



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

**Risk Assessment and
Risk Management Plan for
DIR 084/2008**

**Limited and controlled release of torenia genetically
modified for enhanced phosphate uptake**

Applicant: Florigene Pty Ltd

September 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 three lines of torenia genetically modified for enhanced phosphate uptake into the environment in respect of application DIR 084/2008 from Florigene Pty Ltd (Florigene).

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 public¹.

The application

Florigene applied for a licence for dealings involving the intentional release of three genetically modified (GM) torenia lines on a limited scale and under controlled conditions. The GM torenia lines have been modified to enhance their capacity to absorb phosphate. The release would involve growing a maximum of 400 plants hydroponically at one site in the local government area of Darebin, Victoria, on a maximum total area of 20 m², between October 2008 and May 2009.

The GM torenia lines also contain an antibiotic resistance selectable marker gene, which was used to identify transformed plants during their initial development in the laboratory.

The purpose of the trial is to conduct proof of concept research involving experiments with the GM torenia lines to assess their capacity to absorb phosphate and slow or repress algal overgrowth in the surrounding water.

Florigene proposed a number of controls to restrict the dissemination or persistence of the GM torenia lines and the introduced genetic materials into the environment. These controls have been considered during the evaluation of the application.

Confidential Commercial Information

The identity of one of the promoters used to control expression of the introduced gene in one of the three GM torenia lines has 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 Risk Assessment and Risk Management Plan (RARMP) for this application.

¹ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/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>>.

Risk assessment

The risk assessment considered information contained in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge, and advice received from a wide range of experts, agencies and authorities consulted on the RARMP. No submissions were received 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.

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

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

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

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM torenia 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 eight 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 release to the size, location and duration requested by the applicant, as these were an important part of establishing the context for assessing the risks.

The licence conditions require Florigene to **limit** the duration of the release to between October 2008 to May 2009 on a maximum total area of 20 m² at one site. The **control** measures to restrict the spread and persistence of the GMOs include preventing the use of GM plant materials in human food or animal feed; destroying waste GM plant materials; and transporting GM plant materials in accordance with OGTR transportation guidelines².

² The licence for DIR 084/2008 is available on the OGTR website via the link to DIR 084/2008 (<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir084-2008>>)

Conclusions of the RARMP

The risk assessment concluded that this limited and controlled release of three GM torenia lines on a maximum total area of 20 m² over eight months in the Victorian local government area of Darebin poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMOs and their genetic materials 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.

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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
CaMV	Cauliflower mosaic virus
CCI	Confidential Commercial Information
cv	Cultivar
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
L	Litre
m	Metre
m ²	Square metre
<i>mas</i>	Gene encoding mannopine synthase
mM	Millimolar
mRNA	Messenger Ribonucleic Acid
miRNA	Micro ribonucleic acid
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NLRD	Notifiable Low Risk Dealing
<i>nos</i>	Gene encoding nopaline synthase
<i>nptII</i>	Gene encoding neomycin phosphotransferase type II
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment Level 2
<i>PHR1</i>	Gene encoding phosphate starvation response regulator 1
RARMP	Risk Assessment and Management Plan
the Regulations	Gene Technology Regulations 2001
the (Acting) Regulator	(Acting) Gene Technology Regulator
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
TMV	Tobacco mosaic virus
TGA	Therapeutic Goods Administration

Technical Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence (DIR 084/2008) to Florigene Pty Ltd (Florigene) for dealings involving the limited and controlled release of genetically modified (GM) torenia 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 public³.

The application

Florigene applied for a licence for dealings involving the intentional release of three lines⁴ of torenia (*Torenia x hybrida*) that have been genetically modified for enhanced phosphate uptake on a limited scale and under controlled conditions. The trial is authorised to take place at one site in the local government area of Darebin, Victoria, on a maximum total area of 20 m², between October 2008 and May 2009.

The three GM torenia lines contain the *phosphate starvation response regulator 1 (PHR1)* gene from thale cress (*Arabidopsis thaliana*) under the control of a different promoter in each line. The *PHR1* gene encodes a transcription factor⁵ thought to play a role in plant responses to phosphate deficiency.

In addition, all of the GM torenia lines contain the antibiotic resistance gene, *neomycin phosphotransferase type II (nptII)*. This gene, encoding the enzyme neomycin phosphotransferase, was derived from *Escherichia coli*, and confers kanamycin or neomycin resistance on the GM plant. The *nptII* gene was used as a selective marker to identify transformed plants during their initial development in the laboratory.

The purpose of the trial is to conduct proof of concept research involving experiments with the GM torenia lines to assess their capacity to absorb phosphate and slow or repress algal overgrowth in the surrounding water. The GM torenia plants will not be used for human food or animal feed.

³ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/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>>.

⁴ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

⁵ A transcription factor is any protein required for the recognition, by RNA polymerases, of specific regulatory sequences in genes (eg a promoter)

Florigene proposed a number of controls to restrict the dissemination or persistence of the GM torenia lines and their genetic material into the environment. These controls have been considered during the evaluation of the application.

Confidential Commercial Information

The identity of one of the promoters used to control expression of the introduced gene in one of the three GM torenia lines has 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 considered information contained in the application (including proposed containment measures), 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 with a wide range of prescribed experts, agencies and authorities on the application (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.

No submissions were received from the public.

A reference document on the parent organism, *The Biology of Torenia spp. (torenia)*, was produced to inform the risk assessment process for licence applications involving GM torenia plants. The document is available from the OGTR or from the website < <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

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

Eight events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry, physiology or ecology. 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 eight events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principle reasons for this include:

- ♦ limits on the size and duration of the release proposed by Florigene
- ♦ suitability of controls proposed by Florigene to restrict the dissemination or persistence of the GM torenia plants and their genetic material
- ♦ limited capacity of the GM torenia lines to spread and persist outside the areas proposed for release
- ♦ limited ability and opportunity for the GM torenia lines to transfer the introduced genes to other torenia 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 torenia 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 environment.

Risk management

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

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

Licence conditions to manage this limited and controlled release

The Acting Regulator has imposed a number of licence conditions to limit and control the release, including requirements to:

- ♦ conduct the release on a maximum total area of 20 m² at one site in the local government area of Darebin (Victoria) between October 2008 and May 2009
- ♦ locate the trial site within the perimeter of existing Florigene greenhouse infrastructure, which is surrounded by a 2.1 metre fence and lockable gates
- ♦ grow the plants hydroponically at ground level in 1000 litre plastic tubs
- ♦ visually monitor the site twice per week during the 12-week growing period
- ♦ complete full written inspections of the site: on a monthly basis during the 12-week growing period; and after severe weather; and if any non-compliances are detected during a bi-weekly visual monitoring
- ♦ destroy all plant materials not required for laboratory analysis
- ♦ clean all equipment used in cultivation practices, and
- ♦ not permit any materials from the release to be used in human food or animal feed.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs, July 2007*⁶; *Policy on transport and supply of GMOs, July 2005*⁷). Licence conditions based on these guidelines and policies have also been

⁶Guidelines for the transport of GMOs

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

⁷ Policy on transport and supply of GMOs

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

imposed 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 Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA), the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and the Australian Quarantine and Inspection Service (AQIS)⁸.

