.

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

132. The probability of transferring introduced genes contained in the GM perennial ryegrass and tall fescue plants is no greater than that of transferring any of the native genes. Non-GM perennial ryegrass and tall fescue contain homologues of all of the introduced perennial ryegrass and tall fescue genes, and the marker gene, *hph*, and regulatory sequences are also widespread in the environment. Therefore, these genes and regulatory sequences are already available for transfer via demonstrated natural mechanisms (Chapter 1, Section 6.5).

133. Reports of horizontal gene transfer from plants to bacteria occurring during laboratory experiments have relied not only on the use of highly similar sequences to allow homologous recombination to occur, but also on conditions designed to enhance the selective advantage of gene transfer events (Mercer et al. 1999; Gebhard & Smalla 1998; Nielsen et al. 2000;

Nielsen 1998; De Vries et al. 2001). This suggests that the likelihood of natural transfer is remote.

134. A key consideration in the risk assessment process should be the safety of the protein product(s) resulting from the expression of the introduced gene(s) rather than horizontal gene transfer *per se* (Thomson 2000). If the protein products are not associated with any risk then even in the unlikely event of horizontal transfer occurring, it should not pose any risk to humans, animals or the environment. Events 1–4 associated with the expression of the introduced genes or end products did not represent an identified risk.

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

2.5 Unintended changes in biochemistry, physiology or ecology

136. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy¹⁰ (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such pleiotropic effects may include:

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

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

Event 6. Changes to biochemistry, physiology or ecology of the GM perennial ryegrass and tall fescue lines resulting from expression or random insertion of the introduced genes

138. Some slightly altered physiology has been observed in the GM perennial ryegrass and tall fescue lines under glasshouse conditions. This is outlined in Chapter 1, Section 5.5.2 and discussed in Event 2. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced genes, have already been discussed in Events 1 to 3, and were not considered identified risks.

139. Various biochemical pathways of the GM perennial ryegrass and tall fescue plants could be changed by the altered expression of genes involved in the fundamental processes of lignin and fructan metabolism, resulting in the production of novel compounds or higher levels of endogenous toxins, allergens or anti-nutritional compounds. Non-GM perennial ryegrass and

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

tall fescue, pollen can be allergenic to people who suffer from hayfever, and toxicity to other plants via allelopathy has been observed. For further discussion regarding the toxicity and allergenicity of non-GM perennial ryegrass and tall fescue see *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum Schreb. (tall fescue)* (OGTR 2008).

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

141. Expression or silencing of genes in the lignin biosynthesis pathway may lead to changes in growth and development (Chapter 1, Section 5.2.5). The GM perennial ryegrass and tall fescue lines will be monitored for altered phenotypes by the applicant.

142. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered capacity to deal with environmental stress, production of novel substances, and changes to levels of endogenous substances. However, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

143. The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the scale and duration of the trial would limit the potential for adverse effects. The proposed trial site will be surrounded by a 1 m high fence with access to the trial site being via locked gates. This will reduce inadvertent access by humans and prevent grazing livestock entering the site, which limits exposure of the public and grazing livestock to the GM plant material. Livestock would not be intentionally exposed as the GM plant material will not be used as feed.

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

2.6 Unauthorised activities

Event 7. Use of GMOs outside the proposed licence conditions (non-compliance)

145. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM perennial ryegrass and tall fescue lines outside of the proposed release areas. The adverse outcomes that this event could cause are discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

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

Section 3 Risk estimate process and assessment of significant risk

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

148. Seven events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the

introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

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

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

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

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM perennial ryegrass and tall fescue lines into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment¹¹.

Section 4 Uncertainty

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

152. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹². For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily treating an identified risk.

¹¹ As none of the proposed dealings were considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

¹² A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

153. For DIR 082/2007 which involves proof of concept research, uncertainty exists in relation to the characterisation of:

- Event 1, regarding potential increases in allergenicity or toxicity through contact with plant material containing proteins encoded by the introduced genes or their end products
- Event 2, associated with a potential for increased survival of the GMOs
- Event 3, regarding potentially altered seed persistence or plant architecture
- Event 4, regarding potential effects of transfer and expression of introduced genes in sexually compatible species
- Event 6, regarding the consequences of the end products of the introduced genes being involved in a number of biochemical pathways.

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

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

Chapter 3 Risk management

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

Section 1 Background

155. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment. All licences are required to be subject to three conditions prescribed in the Act.

156. Section 63 requires that each licence holder inform relevant people of their obligations under the licence. Other mandatory statutory conditions contemplate the Regulator maintaining oversight of licensed dealings. For example, section 64 requires the licence holder to provide access to premises to OGTR monitors, and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

157. It is a further requirement that the licence be subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Responsibilities of other Australian regulators

158. Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Health and Medical Research Council (NHMRC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹³.

159. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. *The Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

160. No other approvals are required in the case of this application.

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

Section 3 Risk treatment measures for identified risks

161. The risk assessment of events listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed trial of GM perennial ryegrass and tall fescue. The *Risk Analysis Framework* (OGTR 2007), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

162. These events were considered in the context of the scale of the proposed release (a maximum total area of 800 m² from 2008 –2010 on one site in the shire of Southern Grampians, Victoria, the containment measures (Chapter 1, Section 3), and the receiving environment (Chapter 1, Section 6).

Section 4 General risk management

163. Licence conditions have been imposed to control the dissemination and persistence of the GMOs and their genetic material in the environment and limit the release to the size, location and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are detailed in the licence and summarised in Chapter 3, Section 4.1.2.

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by DPI Victoria

164. Chapter 1, Section 3.2 and Chapter 1, Section 3.3 provide details of the limits and controls proposed by DPI Victoria in their application, and discussed in the characterisation of events in Chapter 2. The appropriateness of these limits and controls are considered further below.

165. The release will be confined to one site, which occurs within the Department of Primary Industries research farm. These stations are staffed by personnel who receive appropriate training in practices relevant to the handling and disposal of GMOs. Additionally, the applicant does not intend to use any of the GM plant material as human food or animal feed. Furthermore, the duration of the proposed release will be limited to two years. These measures will minimise the potential exposure of humans and vertebrates to the GMOs (Event 1) and the potential for the GM perennial ryegrass and tall fescue lines to persist or to establish outside the proposed release site (Event 3).

166. The trial site will be located more than 50 m from the nearest waterway which will minimise the chance of plant material being washed away from the site (Event 3).

167. The applicants proposal to limit gene flow from the GM perennial ryegrass and tall fescue (Event 4) includes removing the plants from the field site prior to flowering, surrounding the site with a border of *Triticale sp.*, which they suggest would act as a physical barrier to pollen flow in the event of flowering, ensuring that the trial site and border rows are free from pasture grasses and growing the GMOs in the field at a time when other grasses in the area are not flowering.

168. The removal of plants prior to flowering should be a very effective measure to prevent gene flow from the GM perennial ryegrass and tall fescue lines. The applicant has proposed weekly monitoring of the trial site to look for indications of flowering. Generally, it takes four weeks from the initiation of a flowering spike to head formation (information supplied by applicant), and a further two weeks until anthesis (Singh et al. 1976). Therefore, weekly monitoring would be adequate to detect flowering before it occurs. However, in the highly

unlikely event that unanticipated flowering occurs in the field, the potential for gene flow has been considered.