FSANZ is responsible for human food safety assessment, including GM food. As the trial involves proof of concept research, the applicant does not intend any material from the GM torenia lines proposed for release to be used in human food. Accordingly, the applicant has not applied to FSANZ to evaluate any of the GM torenia lines. However, the flowers of torenia are reportedly consumed by some people in salads and FSANZ approval would need to be obtained before they could be sold as human food in Australia.

Identification of issues to be addressed for future releases

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

- ♦ characterisation of the genetic material inserted into the plants, including genetic stability
- ♦ characteristics indicative of weediness including altered sexual and asexual reproductive capacity, tolerance to environmental stresses and disease, altered plant growth in soil
- ♦ additional data on the potential toxicity and allergenicity of plant materials from the GM torenia lines.

Suitability of the applicant

The Regulator determined, at the commencement of the assessment process for this application, that Florigene is suitable to hold a DIR licence under the requirements of section 58 of the Act. The Acting Regulator is satisfied that Florigene 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 concluded that this limited and controlled release of three GM torenia lines on a maximum total area of 20 m² over eight months in the Victorian local government area of Darebin poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

⁸ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

The risk management plan concluded 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 materials 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.

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 and the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation⁹, details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant horticultural practices. The parameters for the risk assessment context are summarised in Figure 1.

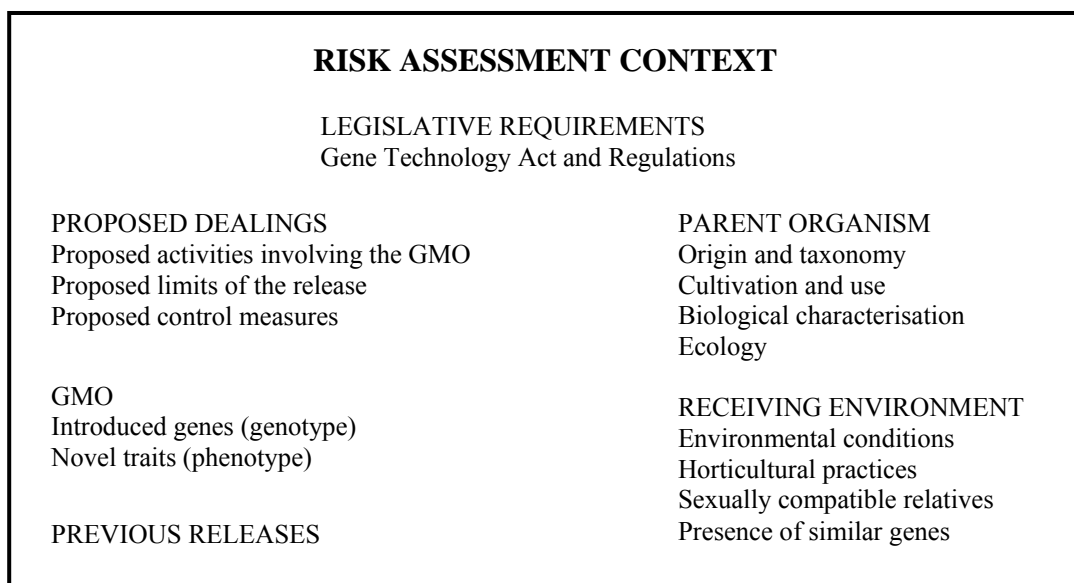


Figure 1 Components of the context considered during the preparation of the risk assessment

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 horticultural practices (Section 6.3)
 - ♦ the presence of related plants in the environment (Section 6.4)
 - ♦ the presence of the introduced or similar genes in the environment (Section 6.5)
 - ♦ any previous releases of these or other GMOs relevant to this application (Section 7)

⁹ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>> and in the *Risk Analysis Framework* (OGTR 2007) <<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 Gene Technology Regulator (the Regulator) must take into account, and with whom she must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions on licence applications. In addition, the Gene Technology Regulations 2001 (the Regulations) outline matters the Regulator must consider when preparing a RARMP.

4. In accordance with section 50A of the Act, the Regulator 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 GMO and its 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 Acting Regulator has prepared a RARMP for this application.

Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public. The advice from the prescribed experts, agencies and authorities, and where it was taken into account, is summarised in Appendix B. No submissions were received from the public.

5. 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

6. Florigene Pty Ltd (Florigene) proposes to release three torenia lines¹⁰ that have been genetically modified for enhanced phosphate uptake. The release would be conducted under limited and controlled conditions.

3.1 The proposed activities

7. Up to four hundred GM torenia plants will be grown hydroponically. The applicant has stated that the principal purpose of the proposed release is to conduct proof of concept research involving experiments with the GM torenia lines to assess their capacity to absorb phosphate and slow or repress algal overgrowth in the surrounding water. The GM torenia plants will not be used for human food or animal feed.

8. Plants will be grown from cuttings derived from GM mother plants currently cultivated in a Physical Containment Level 2 (PC2) greenhouse at the same location. Plants will be hydroponically established in sponge plugs, transferred to purpose-built holders before being placed in 1000 litre impermeable plastic tubs (4-6 in total), which would contain approximately 300 litres of a hydroponic solution. The tubs will be placed on the ground in

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

full sunlight. The tubs are made from heavy-duty plastic and would be unlikely to leak or burst.

9. Plants will be allowed to grow and flower over a single 12 week period within the total proposed duration (see Section 3.2 below) and may be pruned in order to stimulate flowering, to collect flowers for analysis, or to maintain a manageable plant size. A number of parameters will be measured during the trial including water usage, phosphate concentrations in the hydroponic solution, the growth of any opportunistic algal species, and plant growth.

10. All plant materials not required for analysis will be destroyed by placing them into plastic bags and storing them in a lidded waste skip for at least a month on site. Non-viable plant material will then be disposed of via standard methods for non-transgenic plants (eg landfill).

11. Following removal of plants and plant material, hydroponic medium will be disposed of by tipping it onto the ground, where it will soak at the site. Tubs and floats will be washed and treated with bleach for re-use.

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

12. The release is proposed to take place at one site in the local government area of Darebin, Victoria, on a maximum total area of 20 m², between October 2008 and May 2009.

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

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

- ◆ locating the trial site within the perimeter of existing Florigene greenhouse infrastructure, at La Trobe University, Bundoora, which is surrounded by a 2.1 metre fence and lockable gates, and accessible only to trained and authorised staff
- ◆ growing the plants hydroponically at ground level in 1000 litre plastic tubs
- ◆ visually monitoring the site twice per week during the 12-week growing period
- ◆ completing full written inspections of the site: on a monthly basis during the 12-week growing period; and after severe weather; and if any non-compliances are detected during a bi-weekly visual monitoring
- ◆ destroying all plant materials not required for laboratory analysis
- ◆ destroying any volunteers
- ◆ transporting GM plant materials in accordance with OGTR transportation guidelines, and
- ◆ not using the GMOs in human food or animal feed.

14. These controls, and the limits outlined in Section 3.2, have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.

Section 4 The parent organism

The parent organism is the ornamental flowering plant, *Torenia x hybrida* (cv. Summer Wave[®] Blue), which is exotic to Australia but commercially available for planting in home gardens. *T. x hybrida* was specifically bred as a pot plant and is a sterile hybrid that does not set seed or produce viable pollen. Further detailed information about the parent organism is

contained in a reference document, *The Biology of Torenia spp. (torenia)*, that was produced to inform the risk assessment process for licence applications involving GM torenia plants (OGTR 2008). The document is available from the OGTR or from the website <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

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

5.1 Introduction to the GMOs

15. The three GM torenia lines, TT4-1, TT14-7 and TT19-7, contain the *phosphate starvation response regulator 1 (PHR1)* gene from thale cress (*Arabidopsis thaliana*) under the control of a different promoter in each line. In line TT4-1, expression of the *PHR1* gene is controlled by a chimeric promoter (E12 35S Ω) comprising the Cauliflower mosaic virus (CaMV) core promoter and a G-free sequence (Ω sequence) from the 5'-untranslated region of Tobacco mosaic virus (TMV) (Mitsuhara et al. 1996); the terminator region is from the *nopaline synthase (nos)* gene from *Agrobacterium tumefaciens*. In line TT14-7, expression is controlled by another chimeric promoter (MAC) comprising elements from the CaMV 35S promoter and the *mannopine synthase (mas)* gene promoter from *A. tumefaciens* (Comai et al. 1990); the terminator region is also from the *mas* gene. In line TT19-7, expression is controlled by two promoters [MAC and a modified promoter, the details of which have been declared Confidential Commercial Information (CCI)]; the terminator region is from the *mas* gene. The *PHR1* gene is constitutively expressed in each of the three GM torenia lines.

16. The *PHR1* gene encodes a transcription factor¹¹ thought to play a role in plant responses to phosphate deficiency. Each line was generated independently using 1 of 3 different transformation vectors.

17. In addition, all of the GM torenia lines contain the antibiotic resistance selectable marker gene, *neomycin phosphotransferase type II (nptII)*. This gene, encoding for the enzyme, neomycin phosphotransferase, was derived from *Escherichia coli*, and confers kanamycin or neomycin resistance on the GM plant. In each line, expression of the *nptII* gene is under the control of the promoter and terminator regions of the *nos* gene from *A. tumefaciens*.

5.2 Introduction to phosphate uptake in plants

18. Phosphorus is an essential plant nutrient that plays an important role in the biosynthesis of nucleic acids and cell membranes (via the formation of phospholipids), energy metabolism (photosynthesis and respiration) via adenosine triphosphate (ATP), enzyme regulation and signal transduction (Raghothama 1999; Poirier & Bucher 2002; Bucher 2006).

19. Phosphorus is a limiting factor for plant growth and is poorly bio-available from soil (Bucher 2006) because it normally forms insoluble complexes or is bound in organic matter (Poirier & Bucher 2002). Phosphorus (in the form of inorganic orthophosphate) is predominantly taken up at the root periphery through the root epidermis and root hairs (Karandashov & Bucher 2005), and at root and mycorrhiza interfaces via fungal mycelium (Karandashov & Bucher 2005; Bucher 2006). Some plant species also possess cluster (or proteoid) roots, which are specialised structures enabling the acquisition of phosphate from nutrient poor soils (Vance et al. 2003). Phosphate is then 'loaded' into the xylem and distributed to various tissues (Poirier & Bucher 2002). Once inside cells, phosphate enters a

¹¹ A transcription factor is any protein required for the recognition, by RNA polymerases, of specific regulatory sequences in genes (eg a promoter)

number of organelles (eg plastids, mitochondria) and may exchange with other solutes (eg triose-, pentose- or hexose-phosphates, phosphoenolpyruvate) (Poirier & Bucher 2002). Phosphate uptake into plant cells and organelles is mediated by a number of high and low affinity membrane transporters encoded by the *Pht1* gene family [reviewed by Bucher (2006); Karandashov & Bucher (2005); Raghothama (1999)].

20. The vacuole serves as the main intracellular storage site for phosphate in higher plants but under conditions of starvation is localised to the cytosol and chloroplasts (Poirier & Bucher 2002). In this way, phosphorus homeostasis is maintained by ensuring that the metabolic pool of phosphate remains constant.

21. Plants maintain phosphorus homeostasis under limiting conditions by conserving existing phosphate stores and enhancing uptake from the environment (Vance et al. 2003; Ticconi & Abel 2004). The 'phosphate starvation response' has been reviewed by a number of authors (Raghothama 1999; Poirier & Bucher 2002; Davies & Peoples 2003; Vance et al. 2003; Ticconi & Abel 2004; Franco-Zorrilla et al. 2004) and involves:

- ♦ modification of the root system to improve soil exploration for phosphate (restructuring root architecture and increasing the length and density of root hairs to expand the root surface area; proteoid root formation)
- ♦ increased mobilisation of soil phosphate (via the secretion of phosphohydrolases and acid phosphatases that degrade organic matter, and organic acids that release soil-bound or mineralised phosphates)
- ♦ modification of phosphate transport (increased expression of high affinity phosphate transporters; redistribution of phosphate from senescent tissue via acid phosphatases)
- ♦ metabolic adjustments, which recycle and conserve phosphate [use of alternative respiratory pathways; altered carbon metabolism; increased secondary metabolism leading to enhanced flavonoid (eg anthocyanins) and indole alkaloid production], and
- ♦ increased gene expression.

22. There are a number of regulators of the phosphate starvation response in plants including: hormones (Karthikeyan AS et al. 2002); microRNAs (miRNAs) such as miRNA399, which down regulates a ubiquitin-conjugating E2 enzyme (encoded by the *PHO2* gene)(Chiou et al. 2006); enzymes such as the SUMO E3 ligase (Miura et al. 2008); and transcription factors such as BHLH32 (Chen et al. 2007) and PHR1 (see below). However, the overall pathways and their integration remain poorly understood (Bari et al. 2006).

5.3 The introduced genes and their encoded proteins

5.3.1 The *PHR1* gene and encoded protein

23. The *PHR1* gene encodes a transcription factor, which binds as a dimer to an imperfect palindromic sequence of eight base pairs in the promoter region of phosphate starvation-response genes (Rubio et al. 2001). The key role of the PHR1 protein is to co-ordinate the regulation of phosphate starvation response genes including those encoding phosphate transporters, acid phosphatases and ribonucleases [reviewed by Franco-Zorrilla (2004)]. Some of the effects of the PHR1 protein are mediated via the miRNA399/*PHO2* gene pathway leading to an accumulation of phosphate in plant tissue (Bari et al. 2006; Chiou et al. 2006; Chen et al. 2007).

24. The PHR1 protein (from *A. thaliana*) is 409 amino acids and contains a MYB-related domain (characteristic of DNA binding proteins) and a coil-coil domain, which may be

involved in protein-protein interactions (Rubio et al. 2001). Expression of the *PHR1* gene occurs independently of phosphate status but is increased during phosphate starvation, which has led to the hypothesis that PHR1 activity is regulated post-translationally (Rubio et al. 2001; Bari et al. 2006).

25. With regard to orthologous expression, to date the *PHR1* gene has only been expressed in transgenic *Arabidopsis thaliana* knockout mutants (Nilsson et al. 2007) and not in any other species.