169. Differences in pollen flow have been observed between different pollen flow studies in perennial ryegrass and tall fescue. A number of variables, particularly pollen source size, climatic conditions and the difficulty of detecting rare events, could influence the accuracy and reproducibility of these measurements. Low rates of outcrossing occur up to 150 m for both grass species (OECD 2008).

170. Commercial grass seed production standards require an isolation distance of 274 m (Wang & Ge 2006) in the USA, and 200 m for tall fescue (if the area is less than 2 ha) in South Australia (Smith & Baxter 2002).

171. A field trial release of GM tall fescue in USA required a 400 m mown area surrounding the GM tall fescue plants (USDA-APHIS 2006). In France, trials with GM tall fescue plants are required to be cut to prevent flowering and seed set (Directorate General for the Environment & European Commission 2003).

172. For both foundation and registered seed in the USA, the acceptable level of off-types or other cultivars of the same species are 0.1% with significantly less for contamination by other species (Montana Seed Growers Association 2008). The OECD rules relating to the production of basic and certified seed from grasses require an isolation distance of 200 m (if the area is less than 2 ha) for seed multiplication or 100 m if the seed is to be used for fodder (OECD 2008).

173. On the basis of the scientific literature on gene flow, international containment measures for GM perennial ryegrass and tall fescue trials, and the rules for producing seed, gene flow over long distances under Australian conditions is highly unlikely. The *Triticale sp.* border row proposed by the applicant was suggested as a physical barrier. Generally, the use of physical barrier plants alone would not effectively prevent pollen dispersal by wind. However, the *Triticale sp.* will be a dense stand of plants greater than 1 m in height (Singh et al. 1976) which should reduce wind flow and thus may reduce pollen movement. Furthermore, the management of the border rows prior to planting, and the planting of a dense tall crop is likely to suppress weeds including sexually compatible grass species. In the highly unlikely event that any of the GM perennial ryegrass and tall fescue plants flower in the field then the planting of this dense *Triticale sp.* border would act to reduce the number of sexually compatible plants within pollination distance and reduce pollen movement. The applicant originally proposed a 300m *Triticale sp.* border however, during the consultation period they reduced the proposed border width to at least 250 m, due to the trapezoidal shape of the field which they propose to use for the trial. The reduction of the *Triticale sp.* border to 250 m should not affect the efficacy of the border. Therefore, a 250 m *Triticale sp.* border row is included as a licence condition (Event 4).

174. A field trial release of GM tall fescue in USA requires a 4 m fallow border to detect vegetative reproduction (USDA-APHIS 2006). Perennial ryegrass and tall fescue may form short rhizomes or stolons as a means of vegetative spread, although this has been observed over a maximum of 15.5 cm (Sawada 1991). When considering the scientific literature and international containment measures for GM perennial ryegrass and tall fescue trials, a border of at least 2 m, kept free of vegetation that will inhibit the identification of volunteers, surrounding the GM perennial ryegrass and tall fescue is suggested to improve the detection of stolons or rhizomes from the GM grasses which would be hard to detect in the *Triticale sp.* border row. This is important if stolon/rhizome development is enhanced in the GM grasses and it will limit spread of the GM perennial ryegrass and tall fescue plants (Event 3).

175. The applicant proposed to surround the trial with a 1 m high fence with a locked gate. These measures will aid in excluding grazing livestock which may have access to the GMOs. This will limit the potential exposure of vertebrates to the GMOs (Event 1) and the potential dispersal of the GMOs (Event 3).

176. The applicant proposed a number of conditions to minimise the persistence of any GM perennial ryegrass and tall fescue plants and seeds in the seed bank at the release site after harvest of the trial (Event 2). These conditions include removing the plants from the field site prior to flowering and destroying all plant materials remaining at the field site by spraying with herbicide. The applicant also proposed to monitor the release site for 12 months after harvest and to destroy all volunteers.

177. Removal of the plants prior to flowering will effectively reduce the chance of seed production. In the unlikely event that flowering and seed set occurs, monitoring will also ensure that no viable seed bank remains as the grass seeds do not persist for more than one season (OECD 2008). Monitoring for 12 months will identify any remaining plants or stoloniferous growth remaining after herbicide spray. The site will need to be clear of volunteers in the final six month periods of monitoring before an application that inspection conditions no longer apply can be made to the Regulator. These measures will minimise the persistence of the GMOs in the environment (Event 2).

178. The applicant has stated that any plant material taken off-site for experimental analysis will be transported according to the OGTR *Guidelines for the transport of GMOs* (<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/certifications-1>>) and will be destroyed by autoclaving immediately after analysis. These are standard protocols for the handling of GMOs to minimize exposure of the GMO to human and other organisms (Event 1), dispersal into the environment (Event 3), and gene flow/transfer (Events 4 & 5).

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

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

- conduct the release on an area of up to 800 m² per year at one site in the shire of Southern Grampians, Victoria, between August 2008 and July 2010
- establish a 2 m monitoring zone around the trial site that is maintained in a manner that enables the identification of stoloniferous growth
- remove the GM plants from the field before flowering
- surrounding the 800 m² trial site with a 250 m border of *Triticale sp*
- enclose the trial site with a 1 m high fence with a lockable gate
- locate the trial site at least 50 m away from natural waterways
- not permit any materials from the release to be used in human food or animal feed
- following harvest, clean the site, monitoring zone and equipment used on the site
- monitor the site for at least 12 months and destroy any perennial ryegrass and tall fescue plants that may grow until no volunteers are detected for a continuous 6 month period
- transport plant materials in accordance with OGTR transportation guidelines
- at the end of the trial, destroy all plant materials not required for further analysis.

4.1.2 Measures to control other activities associated with the trial

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

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

4.2 Other risk management considerations

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

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

4.2.1 Applicant suitability

183. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act matters that the Regulator must take into account include:

- any relevant convictions of the applicant (both individuals and the body corporate)
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the applicant's history of compliance with previous approved dealings
- the capacity of the applicant to meet the conditions of the licence.

184. On the basis of information submitted by the applicant and records held by the OGTR, the Acting Regulator considers DPI Victoria suitable to hold a licence.

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

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

4.2.2 Compliance and contingency plans

187. Prior to planting the GM perennial ryegrass and tall fescue lines, DPI Victoria is required to submit a plan detailing how it intends to ensure compliance with the licence conditions and to document that compliance. This plan would be required before the planting of the GM perennial ryegrass and tall fescue lines could occur.

188. DPI Victoria is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan would detail measures to be undertaken in the event of any unintended presence of the GM perennial ryegrass and tall fescue lines outside of the permitted areas.

189. DPI Victoria is also required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This detection method is required within 30 days of the issue date of the licence.

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

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

4.2.4 Reporting structures

191. The licence obliges the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the trial
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

192. The licence holder is also be obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

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

- expected and actual dates of planting
- expected and actual dates of commencement of flowering
- expected and actual dates of final destroying and cleaning at the end of the trial.

4.2.5 Monitoring for Compliance

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

195. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

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

Section 5 Issues to be addressed for future releases

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

- characterisation of the introduced genetic material in the plants, including genotypic stability
- additional data on the potential toxicity of plant materials from the GM perennial ryegrass and tall fescue lines
- additional data on the potential allergenicity of pollen from the GM perennial ryegrass and tall fescue
- characteristics indicative of weediness including measurement of altered sexual and asexual reproductive capacity including seed persistence and plant establishment; altered growth rates, tolerance to drought, cold and other environmental stresses; and disease and pest susceptibility
- information on characteristics indicative of weediness which may be conferred by the GM traits if gene transfer occurred to sexually compatible species.

Section 6 Conclusions of the RARMP

198. The risk assessment concludes that this proposed limited and controlled release of up to 500 GM perennial ryegrass and tall fescue lines on an area of 800 m² per year from 2008 - 2010 in the Victorian shire of Southern Grampians poses negligible risks to the health and safety of people or the environment as a result of gene technology.

199. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. If a licence were to be issued, conditions are proposed to limit the dissemination and persistence of the GMOs and their genetic material in the environment and to limit the release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

References

- Amiard, V., Morvan-Bertrand, A., Billard, J.P., Huault, C., Keller, F., Prud'homme, M.P. (2003). Fructans, But Not the Sucrosyl-Galactosides, Raffinose and Loliose, Are Affected by Drought Stress in Perennial Ryegrass. *Plant Physiology* **132**: 2218-2229.
- Arts, J., Mommers, C., de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. *Critical review in Toxicology* **36**: 219-251.
- Bancal, P., Gaudillère, J.P. (1989). Rate of accumulation of fructan oligomers in wheat seedlings (*Triticum aestivum* L.) during the early stages of chilling treatment. *New Phytologist* **112**: 459-463.
- Bianchi, M.W., Damerval, C., Vartanian, N. (2002). Identification of proteins regulated by cross-talk between drought and hormone pathways in *Arabidopsis* wild-type and auxin-insensitive mutants, *axr1* and *axr2*. *Functional Plant Biology* **29**: 55-61.
- Blair, G. (1997). Matching pasture to the Australian environment. Chapter 5. In: JV Lovett, JM Scott, eds. *Pasture Production and Management*, Edition 1. Inkata Press, Victoria. pp 88-109.
- Blattner, F.R., Plunkett, G., Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B., Shao, Y. (1997). The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453-1462.
- Boerjan, W., Ralph, J., Baucher, M. (2003). Lignin biochemistry. *Annual Review of Plant Biology* **54**: 519.
- Boudet, A.M. (2000). Lignins and lignification: Selected issues. *Plant Physiology and Biochemistry* **38**: 81-96.
- Bradford, K.J., van Deynze, A., Gutterson, N., Parrott, W., Strauss, S.H. (2005). Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. *Nature Biotechnology* **23**: 439-444.
- Bruening, G. (1998). Plant gene silencing regularized. *Proceeding of the National Academic of Science, USA* **95**: 13349-13351.
- Cairns, A.J. (2003). Fructan biosynthesis in transgenic plants. *Journal of Experimental Botany* **54**: 549-567.
- Callow, M.N., Lowe, K.F., Bowdler, T.M., Lowe, S.A., Gobius, N.R. (2003). Dry matter yield, forage quality and persistence of tall fescue (*Festuca arundinacea*) cultivars compared with perennial ryegrass (*Lolium perenne*) in a subtropical environment. *Australian Journal of Experimental Agriculture* **43**: 1093-1099.
- Campbell, J.E., Gibson, D.J. (2001). The effect of seeds of exotic species transported via horse dung on vegetation along trail corridors. *Plant Ecology* **157**: 23-35.
- Canturf (2006). Choosing your lawn.
<http://www.canturf.com.au/lawn/choose.html#canberrablend> .

- Casler, M.D., Vogel, K.P. (1999). Accomplishments and Impact from Breeding for Increased Forage Nutritional Value. *Crop Science* **39**: 12-20.
- Chambers, J.C., MacMahon, J.A. (1994). A Day in the Life of a Seed: Movements and Fates of Seeds and Their Implications for Natural and Managed Systems. *Annual Review of Ecology and Systematics* **25**: 263-292.
- Clay, K. (1990). Fungal Endophytes of Grasses. *Annual Review of Ecology and Systematics* **21**: 275-297.
- Clay, K., Schardl, C. (2002). Evolutionary Origins and Ecological Consequences of Endophyte Symbiosis with Grasses. *The American Naturalist* **160**: 99-127.
- Cornish, M.A., Hayward, M.D., Lawrence, M.J. (1979). Self-incompatibility in ryegrass. I. Genetic control in diploid *Lolium perenne* L. *Heredity* **43**: 95-106.
- Costa, P., Bahrman, N., Frigerio, J.M., Kremer, A., Plomion, C. (1998). Water-deficit-responsive proteins in maritime pine. *Plant Molecular Biology* **38**: 587-596.
- Crafts-Brandner, S.J. (2005). Fructans and freezing tolerance. *New Phytologist* **166**: 708-709.
- Cunliffe, K.V., Vecchies, A.C., Jones, E.S., Kearney, G.A., Forster, J.W., Spangenberg, G.C., Smith, K.F.E. (2004). Assessment of gene flow using tetraploid genotypes of perennial ryegrass (*Lolium perenne* L.). *Australian Journal of Agricultural Research* **55**: 389-396.
- Damiani, C.R., Volterrani, M., Lercari, S., Stefanini, S. (2004). Comparative study on fructan accumulation ability in seventeen tall fescue varieties. *Acta Horticulture* **661**: 217-225.
- Darwen, C.W.E., John, P. (1989). Localization of the Enzymes of Fructan Metabolism in Vacuoles Isolated by a Mechanical Method from Tubers of Jerusalem Artichoke (*Helianthus tuberosus* L.). *Plant Physiology* **89**: 658-663.
- De Ascensao, A.R.D.C., Dubery, I.A. (2000). Panama Disease: Cell Wall Reinforcement in Banana Roots in Response to Elicitors from *Fusarium oxysporum* f. sp. *cubense* Race Four. *Phytopathology* **90**: 1173-1180.
- De Roover, J., Vandenbranden, K., Van Laere, A., Van den, E.W. (2000). Drought induces fructan synthesis and 1-SST (sucrose:sucrose fructosyltransferase) in roots and leaves of chicory seedlings (*Cichorium intybus* L.). *Planta* **210**: 808-814.
- De Vries, J., Meier, P., Wackernagel, W. (2001). The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. *FEMS Microbiology Letters* **195**: 211-215.
- Department of the Environment and Heritage (2004). Weeds of National Significance. <http://www.deh.gov.au/biodiversity/invasive/weeds/wons.html> .
- Directorate General for the Environment and European Commission (2003). Notification report - Field experiment of genetically modified hypolignified tall fescue - France 2003. Report No. B/FR/03/02/07, Joint Research Centre of the European Commission, available at http://gmoinfo.jrc.it/gmp_report.aspx?CurNot=B/FR/03/02/07.