5.3.2 Toxicity/allergenicity of the PHR1 protein

26. The PHR1 protein has functional homology with the Phosphate Starvation Response 1 (PSR1) protein from *Chlamydomonas reinhardtii* (a unicellular green alga) (Sims 1994) and there are homologous genes *OsPHR1* and *OsPHR2* in rice (Yi et al. 2005) although only *OsPHR2* overexpression results in accumulation of phosphate under conditions of phosphate starvation (Zhou et al. 2008). Such MYB-based regulators of phosphate starvation are likely to be common to vascular plants and unicellular green algae (Rubio et al. 2001) if not all photosynthetic organisms. On this basis, people and other organisms have a long history of exposure to PHR1-like proteins. In addition, the three GM torenia lines have been grown by Florigene staff in a contained glasshouse, with no report of any adverse reactions resulting from handling plant material.

27. No toxicity/allergenicity tests have been performed on the PHR1 protein as the proposed trial is still at proof of concept stage. Such tests may have to be conducted if approval was sought for the GMOs to be considered for human consumption in Australia (see discussion in Section 7.1.2).

28. 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 amino acid sequence of the PHR1 protein from *Arabidopsis* was compared to databases of known toxins and allergens. The results of these analyses did not indicate that the PHR1 protein shared any significant sequence homology with any known toxins or allergens.

29. A comprehensive search of the scientific literature also yielded no information to suggest that the PHR1 protein is toxic or allergenic to people, or toxic to other organisms. In addition, transcription factors per se are not known to be toxic or allergenic.

5.3.3 Adverse effects of phosphorus

30. Phosphorus is essential for all living organisms but may be toxic at high enough levels. In people, the upper level of (dietary) intake of phosphorus is 3000-4000 mg/day, with no evidence to indicate that intakes above these limits cause harm (NH&MRC 2006). In plants adapted to low nutrient environments, such as members of the *Proteaceae* family, high levels of phosphorus may cause toxicity, manifesting as growth inhibition, early leaf senescence and chlorotic and/or necrotic regions on the leaves (Shane et al. 2004). The run-off of phosphorus from natural and artificial sources can contribute to the eutrophication of waters resulting in algal blooms, which can cause deoxygenation, toxicity, loss of amenity and biodiversity, and affect water for drinking and recreational use (Drewry et al. 2006).

5.3.4 The selectable marker gene (*nptII*) and the encoded protein

31. All of the GM torenia lines contain the antibiotic resistance selectable marker gene, *nptII*. This gene, encoding for the enzyme, NPTII, was derived from *Escherichia coli* and

confers kanamycin or neomycin resistance on the GM plant. The *nptII* gene was used as a selective marker to identify transformed plant tissue during initial development of GM plants in the laboratory.

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

5.4 The regulatory sequences

33. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. Information on the promoters and terminators used in the proposed release is given in Section 5.1.

34. While some of the regulatory sequences are derived from plant pathogens (*Agrobacterium tumefaciens*, CaMV, TMV), the sequences are not pathogenic in themselves nor do they cause any disease symptoms in the GM plants.

5.5 Method of genetic modification

35. The three GM torenia lines, TT4-1, TT14-7 and TT19-7 were generated by *Agrobacterium tumefaciens*-mediated transformation using binary vectors pSPS1898, pSPB2314 and pSPB2376, respectively. The transformation vectors and tissue cultures were produced by Suntory Ltd Research Centre (Osaka, Japan), with the tissue cultures imported into Australia under AQIS Permit 200319814. Construction of the binary vectors was based on pBINPLUS (van Engelen et al. 1995). The three lines were generated from independent transformation events using disarmed strains of *A. tumefaciens* to transform plant cells in culture (Aida & Shibata 1995). This method of transformation is used extensively to genetically modify plants (Valentine 2003) and has been discussed in previous RARMPs [most comprehensively for DIR 060/2005 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir060-2005>) or by contacting the OGTR].

5.6 Characterisation of the GMOs

5.6.1 Stability and molecular characterisation

36. The sequence and function of all introduced genetic elements is known. The presence of the *PHR1* gene in the three torenia lines has been confirmed by reverse transcription polymerase chain reaction (RT-PCR). Southern analysis indicated a single insertion site for the *PHR1* gene in line TT4-1, while multiple integrations have been determined for line TT14-7. No Southern analysis has been conducted on line TT19-7.

5.6.2 Characterisation of the phenotype of the GMOs

37. Data provided in the application indicated that under greenhouse conditions, lines TT4-1 and TT19-7 depleted phosphate from hydroponic media more rapidly than non-GM torenia. The observed enhancement of phosphate uptake in lines TT4-1 and TT19-7 is consistent with

that observed in *A. thaliana* plants over-expressing the *PHR1* gene (Nilsson et al. 2007). In contrast, depletion of phosphate from hydroponic media in line TT14-7 was comparable to non-GM torenia. On this basis, line TT14-7 would be used as a comparator line in the proposed limited and controlled release.

38. The applicant does not expect expression of the *PHR1* gene to alter the morphology or to effect physiological processes in the GM torenia lines. During contained hydroponic experiments, no apparent differences in appearance or growth were observed between the GM torenia lines, mother plants and non-GM parent (*T. x hybrida*).

39. The applicant stated that the enhanced phosphate uptake phenotype has been shown to be stable for four years in experiments conducted in Australia and Japan.

Section 6 The receiving environment

40. 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 horticultural 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

41. The abiotic factors relevant to the growth and distribution of torenia plants in Australia are discussed in the *Biology of Torenia spp.* document (OGTR 2008).

42. The release is proposed to take place at a single site in the City of Darebin, Victoria, from October 2008 to May 2009. This region is in the temperate climatic type, with no dry season and a warm summer (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology). Table 1 summarises the average temperature and rainfall data at Bundoora, Victoria, over the proposed trial duration.

Table 1: Climatic data for Bundoora, Victoria

Parameter	Oct	Nov	Dec	Jan	Feb	Mar	April	May
Average daily max temperature (°C)	19.1	22.0	24.5	26.1	26.9	23.9	20.2	16.6
Average daily min temperature (°C)	8.5	10.3	12.2	13.8	14.0	12.2	9.6	7.8
Average monthly rainfall (mm)	70.8	64.9	64.9	50.3	38.4	44.8	58.1	54.2

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

43. It is proposed to locate the tubs on flat gravel within approximately 7 m of two concrete-edge, grated, storm water drains that are raised above the gravel. In the unlikely event of the tubs bursting or overflowing the solution from the tubs would seep into the gravel before reaching the drains and any plant material in the tubs would remain on the ground.

6.2 Relevant biotic factors

44. The biotic factors pertaining to the growth and distribution of commercial torenia plants in Australia are discussed in the *Biology of Torenia spp.* document (OGTR 2006).

6.3 Relevant horticultural practices

45. The GM torenia plants will be grown hydroponically as described in Section 3.1 of this Chapter. Plants would be allowed to grow and flower over a single 12 week period within the

total proposed duration and may be pruned in order to stimulate flowering, to collect flowers for analysis or to maintain a manageable plant size (see Section 3.1). The size, location and duration of the proposed limited and controlled release of the GM torenia lines are outlined in Section 3.2 of this Chapter.