- Dixon, R.A., Achnine, L., Kota, P., Liu, C.J., Reddy, M.S.S., Wang, L. (2002). The phenylpropanoid pathway and plant defence-a genomics perspective. *Molecular Plant Pathology* **3**: 371-390.
- Dixon, R.A., Paiva, N.L. (1995). Stress-Induced Phenylpropanoid Metabolism. *The Plant Cell* **7**: 1085-1097.
- Donaghy, D. J. (2001). Stolon formation in perennial ryegrass may aid persistence. The Australian Society of Agronomy. Proceedings of the 10th Australian Agronomy Conference, Hobart, 29 January - 1 February, 2001, available online at <http://www.regional.org.au/au/asa/2001/3/d/donaghy.htm>.
- EFSA (2004). Opinion of the scientific panel on genetically modified organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. *The EFSA Journal* **48**: 1-18.
- Fearon, C.H., Hayward, M.D., Lawrence, M.J. (1983). Self-incompatibility in ryegrass. V. Genetic control, linkage and seed-set in diploid *Lolium multiflorum* Lam. *Heredity* **50**: 35-45.
- Fellows, G.M., Roeth, F.W. (1992). Factors affecting Shattercane (*Sorghum bicolor*) seed survival. *Weed Science* **40**: 434-440.
- Felsot, A.S. (2000). Insecticidal genes. Part 2: Human health hoopla. *Agrichemical & Environmental News* **168**: 1-7.
- Fiers, M.W.E.J., Kleter, G.A., Nijland, H., Peijnenburg, A.A.C.M., Nap, J.P., van Ham, R.C.H.J. (2004). Allermatch, a webtool for the prediction of potential allergenicity according to current FAO/WHO codex alimentarius guidelines. *BMC Bioinformatics* **5**: 1-6.
- Fischer, S.F., Poschlod, P., Beinlich, B. (1996). Experimental Studies on the Dispersal of Plants and Animals on Sheep in Calcareous Grasslands. *The Journal of Applied Ecology* **33**: 1206-1222.
- Funnell, D.L., Pedersen, J.F. (2006). Reaction of Sorghum Lines Genetically Modified for Reduced Lignin Content to Infection by *Fusarium* and *Alternaria* spp. *Plant Disease* **90**: 331-338.
- Gadegaard, G., Didion, T., Folling, M., Storgaard, M., Andersen, C.H., Nielsen, K.K. (2008). Improved fructan accumulation in perennial ryegrass transformed with the onion fructosyltransferase genes 1-SST and 6G-FFT. *Journal of Plant Physiology* **In Press**, **Corrected Proof**:
- Gardenet (2006). Horticultural Information Service - <http://www.gardenet.com.au/>.
- Gardner, R.C., Howarth, A.J., Hahn, P., Brown-Luedi, M., Shepherd, R.J., Messing, J. (1981). The complete nucleotide sequence of an infectious clone of cauliflower mosaic virus by M13mp7 shotgun sequencing. *Nucleic Acids Research* **9**: 2871-2888.
- Gebhard, F., Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* **64**: 1550-1554.

- Glover, J. (2002). Gene flow study: Implications for the release of genetically modified crops in Australia. Bureau of Rural Sciences, Australian Government Department of Agriculture, Fisheries and Forestry, Canberra.
- Goodman, R.E., Vieths, S., Sampson, H.A., Hill, D., Ebisawa, M., Taylor, S.L., van Ree, R. (2008). Allergenicity assessment of genetically modified crops - what makes sense? *Nature Biotechnology* **26**: 73-81.
- Grand, C., Sarni, F., Lamb, C.J. (1987). Rapid induction by fungal elicitor of the synthesis of cinnamyl-alcohol dehydrogenase, a specific enzyme of lignin synthesis. *European Journal of Biochemistry* **169**: 73-77.
- Gregersen, P.L., Christensen, A.B., Sommer-Knudsen, J., Collinge, D.B. (1994). A putative O-methyltransferase from barley is induced by fungal pathogens and UV light. *Plant Molecular Biology* **26**: 1797-1806.
- Grewal, P., Richmond, D. (2003). Benefits of endophytic grasses - By infecting grasses, endophytes provide enhanced resistance to insects and weeds. *GCSAA's Golf Course Management magazine*
- Halpin, C. (2004). Investigating and Manipulating Lignin Biosynthesis in the Postgenomic Era. In: JA Callow, ed. *Advances in Botanical Research Incorporating Advances in Plant Pathology*, Edition Volume 41. Academic Press, pp 63-106.
- Hano, C., Addi, M., Bensaddek, L., Crônier, D., Baltora-Rosset, S., Doussot, J., Maury, S., Mesnard, F., Chabbert, B., Hawkins, S., Lainé, E., Lamblin, F. (2006). Differential accumulation of monolignol-derived compounds in elicited flax (*Linum usitatissimum*) cell suspension cultures. *Planta* **223**: 975-989.
- Haslberger, A.G. (2003). Codex guidelines for GM foods include the analysis of unintended effects. *Nature Biotechnology* **21**: 739-741.
- He, X., Hall, M.B., Gallo-Meagher, M., Smith, R.L. (2003). Improvement of Forage Quality by Downregulation of Maize O-Methyltransferase. *Crop Science* **43**: 2240-2251.
- Hendry, G.A.F., Wallace, R.K. (2008). The origin, distribution and evolutionary significance of fructans. Chapter 4. In: M Suzuki, NJ Chatterton, eds. *Science and Technology of Fructans*. CRC Press, pp 119-140.
- Hill, M.J., Pearson, C.J. (1985). Primary growth and regrowth responses of temperate grasses to different temperatures and cutting frequencies. *Australian Journal of Agricultural Research* **36**: 25-34.
- Hodkinson, D.J., Thompson, K. (1997). Plant Dispersal: The Role of Man. *The Journal of Applied Ecology* **34**: 1484-1496.
- Hopkins, D.W., Webster, E.A., Chudek, J.A., Halpin, C. (2001). Decomposition in soil of tobacco plants with genetic modifications to lignin biosynthesis. *Soil Biology and Biochemistry* **33**: 1455-1462.

- Hu, W.J., Harding, S.A., Lung, J., Popko, J.L., Ralph, J., Stokke, D.D., Tsai, C.J., Chiang, V.L. (1999). Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. *Nat Biotechnol* **17**: 808-812.
- Hulme, P.E. (1994). Post-Dispersal Seed Predation in Grassland: Its Magnitude and Sources of Variation. *The Journal of Ecology* **82**: 645-652.
- Janzen, D.H. (1984). Dispersal of Small Seeds by Big Herbivores: Foliage is the Fruit. *The American Naturalist* **123**: 338-353.
- Jeong, B.R., Housley, T.L. (1990). Fructan Metabolism in Wheat in Alternating Warm and Cold Temperatures. *Plant Physiol* **93**: 902-906.
- Ji, X., Van den Ende, W., Schroeven, L., Clerens, S., Geuten, K., Cheng, S., Bennett, J. (2007). The rice genome encodes two vacuolar invertases with fructan exohydrolase activity but lacks the related fructan biosynthesis genes of the Pooideae. *New Phytologist* **173**: 50-62.
- Johns, G.G., Greenup, L.R. (1976). Pasture seed theft by ants in northern New South Wales. *Australian Journal of Experimental Agriculture and Animal Husbandry* **16**: 249-256.
- Jones, W.E. (2004). Laminitis and fructan consumption. *Journal of Equine Veterinary Science* **24**: 254.
- Joost, R.E. (1995). *Acremonium* in fescue and ryegrass: boon or bane? A review. *Journal of Animal Science* **73**: 881-888.
- Kahl, G. (2001). *The dictionary of gene technology: genomics, transcriptomics, proteomics*. Wiley-VCH, Weinheim, Germany. pp 1-941.
- Kawakami, A., Sato, Y., Yoshida, M. (2008). Genetic engineering of rice capable of synthesizing fructans and enhancing chilling tolerance. *J Exp Bot* **59**: 793-802.
- Kawakami, A., Yoshida, M. (2002). Molecular Characterization of Sucrose:Sucrose 1-Fructosyltransferase and Sucrose:Fructan 6-Fructosyltransferase Associated with Fructan Accumulation in Winter Wheat during Cold Hardening. *Bioscience, Biotechnology, and Biochemistry* **66**: 2297-2305.
- Kemp, H., Bourke, C., Wheatley, W. (2007). NSW DPI, Endophytes of perennial ryegrass and tall fescue.
- King, C., Mansmann, R.A. (2004). Preventing laminitis in horses: Dietary strategies for horse owners. *Clinical Techniques in Equine Practice* **3**: 96-102.
- Kneusel, R.E., Matern, U., Nicolay, K. (1989). Formation of trans-caffeoyl-CoA from trans-4-coumaroyl-CoA by Zn²⁺-dependent enzymes in cultured plant cells and its activation by an elicitor-induced pH shift. *Archives of Biochemistry and Biophysics* **269**: 455-462.
- Konstantinova, T., Parvanova, D., Atanassov, A., Djilianov, D. (2002). Freezing tolerant tobacco, transformed to accumulate osmoprotectants. *Plant Science* **163**: 157-164.

- Kurland, C.G., Canback, B., Berg, O.G. (2003). Horizontal gene transfer: a critical view. *Proceedings of the National Academy of Science of the United States of America* **100**: 9658-9662.
- Lamp, C.A., Forbes, S.J., Cade, J.W. (2001). *Grasses of temperate Australia - A field guide*. Inkata Press (1st Edition) and CH Jerram & Associates Science Publishers (Revised Edition).
- Landry, L.G., Chapple, C.C.S., Last, R.L. (1995). Arabidopsis Mutants Lacking Phenolic Sunscreens Exhibit Enhanced Ultraviolet-B Injury and Oxidative Damage. *Plant Physiology* **109**: 1159-1166.
- Lazenby, A. (1997). Selection and breeding of pasture plants. Chapter 7. In: JV Lovett, JM Scott, eds. *Pasture Production and Management*, Edition 1. Inkata Press, Victoria. pp 133-154.
- Lee, M.R.F., Merry, R.J., Davies, D.R., Moorby, J.M., Humphreys, M.O., Theodorou, M.K., MacRae, J.C., Scollan, N.D. (2003). Effect of increasing availability of water-soluble carbohydrates on in vitro rumen fermentation. *Animal Feed Science and Technology* **104**: 59-70.
- Lewis, N.G., Yamamoto, E. (1990). Lignin: occurrence, biogenesis and biodegradation. *Annu Rev Plant Physiol Plant Mol Biol* **41**: 455-496.
- Li, H.J., Yang, A.F., Zhang, X.C., Gao, F., Zhang, J.R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: sucrose 1-fructosyltransferase gene from *Lactuca sativa*. *Plant Cell, Tissue and Organ Culture* **89**: 37-48.
- Lodge, G.M. (2004). Seed dormancy, germination, seedling emergence, and survival of some temperate perennial pasture grasses in northern New South Wales. *Australian Journal of Agricultural Research* **55**: 345-355.
- Lu, Y., Xu, W., Kang, A., Luo, Y., Guo, F., Yang, R., Zhang, J., Huang, K. (2007). Prokaryotic Expression and Allergenicity Assessment of Hygromycin B Phosphotransferase Protein Derived from Genetically Modified Plants. *Journal of Food Science* **72**: M228-M232.
- Maury, S., Geoffroy, P., Legrand, M. (1999). Tobacco O-Methyltransferases Involved in Phenylpropanoid Metabolism. The Different Caffeoyl-Coenzyme A/5-Hydroxyferuloyl-Coenzyme A 3/5-O-Methyltransferase and Caffeic Acid/5-Hydroxyferulic Acid 3/5-O-Methyltransferase Classes Have Distinct Substrate Specificities and Expression Patterns. *Plant Physiology* **121**: 215-224.
- McElroy, D., Zhang, W., Cao, J., Wu, R. (1990). Isolation of an Efficient Actin Promoter for Use in Rice Transformation. *The Plant Cell* **2**: 163-171.
- Menden, B., Kohlhoff, M., Moerschbacher, B.M. (2007). Wheat cells accumulate a syringyl-rich lignin during the hypersensitive resistance response. *Phytochemistry* **68**: 513-520.
- Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A., Flint, H.J. (1999). Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* **65**: 6-10.

- Meyer, W.A., Watkins, E. (2003). Tall Fescue (*Festuca arundinacea*). Chapter 8. In: MD Casler, RR Duncan, eds. *Turfgrass Biology Genetics and Breeding*, Edition 1. John Wiley & Sons, New Jersey & Canada. pp 107-127.
- Miki, B., McHugh, S. (2004). Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *Journal of Biotechnology* **107**: 193-232.
- Miller, L.A., Moorby, J.M., Davies, D.R., Humphreys, M.O., Scollan, N.D., MacRae, J.C., Theodorou, M.K. (2001). Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass and Forage Science* **56**: 383-394.
- Milne, G. (2005). Management - Southern Hemisphere. In: HA Fribourg, DB Hannaway, eds. *Tall Fescue - Online Monograph*, available online at <http://forages.oregonstate.edu/is/tfis/book/tf21c/management-south-hemisphere.pdf>. Oregon State University,
- Mitchell, H.J., Hall, J.L., Barber, M.S. (1994). Elicitor-Induced Cinnamyl Alcohol Dehydrogenase Activity in Lignifying Wheat (*Triticum aestivum* L.) Leaves. *Plant Physiology* **104**: 551-556.
- Mitchell, H.J., Hall, S.A., Stratford, R., Hall, J.L., Barber, M.S. (1999). Differential induction of cinnamyl alcohol dehydrogenase during defensive lignification in wheat (*Triticum aestivum* L.): characterisation of the major inducible form. *Planta* **208**: 31-37.
- Moerschbacher, B.M., Noll, U., Gorrichon, L., Reisener, H.J. (1990). Specific Inhibition of Lignification Breaks Hypersensitive Resistance of Wheat to Stem Rust. *Plant Physiology* **93**: 465-470.
- Montana Seed Growers Association (2008). Seed standards - Grass. <http://ag.montana.edu/msga/Seed%20Standards/grass%20standards.pdf>.
- Nagarathna, K.C., Shetty, S.A., Shetty, H.S. (1993). Phenylalanine Ammonia Lyase Activity in Pearl Millet Seedlings and its Relation to Downy Mildew Disease Resistance. *Journal of Experimental Botany* **44**: 1291-1296.
- Nielsen, K.M. (1998). Barriers to horizontal gene transfer by natural transformation in soil bacteria. *Acta pathologica, microbiologica, et immunologica Scandinavica* **106**: 77-84.
- Nielsen, K.M., van Elsas, J.D., Smalla, K. (2000). Transformation of *Acinetobacter* sp strain BD413(pFG4 Delta nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Applied and Environmental Microbiology* **66**: 1237-1242.
- OECD (2008). OECD Seed Schemes 2008. Organisation for Economic Co-operation and Development,
- OGTR (2005). *Risk Analysis Framework*. Australian Government, Canberra, ACT.
- OGTR (2007). Risk Analysis Framework. Report No. Version 2.2, Document produced by the Australian Government Office of the Gene Technology Regulator, available online from <http://www.ogtr.gov.au/>,

OGTR (2008). The Biology of *Lolium multiflorum* Lam. (Italian ryegrass), *Lolium perenne* L. (perennial ryegrass) and *Lolium arundinaceum* (Schreb.) Darbysh. (tall fescue). Document prepared by the Australian Government Office of the Gene Technology Regulator, Canberra, available online at <http://www.ogtr.gov.au>.