6.4 Presence of related plants in the receiving environment

46. There are a number of wildlife reserves within proximity to the trial site including the Melbourne Wildlife Sanctuary, Gresswell Forest Reserve, Gresswell Habitat Link and Gresswell Hill.
47. A botanical survey of the general area of the trial site conducted by BIOSIS Research Melbourne recorded fifty-seven plant species. No species from the genus *Torenia* were found, while the only member of the Scrophulariaceae family observed was *Veronica gracilis*. There is no information to suggest that *T. x hybrida* is able to cross with this species.
48. A survey of the Gresswell Forest Nature Reserve, which is located approximately one km from the trial site, recorded approximately 250 plant species, with only two of these from the Scrophulariaceae family (*Gratiola latifolia* and *Veronica gracilis*) (http://www.LaTrobe.edu.au/wildlife/APP_1.pdf). There is no information to suggest that *T. x hybrida* is able to cross with these species.
49. A residential area located approximately 200m from the trial site may have *Torenia* species growing in some of the gardens.
50. GM torenia plants with altered flower colour are currently being grown in hanging baskets at the same location under Licence DIR 068/2006¹².

6.5 Presence of the *PHR1* gene, PHR1 protein and phosphate in the environment

51. Homologues of the *PHR1* gene occur naturally in vascular plants and unicellular algae (Rubio et al. 2001). Therefore, it is expected that humans and other organisms routinely encounter the PHR1 protein, or its homologues, through contact with plants and food derived from plants. This information forms the baseline data for assessing the risks from exposure to these enzymes as a result of the trial of the GM torenia lines.
52. The *nptII* 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.
53. Phosphorus is an essential element required by all living organisms and occurs naturally in the environment by virtue of its presence in both organic and inorganic material. The concentration of available phosphorus (as phosphates) in soils is approximately 2 µM, while it is several orders of magnitude higher in plants (5-20 mM) (Raghothama 1999). On a dry weight basis, the concentration of phosphorus in plants ranges from 0.05 to 0.50% (Vance et al. 2003). Relatively high levels of phosphates are found naturally in fish (4000 mg/kg), poultry (2100 mg/kg), red meats (1600 mg/kg), dairy and cereals (>900 mg/kg) (Expert Group on Vitamins and Minerals 2003).

¹² Information on DIR 068/2006, including the licence conditions is available on the OGTR website at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir068-2006>

Section 7 Australian and international approvals

7.1 Australian approvals of the GM torenia lines

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

54. There has been no release of these GM torenia lines in Australia. The three GM torenia lines were developed in Japan and brought to Australia where they have been grown in a PC2 greenhouse at the proposed site under Notifiable Low Risk Dealing (NLRD) 15 (OGTR Reference 1611/2005).

55. GM torenia lines with altered flower colour, derived from *T. x hybrida*, were previously approved for limited and controlled release under Licence DIR 068/2006.

7.1.2 Approvals by other Australian government agencies

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

FSANZ is responsible for human food safety assessment and food labelling, including GM food. The applicant does not intend to use materials from the GM torenia lines in human food, accordingly an application to FSANZ has not been submitted. However, the flowers of torenia are reportedly consumed by some people in salads and FSANZ approval would need to be obtained before they could be sold as human food in Australia.

7.2 International approvals

57. There has been no release of these GM torenia lines in any other country.

Chapter 2 Risk assessment

Section 1 Introduction

58. 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.

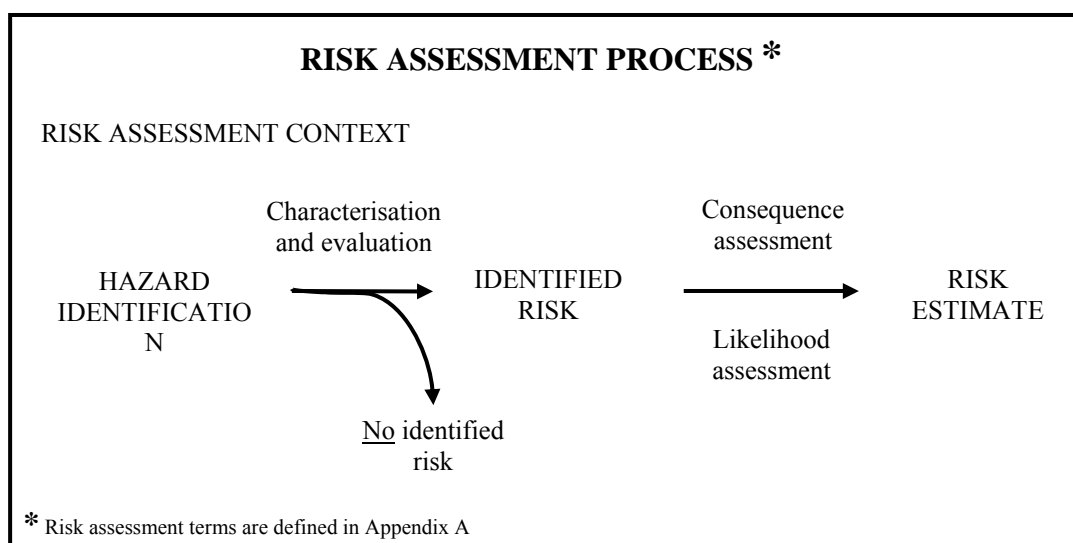


Figure 4 The risk assessment process

59. 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.

60. 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 may be an event, a substance or an organism (OGTR 2007).

61. 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.

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

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

Section 2 Hazard characterisation and the identification of risk

64. 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.

65. 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.

66. 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 gene product(s)
- ♦ potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs¹³
- ♦ potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- ♦ the biotic and abiotic factors at the site of release
- ♦ horticultural management practices for the GMOs.

67. The eight events that were characterised are discussed in detail later in this Section. They are summarised in Table 2 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.

¹³ As discussed in Sections 5.3.4 of Chapter 1, the *npIII* gene and its product has already been considered in detail in previous RARMPs and by other regulators. It has not been found to pose risks to either people or the environment and will not be considered further.

Table 2 Summary of events that may give rise to an adverse outcome through the expression of the introduced gene for enhanced phosphate uptake

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 or allergenic to people, or toxic to other organisms	1. Exposure to GM plant materials containing the PHR1 protein and phosphate.	Allergic reactions in people, or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The PHR1 protein is widespread in the environment and unlikely to be toxic/allergenic to people or toxic to other organisms. The level of phosphate acquired by the GM plants is unlikely to be toxic. The proposed release is limited and controlled, further reducing exposure to the PHR1 protein and any end products.
Section 2.2 Spread and persistence (weediness) of the GM torenia lines in the environment	2. Expression of the introduced gene improving the survival of GM torenia plants.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The genetic modification is not expected to affect the survival or low weediness potential of the GM lines. The proposed limits and controls would minimise persistence.
	3. Dispersal of viable GM plant materials.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The genetic modification is not expected to affect the dispersal or low weediness potential of the GM lines. The proposed limits and controls would minimise dispersal. Events 1 and 2 associated with potential for toxicity/allergenicity and enhanced survival of the GM plants were not considered to give rise to an identified risk
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced gene or regulatory sequences in sexually compatible plants.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The male (and female) infertility of non-GM torenia is not expected to be altered by the introduced gene. Thus it is highly unlikely that crossing with sexually compatible plants would occur. The proposed limits and controls would minimise transfer.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence of the introduced gene, or regulatory sequences, in unrelated organisms as a result of gene transfer.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The introduced gene or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via natural mechanisms. Events 1–3 associated with potential for toxicity, allergenicity or weediness as a result of expression of the introduced gene were not considered to give rise to identified risks..
Section 2.5 Unintended changes in biochemistry, physiology or ecology	6. Changes to biochemistry (including innate toxic or allergenic compounds), physiology or ecology of the GM torenia lines resulting from altered expression or random insertion of the introduced gene.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	<ul style="list-style-type: none"> Unintended changes are likely to be detected and eliminated during the selection process. The potential for adverse effects would be minimised by the proposed limits and controls.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.6 Unintended presence in the environment of <i>A. tumefaciens</i> containing the introduced genes	7. Transfer of the introduced genes from <i>A. tumefaciens</i> to other organisms.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	<ul style="list-style-type: none"> It is highly unlikely that <i>A. tumefaciens</i> could conjugate with other <i>A. tumefaciens</i> strains or other bacteria naturally present at the site. It is highly unlikely that the <i>A. tumefaciens</i> would infect other plants at the site. Events 1–3, 5 and 6 associated with potential for toxicity, allergenicity, weediness and expression of the introduced genes in other organisms were not considered to give rise to identified risks.
Section 2.7 Unauthorised activities	8. Use of the GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.6	No	<ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

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

68. Toxicity is the adverse effect(s) of exposure to a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000). Allergenicity is the potential of a protein to elicit an immunological reaction following exposure, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

69. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced gene for enhanced phosphate uptake. Workers cultivating the torenia plants would be exposed to all plant parts. Organisms may be exposed directly to the proteins through biotic interactions with GM torenia plants (vertebrates, insects, symbiotic microorganisms and/or pathogenic fungi, and algae in the hydroponic media) or through contact with dead plant material. Indirect exposure would include organisms that feed on organisms that feed on GM torenia plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

Event 1: Exposure to GM plant materials containing the PHR1 protein and phosphate.

70. There are no known toxic or allergenic properties of non-GM torenia (OGTR 2008). Although no toxicity/allergenicity tests have been performed on the pHR1 protein, it is unlikely that the introduction of the *PHR1* gene would alter this on the basis of the following considerations:

- The *PHR1* gene is present in vascular plants and therefore an orthologous gene is already likely to be present in non-GM torenia (Rubio et al. 2001);
- The encoded PHR1 protein is unlikely to be toxic or allergenic to people or other organisms (see Section 5.3.2 of Chapter 1); and
- GM torenia plants are unlikely to acquire phosphate to a level that is toxic to people or other organisms (see Section 5.3.3 and 6.5 of Chapter 1).

71. People and other organisms are already exposed to the encoded PHR1 protein and phosphates by virtue of their natural and widespread presence in the environment.

72. The proposed limits and controls of the trial would minimise the likelihood of exposure of people and other organisms to GM plant materials. Exposure of people would be limited to trained and authorised staff associated with the proposed trial because only they would have access to the site and as no plant material will be used as food, animal feed or plant products. Exposure of other organisms would be limited to algae and other micro-organisms growing in the hydroponic media or birds or flying insects that could access the plants growing hydroponically in the tubs.

73. **Conclusion:** The potential for allergic reactions in people or toxicity in people or other organisms as a result of exposure to GM plant materials containing the PHR1 protein is **not an identified risk** and will not be assessed further.

2.2 Spread and persistence (weediness) of the GM torenia lines in the environment

74. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM torenia plants in particular, is provided in the *Biology of Torenia spp.* document (OGTR 2008). In summary, *T. x hybrida* does not possess characteristics that are usually associated with weediness and does not pose a weed problem in Australia.

75. Scenarios that could lead to increased spread and persistence of the GM torenia lines include expression of the introduced gene conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These events could lead to increased exposure of people and other organisms to the encoded protein and any end products.

Event 2: Expression of the introduced gene improving the survival of the GM torenia plants

76. Baseline information on the weediness of *T. x hybrida* is provided in the *Biology of Torenia spp.* document (OGTR 2008). *T. x hybrida* is a sterile hybrid that is incapable of sexual reproduction. As outlined in the RARMP prepared in relation to Licence DIR 068/2006, *T. x hybrida* was bred as a pot plant, rather than an in-ground plant. In this habitat, *T. x hybrida* is typically an annual that dies back during the winter in temperate regions or as a result of disease, insect infestation or general deterioration. *T. x hybrida* does not produce seed or long-term survival structures (such as stolons, runners or rhizomes) and detached stems do not have the propensity to form roots (and possibly new plants) except through deliberate horticultural intervention. Supplementary information provided in relation to an application to vary Licence DIR 068/2006 indicated that *T. x hybrida* does not regenerate from roots and cannot be propagated by root cuttings. Results from a preliminary experiment indicated that leaves and stem pieces are readily desiccated after 23 days when left on the ground or under mulch. Introduction of the *PHR1* gene is unlikely to alter these characteristics.

77. Data provided in the application indicated that introduction of the *PHR1* gene into two of the three torenia lines (lines TT4-1 and TT19-7) enhanced phosphate uptake from hydroponic media relative to non-GM *T. x hybrida*. It is a reasonable expectation that these same lines would show enhanced phosphate uptake when grown in soil. On this basis, these two lines could show improved survival and therefore have a selective advantage in soils where phosphorus is a limiting factor for plant growth. Further, the lines could deplete phosphorus from soil and ‘starve’ surrounding vegetation. However, given that *T. x hybrida* behaves as an annual in the cool-winter Victorian climate and has no capacity to reproduce sexually and only very limited capacity to reproduce asexually, any such survival advantage would be short lived and not expected to increase the weediness of the GM torenia lines.

78. Given that the phosphate starvation response in plants involves the modification of the root system to improve soil exploration for phosphate (Vance et al. 2003; Ticconi & Abel 2004), introduction of the *PHR1* gene could result in changes to the morphology of the root system of GM torenia plants. The applicant stated that there were no differences observed between GM and non-GM *T. x hybrida* during contained hydroponic dealings with the three GM torenia lines. However, it remains to be determined whether root morphology would be altered in GM torenia plants grown in soil and whether this would have implications for establishment, survival and persistence in the environment.

79. The main purpose of the proposed release is to conduct proof of concept experiments with the GM torenia lines to assess their capacity to absorb phosphate and slow or repress opportunistic algal overgrowth in the surrounding water. Thus, any characteristics that may impact on the survivability of the GM plants will be closely monitored during the proposed trial.

80. In the unlikely event that survival was improved, GM torenia plants are unlikely to spread and persist in the environment because of their sterility and limited capacity to reproduce asexually. Furthermore, the proposed control measures, such as growing the GM plants in tubs, visually monitoring the trial site twice per week during the 12-week growing period and completing full written inspections (on a monthly basis during the 12-week growing period; and after severe weather; and if any non-compliances are detected during a bi-weekly visual monitoring) would assist in ensuring that plants or plant material are not dispersed into the environment.

81. **Conclusion:** The potential for the increased survival of the GM torenia lines as a result of the expression of the introduced gene for enhanced phosphate uptake is **not an identified risk** and will not be assessed further.