Oregon State University (2000). *Grass Growth and Regrowth for Improved Management*. Oregon State University, online project publication available at <http://forages.oregonstate.edu/projects/regrowth/default.cfm>.

Pakusch, A.E., Kneusel, R.E., Matern, U. (1989). S-adenosyl-L-methionine:trans-caffeoyl-coenzyme A 3-O-methyltransferase from elicitor-treated parsley cell suspension cultures. *Archives of Biochemistry and Biophysics* **271**: 488-494.

Pavis, N., Boucaud, J., Prud'homme, M.P. (2001a). Fructans and fructan-metabolizing enzymes in leaves of *Lolium perenne*. *New Phytologist* **150**: 97-109.

Pavis, N., Chatterton, N.J., Harrison, P.A., Baumgartner, S., Praznik, W., Boucaud, J., Prud'homme, M.P. (2001b). Structure of fructans in roots and leaf tissues of *Lolium perenne*. *New Phytologist* **150**: 83-95.

Pedersen, J.F., Vogel, K.P., Funnell, D.L. (2005). Impact of Reduced Lignin on Plant Fitness. *Crop Science* **45**: 812-819.

Pellegrini, L., Geoffroy, P., Fritig, B., Legrand, M. (1993). Molecular Cloning and Expression of a New Class of Ortho-Diphenol-O-Methyltransferases Induced in Tobacco (*Nicotiana tabacum* L.) Leaves by Infection or Elicitor Treatment. *Plant Physiology* **103**: 509-517.

Pilate, G., Guiney, E., Holt, K., Petit-Conil, M., Lapiere, C., Leple, J.C., Pollet, B., Mila, I., Webster, E.A., Marstorp, H.G., Hopkins, D.W., Jouanin, L., Boerjan, W., Schuch, W., Cornu, D., Halpin, C. (2002). Field and pulping performances of transgenic trees with altered lignification. *Nat Biotechnol* **20**: 607-612.

Pilon-Smits, E.A.H., Ebskamp, M.J.M., Paul, M.J., Jeuken, M.J.W., Weisbeek, P.J., Smeekens, S.C.M. (1995). Improved Performance of Transgenic Fructan-Accumulating Tobacco under Drought Stress. *Plant Physiology* **107**: 125-130.

Pollock, C.J. (1984). Sucrose accumulation and the initiation of fructan biosynthesis in *Lolium temulentum* L. *New Phytologist* **96**: 527-534.

Pollock, C.J., Jones, T. (1979). Seasonal patterns of fructan metabolism in forage grasses. *New Phytologist* **83**: 9-15.

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

Rao, R.N., Allen, N.E., Hobbs, J.N.J., Alborn, W.E.Jr., Kirst, H.A., Paschal, J.W. (1983). Genetic and enzymatic basis of hygromycin B resistance in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **24**: 689-695.

Rastogi, S., Dwivedi, U.N. (2008). Manipulation of lignin in plants with special reference to O-methyltransferase. *Plant Science* **174**: 264-277.

- Rengel, Z., Graham, R.D., Pedler, J.F. (1994). Time-course of Biosynthesis of Phenolics and Lignin in Roots of Wheat Genotypes Differing in Manganese Efficiency and Resistance to Take-all Fungus. *Annals of Botany* **74**: 471-477.
- Reymond, P., Bodenhausen, N., Van Poecke, R.M.P., Krishnamurthy, V., Dicke, M., Farmer, E.E. (2004). A Conserved Transcript Pattern in Response to a Specialist and a Generalist Herbivore. *The Plant Cell* **16**: 3132-3147.
- Ritsema, T., Smeekens, S. (2003). Fructans: beneficial for plants and humans. *Current Opinion in Plant Biology* **6**: 223-230.
- Sawada, H. (1991) Aerial tillering as a potential route of vegetative reproduction in perennial ryegrass (*Lolium perenne* L.) pastures. *Grassland Science* **36** (4): 370-375.
- Schardl, C.L., Leuchtman, A., Chung, K.R., Penny, D., Siegel, M.R. (1997). Coevolution by common descent of fungal symbionts (*Epichloë* spp.) and grass hosts. *Molecular Biology and Evolution* **14**: 133-143.
- Schardl, C.L., Leuchtman, A., Spiering, M.J. (2004). Symbioses of grasses with seedbourne fungal endophytes. *Annual Review of Plant Biology* **55**: 315-340.
- Schardl, C.L., Phillips, T.D. (1997). Protective grass endophytes: Where are they from and where are they going? *Plant Disease* **81**: 430-438.
- Schellenbaum, L., Sprenger, N., Schuepp, H., Wiemken, A., Boller, T. (1999). Effects of drought, transgenic expression of a fructan synthesizing enzyme and of mycorrhizal symbiosis on growth and soluble carbohydrate pools in tobacco plants. *New Phytologist* **142**: 67-77.
- Schmitt, D., Pakusch, A.E., Matern, U. (1991). Molecular cloning, induction and taxonomic distribution of caffeoyl-CoA 3-O-methyltransferase, an enzyme involved in disease resistance. *Journal of Biological Chemistry* **266**: 17416-17423.
- Singh, B., Sapra, V.T., Patel, J.A. (1976). Nitrate reductase and its relationship to protein and yield characteristic of triticale. *Euphytica* **25**: 193-199.
- Smith, P. and Baxter, L. (2002). South Australian Seed Certification Scheme - Procedures and Standards Manual. Seed Services, Primary Industries & Resources South Australia, Plant Research Centre, Hartley Grove, Urrbrae, SA 5064, available online at http://www.ruralsolutions.sa.gov.au/_data/assets/pdf_file/0005/43349/seeds_manual.pdf.
- Sprenger, N., Schellenbaum, L., van Dun, K., Boller, T., Wiemken, A. (1997). Fructan synthesis in transgenic tobacco and chicory plants expressing barley sucrose: fructan 6-fructosyltransferase. *FEBS Letters* **400**: 355-358.
- Thomson, J.A. (2000). Horizontal transfer of DNA from GM crops to bacteria and to mammalian cells. *Journal of Food Science* **66**: 188-193.
- Thorogood, D. (2003). Perennial ryegrass (*Lolium perenne* L.). Chapter 7. In: MD Casler, RR Duncan, eds. *Turfgrass Biology Genetics and Breeding*, Edition 1. John Wiley & Sons, New Jersey & Canada. pp 75-105.