Event 3: Dispersal of viable GM plant materials

82. The limits and controls on the proposed release (outlined in Chapter 1, Sections 3.2 and 3.3) will ensure that dispersal of plant material is minimised.

83. The proposed release would be confined to one site, within the fenced perimeter of existing Florigene greenhouse infrastructure at La Trobe University, Bundoora, Victoria. The site is at the same location where GM torenia with altered flower colour are currently being grown under Licence DIR 068/2006. The site is surrounded by a 2.1 m fence, which has lockable gates. These gates remain locked at all times except when personnel or vehicular access is required. Only trained and authorised staff will be permitted access to the site and to handle the GMOs. This minimizes the potential for GM torenia plants to disperse into the environment or come into contact with the public or large animals. Limiting the duration of the release to a one year period would also restrict the opportunity for GM plants to disperse outside the site.

84. Growing the plants hydroponically at ground level in 1000 litre plastic tubs would minimise the potential for dispersal into the environment because plants will not be in contact with soil. There may be some potential for very heavy rain to cause the plastic tubs to overflow thereby dispersing viable plant material into the environment. However, this scenario is highly unlikely as the 1000 litre tubs would only contain approximately 300 litres of hydroponic medium; tubs would need to be filled with approximately 700 litres of rain to overflow. Since the GM plants will only be grown in one 12-week period, it is unlikely that sufficient rain would occur during this period to fill the tubs. The risk of overflow is further mitigated by the applicant's proposal to monitor the site as soon as possible after severe

weather. The likelihood of the tubs bursting is also low given their construction from heavy-duty plastic.

85. As discussed in paragraph 43 the tubs would be located within 7 m of two storm water drains. The likelihood of plant material from the tubs entering the drains in the event that the tubs overflow or burst is low.

86. Dispersal of plant material is greatly limited by both the female sterility of flowers, which therefore do not produce seed, and the poor ability for vegetative reproduction outside a managed horticultural environment.

87. Dispersal of plant material by animals is unlikely because of the location of the tubs within a high fence. Dispersal by birds is also unlikely. The nearest major body of water is Upper Lakes, about 500 m from the location, within the main campus of La Trobe University. Such a body of water would be a habitat for water birds that may be attracted to the trial. However, in testing of the hydroponic system at the location using non-GM plants there was no observation or indication of bird visits. The applicant has observed Noisy Miner birds (*Manorina melanocephala*) visiting the flowers of GM torenia plants in hanging pots associated with DIR 068/2006 but the birds did not appear to remove any plant material.

88. In the unlikely event that material is dispersed away from the proposed site it is unlikely to be a source of potential harm because the GM lines are unlikely to establish outside of the release site, or to be toxic/allergenic (see Events 1 and 2).

89. **Conclusion:** The potential for dispersal of viable GM plant materials is **not an identified risk** and will not be assessed further.

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

90. 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 plants, vertical gene flow could therefore occur via successful cross pollination between the plant and neighbouring plants, related weeds or native plants (Glover 2002).

91. Baseline information on vertical gene transfer associated with non-GM torenia plants is provided in the *Biology of Torenia spp.* document (OGTR 2008). In summary, *T. x hybrida* is sterile and therefore the chances of natural hybridisation either within the same species or between different *Torenia* species are remote.

Event 4: Expression of the introduced genes and regulatory sequences in sexually compatible plants

92. Data presented in the RARMP prepared in relation to Licence DIR 068/2006 indicated that *T. x hybrida* produces pollen grains that are misshapen and do not germinate. It is unlikely that introduction of the *PHR1* gene and subsequent expression of the PHR1 protein would alter pollen viability in GM torenia plants because the role of this transcription factor does not include any involvement in plant reproduction. On this basis it is highly unlikely that the *PHR1* gene or regulatory sequences could be transferred to sexually compatible plants via pollen.

93. Nine lines of GM *T. x hybrida* with altered flower colour are currently authorised for growing in hanging baskets at the same location under Licence DIR 068/2006. Outcrossing with these lines to produce a ‘stacked’ GMO is highly unlikely because they are also male and female sterile. As discussed in Section 6.4 of Chapter 1, a survey of the immediate area of the proposed release site, in addition to a nature reserve one kilometre away, did not identify any

sexually compatible species. While it is not known whether sexually compatible plants would be growing (and flowering) in a residential area approximately 200 m from the site, the likelihood of crossing is remote because the GM torenia plants would not produce viable pollen.

94. While it is unlikely that the GM torenia plants will differ in their sexual reproduction characteristics from the parent organism, the likelihood of vertical gene transfer occurring will be further reduced by the close monitoring of the GM torenia plants during the proposed trial. In addition, the very small size, single location and short duration of the trial would minimise the likelihood of vertical gene transfer occurring.

95. All of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to torenia 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 and, even if it did occur, the chance of an adverse effect to people or the environment is highly unlikely.

96. As described in Sections 5.1 and 5.4 of Chapter 1, the GM torenia lines contain a number of regulatory elements derived from pathogenic species including *E. coli*, CaMV, TMV and *A. tumefaciens*. However, the regulatory elements represent only a small proportion of the parent organism's genetic material and have not been reported to cause any adverse effects.

97. **Conclusion:** The potential for the expression of the introduced genes and regulatory sequences in 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

98. 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 data that alter this conclusion.

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

99. The probability of transferring the *PHRI* gene contained in the GM torenia plants is no greater than that of transferring the native gene. Non-GM torenia is likely to contain a homologue of the *PHRI* gene and therefore is already available for transfer via demonstrated natural mechanisms. In addition, homologues of the gene occur in all vascular plants and unicellular algae (Rubio et al. 2001) and thus is widespread in the environment.

100. 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 horizontal gene transfer is remote (see also discussion in Event 7).

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

102. **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

103. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy¹⁴ (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 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, and
- ♦ secondary effects arising from altered substrate or product levels in the biochemical pathway incorporating the protein encoded by the introduced gene.

104. Such unintended pleiotropic effects might result in adverse outcomes such as toxicity or allergenicity; weediness, altered pest or disease burden 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 torenia lines resulting from altered expression or random insertion of the introduced gene

105. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced genes, are already discussed for Events 1 and 2, which were not considered to give rise to identified risks.

106. As described in Section 5.3.1 of Chapter 1, the PHR1 protein is a transcription factor, which binds as a dimer to an imperfect palindromic sequence of eight base pairs in the promoter region of phosphate starvation-response genes (Rubio et al. 2001). While there is no evidence in the scientific literature that the *PHR1* protein regulates other types of genes, the possibility that the same eight base pair is present in the promoter region of non-phosphate starvation response genes cannot be completely ruled out. However, the likelihood of any pleiotropic effects causing adverse effects is minimised by the limits and controls outlined above.

¹⁴ Pleiotropy is the effect of one particular gene on the expression of other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

107. Activation of the phosphate starvation response in plants generally increases secondary metabolism leading to enhanced flavonoid (eg anthocyanins) and indole alkaloid production (Raghothama 1999; Poirier & Bucher 2002; Davies & Peoples 2003; Vance et al. 2003; Ticconi & Abel 2004). Flavonoids in particular are associated with a plant's response to environmental stressors (Koes et al. 1994) and therefore it is possible that the GM torenia lines could display enhanced pest and disease resistance or tolerance to stress relative to non-GM *T. x hybrida*. However, as plants would be monitored twice per week any such effects should be detectable by the trained staff involved in the trial.