- Tilston, E.L., Halpin, C., Hopkins, D.W. (2004). Genetic modifications to lignin biosynthesis in field-grown poplar trees have inconsistent effects on the rate of woody trunk decomposition. *Soil Biology and Biochemistry* **36**: 1903-1906.
- Toquin, V., Grausem, B., Geoffroy, P., Legrand, M. (2003). Structure of the tobacco caffeic acid O-methyltransferase (COMT) II gene: identification of promoter sequences involved in gene inducibility by various stimuli. *Plant Molecular Biology* **52**: 495-509.
- Tosso, T.J., Ferreyra, E.R., Muñoz, S.L. (1986) Weed seed transported by irrigation water. II. Identification, germination and distribution of the species, through one irrigation season. *Agricultura Técnica* 46 (2): 125-129.
- Uchida, T., Arasea, T. (2005). Weeds control by cutting: is it effective? In "*Increasing work efficiency in agriculture, horticulture and forestry. XXXI CIOSTA-CIGR V Congress Proceedings.*", Krause, M. eds, Institute of Agricultural Engineering, University of Hohenheim, Stuttgart, Germany. pp. 368-375.
- Uhlmann, A., Ebel, J. (1993). Molecular cloning and expression of 4-Coumarate:Coenzyme A Ligase, an enzyme involved in the resistance response of soybean (*Glycine max* L.) against pathogen attack. *Plant Physiology* **102**: 1147-1156.
- USDA-APHIS (2006). USDA/APHIS permit 94-278-01r and 05-278-02r for field testing genetically engineered tall fescue and italian ryegrass plants.
- Valliyodan, B., Nguyen, H.T. (2006). Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Current Opinion in Plant Biology* **9**: 189-195.
- Vance, C.P., Kirk, T.K., Sherwood, R.T. (1980). Lignification as a Mechanism of Disease Resistance. *Annual Review of Phytopathology* **18**: 259-288.
- Vermerris, W., McIntyre, L.M. (1999). Time to flowering in brown midrib mutants of maize: an alternative approach to the analysis of developmental traits. *Heredity* **83 (Pt 2)**: 171-178.
- Vermerris, W., Thompson, K.J., McIntyre, L.M. (2002a). The maize *Brown midrib1* locus affects cell wall composition and plant development in a dose-dependent manner. *Heredity* **88**: 450-457.
- Vermerris, W., Thompson, K.J., McIntyre, L.M., Axtell, J.D. (2002b). Evidence for an evolutionarily conserved interaction between cell wall biosynthesis and flowering in maize and sorghum. *BMC Evol Biol* **2**: 2.
- Vincent, D., Lapierre, C., Pollet, B., Cornic, G., Negroni, L., Zivy, M. (2005). Water Deficits Affect Caffeate O-Methyltransferase, Lignification, and Related Enzymes in Maize Leaves. A Proteomic Investigation. *Plant Physiology* **137**: 949-960.
- Vogel, K.P., Jung, H.J. (2001). Genetic Modification of Herbaceous Plants for Feed and Fuel. *Critical Reviews in Plant Sciences* **20**: 15-49.
- Waines, J.G., Hedge, S.G. (2003). Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. *Crop Science* **43**: 451-463.

- Walter, M.H., Grima-Pettenati, J., Grand, C., Boudet, A.M., Lamb, C.J. (1988). Cinnamyl-alcohol dehydrogenase, a molecular marker specific for lignin synthesis: cDNA cloning and mRNA induction by fungal elicitor. *Proc Natl Acad Sci U S A* **85**: 5546-5550.
- Wang, Z.Y., Ge, Y. (2006). Invited Review: Recent advances in genetic transformation of forage and turf grasses. *In Vitro Cellular and Developmental Biology - Plant* **42**: 1-18.
- Wang, Z.Y., Lawrence, R., Hopkins, A., Bell, J., Scott, M. (2004). Pollen-mediated transgene flow in the wind-pollinated grass species tall fescue (*Festuca arundinacea* Schreb.). *Molecular Breeding* **14**: 47-60.
- Waterhouse, P.M., Helliwell, C.A. (2003). Exploring Plant Genomes by RNA-induced Gene Silencing. *Nature Reviews Genetics* **4**: 29-38.
- Watts, K.A., Chatterton, N.J. (2004). A review of factors affecting carbohydrate levels in forage. *Journal of Equine Veterinary Science* **24**: 84-86.
- Webster, E.A., Halpin, C., Chudek, J.A., Tilston, E.L., Hopkins, D.W. (2005). Decomposition in soil of soluble, insoluble and lignin-rich fractions of plant material from tobacco with genetic modifications to lignin biosynthesis. *Soil Biology and Biochemistry* **37**: 751-760.
- Weyens, G., Ritsema, T., van Dun, K., Meyer, D., Lommel, M., Lathouwers, J., Rosquin, I., Denys, P., Tossens, A., Nijs, M., Turk, S., Gerrits, N., Bink, S., Walraven, B., Lefebvre, M., Smeekens, S. (2004). Production of tailor-made fructans in sugar beet by expression of onion fructosyltransferase genes. *Plant Biotechnol J* **2**: 321-327.
- Yamada, T., Kawaguchi, T. (1972). Dissemination of pasture plants by livestock. 2. Recovery, viability and emergence of some pasture plant seeds passed through the digestive tract of the dairy cow. *Journal of Japanese Society of Grassland Science* **18**: 8-15.
- Yamada, T., Matsuo, S., Tamura, K. (1972) Dissemination of pasture plants by livestock. 3. Recovery of some pasture plant seeds passed through the digestive tract of beef cattle and emergence of seeds recovered from the faeces. *Journal of Japanese Society of Grassland Science* **18** (1): 16-27.
- Yang, J., Zhang, J., Wang, Z., Zhu, Q., Liu, L. (2004). Activities of fructan- and sucrose-metabolizing enzymes in wheat stems subjected to water stress during grain filling. *Planta* **220**: 331-343.
- Yates (2006). Lawns - <http://www.yates.com.au/GardenGuide/PlantCare/lawnss.asp>.
<http://www.yates.com.au/GardenGuide/PlantCare/lawnss.asp> .
- Ye, X.D., Wu, X.L., Zhao, H., Frehner, M., Nösberger, J., Potrykus, I., Spangenberg, G. (2001). Altered fructan accumulation in transgenic *Lolium multiflorum* plants expressing a *Bacillus subtilis sacB* gene. *Plant Cell Reports* **20**: 205-212.

Appendix A Definitions of terms in the *Risk Analysis Framework* used by the Regulator

(* terms defined as in Australia New Zealand Risk Management Standard AS/NZS 4360:2004)

Consequence

outcome or impact of an adverse event

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

Event*

occurrence of a particular set of circumstances

Hazard*

source of potential harm

Hazard identification

the process of analysing hazards and the events that may give rise to harm

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

to check, audit, review and evaluate the progress of an activity, process or system on an ongoing basis to identify change from the performance level required or expected and opportunities for improvement

Risk

the chance of something happening that will have an undesired impact

Negligible: risk is insubstantial and there is no present need to invoke actions for mitigation

Low: risk is minimal but may invoke actions for mitigation beyond normal practices

Moderate: risk is of marked concern requiring mitigation actions demonstrated to be effective

High: risk is unacceptable unless actions for mitigation are highly feasible and effective

Risk analysis

the overall process of risk assessment, risk management and risk communication

Risk analysis framework

systematic application of legislation, policies, procedures and practices to analyse risks

Risk assessment

the overall process of hazard identification and risk estimation

Risk communication

the culture, processes and structures to communicate and consult with stakeholders about risks

Risk Context

parameters within which risk must be managed, including the scope and boundaries for the risk assessment and risk management process

Risk estimate

a measure of risk in terms of a combination of consequence and likelihood assessments

Risk evaluation

the process of determining risks that require treatment

Risk management

the overall process of risk evaluation, risk treatment and decision making to manage potential adverse impacts

Risk management plan

integrates risk evaluation and risk treatment with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

those people and organisations who may affect, be affected by, or perceive themselves to be affected by a decision, activity or risk

States

includes all State governments, the Australian Capital Territory and the Northern Territory governments

Uncertainty

imperfect ability to assign a character state to a thing or process; a form or source of doubt

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities on the consultation RARMP for DIR 082/2007

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

Summary of issues raised	Comment
<p>The application lacks details about trial procedures in the second year. Specifically:</p> <ul style="list-style-type: none"> • Will the transgenic plants be moved into an OGTR-certified PC2 glasshouse for flowering? • Are transgenic seeds planted in the second year? If seeds are planted, what steps are taken to ensure viable seed does not persist at the trial site? 	<p>This is explained on Chapter 1, Section 6.3. All GM perennial ryegrass or tall fescue plants will be moved to a PC2 glasshouse prior to flowering, in both seasons of the trial. Seeds will be germinated in the PC2 glasshouse and no seeds will be planted directly into the field. Since seed will not be planted and GM plants will not be allowed to flower, there will be no seed persisting at the trial site.</p>
<p>Suggests that a risk should be identified for section 2.2 “Spread and persistence of the GM perennial ryegrass and tall fescue in the environment” related to transfer of the introduced genes into wild relatives resulting in an increase in weediness.</p> <p>Suggests additional monitoring and control measures. The two risks identified include:</p>	<p>Weediness is discussed in Events 2 and 3 and gene transfer in Event 4 and was not considered to be an identified risk. The RARMP concludes that this limited and controlled release poses negligible risks which do not require specific risk treatment measures (see discussion below).</p>
<p>1) Abiotic stress tolerance</p> <p>Accumulation of more water soluble sugars in the fructan modified plants could increase their tolerance to drought and reduced temperature. This is likely to provide a survival advantage in the environment, and the altered fructan and lignin content could increase dispersal in the environment. While this aspect of the application is discussed in the RARMP, it is not identified as a risk.</p>	<p>The effects of altered fructan content on disease resistance, drought and cold tolerance and the effects of altered lignin content on disease susceptibility and seed survival was discussed in Event 2. No risk was identified due to the containment measures proposed by the applicant and the small scale and short duration of the release. Data on potential for increased weediness would be required for a larger scale release or if reduced containment measures were proposed.</p>
<p>2) Weediness</p> <p>Lolium species complex are wind pollinated, highly outcrossing grasses and are considered to be serious weeds of environmental and agricultural systems. If the GM plants were to flower, the introduced genes could be transferred to either volunteer perennial ryegrass plants or to other members of the Lolium species complex. The effect would be to provide the weedy species with a selective advantage over non-GM plants that could lead to increased spread and persistence in the environment.</p>	<p>Event 4 discusses the expression of the introduced genes in other perennial ryegrass and tall fescue plants or other sexually compatible plants and was not considered to be an identified risk. The proposed limits and controls of the trial will restrict the potential for pollen flow and gene transfer to sexually compatible plants. The licence requires frequent (weekly) inspection of the GM plants to identify changes that indicate that flowers are beginning to develop and the GM plants must be removed from the field prior to flowering.</p>
<p>The major control measure for this trial is the removal of plants from the trial site before flowering and it is likely to be effective. However, some consideration should be given to the effectiveness of the secondary measures if the primary</p>	<p>Noted.</p> <p>The licence conditions require weekly monitoring of the GM perennial ryegrass and tall fescue plants for flowering and removal of plants to the PC2 glasshouse prior to flowering.</p>

<p>one fails through human error, plant biology or extreme weather.</p>	<p>Weekly monitoring will be adequate to detect flowering. It is unlikely that adverse weather would impact on the applicant's ability to fulfil this monitoring requirement. In the unlikely event of the GM perennial ryegrass and tall fescue plants flowering at the trial site, the presence of the <i>Triticale</i> sp. border around the trial site, would reduce the likelihood of gene flow to sexually compatible plants (if any were present and flowering at the same time). Licence conditions also require that the applicant informs the Regulator immediately of any unintended presence of GMOs or Plant Material outside of the Location.</p>
<p>The herbicide application and strategy used in the buffer zone should be imposed in the licence, to maximise the effectiveness of the buffer zone.</p>	<p>The applicant has stated that they used a weed reduction program in the 2 years prior to the GM trial commencing. The Monitoring Zone is required to be kept free of plants that would inhibit the identification of stoloniferous growth and this can be achieved by a number of methods but is not limited to herbicide treatment.</p>
<p>The buffer zone (<i>Triticale</i> sp. border) should be monitored for at least 12 months after completion of the trial until volunteers are not detected for a continuous 6 month period. Should post-trial monitoring of the buffer zone identify any <i>Lolium</i> sp. volunteers, these and any seeds that they have set should be tested for the presence of transgenes by PCR. This information would provide an indication of the efficacy of the proposed containment measures as well as proof of compliance.</p>	<p>There is no requirement in the licence conditions to monitor the isolation zone after completion of the trial. The GM perennial ryegrass and tall fescue plants are not permitted to flower in the field so there is effectively no opportunity for gene flow to sexually compatible plants that might be present in the surrounding <i>Triticale</i> sp. border.</p> <p>Although considered highly unlikely, if the GM plants do flower in the field, this event must be immediately reported to the Regulator and measures would be imposed to minimise any risks, taking into account the specific circumstances at the time.</p>
<ul style="list-style-type: none"> • Suggested areas of further research before future releases of the lines are considered: • The relative growth rate of the plants containing the traits both alone and in combination for all lines tested. • The biomass accumulation data for each line together with the harvest index • The effect on tillering • The effect on rhizomatic growth • The tolerance of each plant line to abiotic stress both alone and in combination • The structure and location of the integration sites • The level of accumulation of sugars from the fructan and lignin lines • The reduction in lignin achieved and relative digestibility of the plant • The suitability of the plants for colonisation of insects eg lawngnubs • The flowering time of the altered plants relative to the non GM parental lines • Testing of the effect on native animals likely to graze on the altered plants • Testing of a BC6 (or greater) backcross of the traits into <i>L. rigidum</i> both alone and in combination for growth rate, biomass accumulation, harvest index, resilience to nutrient deprivation, cold, heat and light stress and seed dormancy. 	<p>Chapter 3, Section 5 of the RARMP identified additional information that may be required by the OGTR to assess an application for a larger scale or commercial release or to justify a reduction in containment measures of these GM perennial ryegrass and tall fescue lines. The additional information identified in the RARMP is similar to most of the issues raised in this submission. In addition, some of the suggested areas of research have been amended to include suggested research. For example, altered characteristics which might lead to weediness now specifically includes growth rate, and disease susceptibility has been broadened to include pest susceptibility and data on characteristics of weediness in sexually compatible relatives has been added. However, a data requirement for combinations of traits has not been included at this stage as stacking would depend on which lines were chosen for future research, and the design of any future trials.</p>

Appendix C Summary of issues raised in submissions received from the public for the consultation RARMP for DIR 082/2007

The Acting Regulator received one submission from the public on the consultation RARMP. This submission, summarised in the table below, was considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Acting Regulator's decision to issue the licence

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

Issues raised: **EN:** Environmental risks, **H:** Human health and safety,

Other abbreviations: **GM:** Genetically Modified; **GMO:** Genetically Modified Organism

Type: **I:** individual

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	x	EN,H	Objects to the release of GM perennial ryegrass and tall fescue into the Australian environment because of lack of published research into effects on humans and environmental systems	Risks to human health and safety and to the environment were assessed as negligible following extensive review of published literature of the non-GM parent and GM trait and consideration of the proposed trial arrangements. The Regulator has imposed a range of measures to restrict the dissemination and persistence of the GMOs and their genetic material in the environment and to limit the trial to the proposed size, location and duration.