108. Observations made during contained hydroponic experiments revealed no apparent differences in appearance or growth between the GM torenia lines, mother plants and non-GM *T. x hybrida*. On this basis, there is no evidence to date of any gross unintended changes in the GM torenia lines. However, there is uncertainty with regard to how the GM torenia plants might behave when grown in soil. While this is not necessarily a relevant consideration for the current hydroponic trial, it may be an issue that needs to be addressed for any future releases where plants may be grown in soil.

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

2.6 Unintended presence of *Agrobacterium tumefaciens* containing the introduced genes, during release

110. *Agrobacterium tumefaciens* is a soil-borne, Gram-negative bacterium that, in nature, causes crown gall on plants. For genetic modification, 'disarmed' strains of *A. tumefaciens* that cannot cause crown gall are used to transfer DNA to plant cells under controlled, optimized laboratory conditions. The strains used for genetic modification may also contain hypervirulent, attenuated tumour-inducing plasmids to increase cell transformation rates. *Agrobacterium tumefaciens* has been shown to be persistent in *in vitro* plant tissues and shoots. Broad spectrum antibacterial compounds tend to have a bacteriostatic effect, suppressing, but not eliminating bacterial growth and when removed the bacteria may resume growth. In particular, Gram-negative bacteria (such as *A. tumefaciens*) are considered to be difficult to eradicate completely from *in vitro* cultures (Barrett et al. 1997; Leifert & Cassells 2001), although persistence of *A. tumefaciens* in some transgenic plants has not been detected (Charity & Klimaszewska 2005).

111. During *Agrobacterium*-mediated transformation of plant cells, the *A. tumefaciens* attaches to plant cell walls and a virulence system is activated in the bacterium, ultimately allowing the transfer and integration of bacterial DNA into the plant DNA (de la Riva et al. 1998). As with most bacterial endophytes, disarmed strains of *A. tumefaciens* would be expected to inhabit the intercellular spaces and xylem vessels of plant tissue (Rosenblueth & Martínez-Romero 2006) via the formation of surface-associated biofilms (Danhorn et al. 2008). This means it is highly unlikely that *A. tumefaciens* would be incorporated into plant reproductive cells. For this reason, *A. tumefaciens* may persist in vegetatively propagated GM plants (such as *T. x hybrida*) since there would be no opportunity for elimination of the *A. tumefaciens* in sexually produced generations.

112. The transfer of GM torenia plants, carrying *A. tumefaciens*, into the environment could result in the transfer of genes to non-target plants or other microorganisms (Leifert 2000). Possible risks associated with the use of *A. tumefaciens* for genetic modification of plants under laboratory conditions have also been considered in previous RARMPs concerning GM rose (see DIR 060/2005 - available at

< <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir060-2005>> or by contacting the OGTR) and GM bananas (see DIR 076/2007 - available at

< <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir076-2007>> or by contacting the OGTR). Of relevance to the current torenia trial is DIR 060/2005 in which GM roses were grown hydroponically in pots above the soil and no risk was identified as a result of the unintended presence of *A. tumefaciens*.

Event 7: Transfer of the introduced gene from *A. tumefaciens* to other organisms

113. The GM torenia lines proposed for release were generated by *Agrobacterium*-mediated genetic modification (Section 5.5, Chapter 1). It is worth noting that one of the AQIS requirements for the importation of the GM torenia lines into Australia is that plant cultures are free from bacterial or fungal contamination or other disease symptoms. Information supplied by the applicant has indicated that testing of plants (in Japan) from each of the transgenic lines, by plating an aqueous slurry of plant material onto YEB medium (containing rifampicillin and tetracycline), did not result in the growth of any *A. tumefaciens* colonies.

114. If *A. tumefaciens* cells containing one of the three gene constructs were present in the cells of GM torenia plants they could transfer the introduced gene via conjugation with a wild type strain or other bacteria and yeast naturally present at the site (Hammerschlag et al. 2000). They could also genetically modify cells of other plants. This general possibility of horizontal gene transfer has already been discussed in Event 5 and was not considered to be an identified risk.

115. As GM torenia plants would be grown hydroponically, the range of organisms contacting any residual *A. tumefaciens* would be limited predominantly to algae and bacteria. It is highly unlikely that other strains of *A. tumefaciens* would be present in the hydroponic media and even more unlikely that contact with other plants would occur.

116. Recent evidence would suggest that *Chlamydomonas reinhardtii* and possibly other related algae could be within the natural host range of *A. tumefaciens*. *A. tumefaciens* cells are able to attach to *Chlamydomonas* cells, a prerequisite for infection, and *Chlamydomonas* cells are also able to induce the *A. tumefaciens* *vir* genes (Kumar & Rajam 2007). Under laboratory conditions, *A. tumefaciens* can be used to introduce foreign genes into green algae (Ausich 1984; Kumar et al. 2004). Since it is unlikely that cells of *A. tumefaciens* are persistent in the GM torenia lines (see paragraph 113 above), it is unlikely that there would be any *Agrobacterium*-mediated gene transfer to any algae that might grow in the hydroponic solutions. It is also pertinent to note that a homologue of the *PHR1* gene is present in at least unicellular green algae (see Section 5.3.2, Chapter 1).

117. Persistence of *A. tumefaciens* in the environment is unlikely because the duration of the trial is short and plant material would be destroyed at the end of the trial.

118. Even if the *PHR1* gene were transferred to another organisms it is unlikely to be a source of potential harm (see Events 1-6).

119. **Conclusion:** The potential for an adverse outcome resulting from the persistence in the environment of *A. tumefaciens* containing the introduced genes is not an identified risk and will not be assessed further.

2.7 Unauthorised Activities

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

120. 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 torenia 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.

121. **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

122. 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.

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

124. 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.

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

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

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM torenia 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 environment.

Section 4 Uncertainty

126. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. It is recognised that both dimensions of risk (ie consequence and likelihood) are always uncertain to some degree.

127. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹⁵. 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.

128. For DIR 084/2008 that involves proof of concept research, uncertainty exists in relation to the characterisation of:

- ♦ Event 1, associated with toxicity/allergenicity
- ♦ Event 2, associated with the potential for increased survival of the GMOs, and
- ♦ Event 6, associated with unintended changes.

Additional data including information to address this uncertainty would be required to assess possible future applications for a larger scale trial, reduced control measures, or the commercial release of any of these GM torenia lines that may be selected for further development.

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

¹⁵ 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.

Chapter 3 Risk management

130. 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

131. 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.

132. Section 63 of the Act 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.

133. 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

134. 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, TGA, NICNAS and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹⁶.

135. 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.

136. FSANZ is responsible for human food safety assessment, including GM food. As the trial involves proof of concept research, the applicant does not intend any material from these GM torenia lines to be used in human food. Accordingly the applicant has not applied to

¹⁶ 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>>

FSANZ for evaluation of any of the GM torenia lines for use in human food. FSANZ approval would need to be obtained before they could be sold as food.

137. No other approvals are required.

Section 3 Risk treatment measures for identified risks

138. The risk assessment of events listed in Chapter 2 concluded that there are **negligible** risks to people or the environment from the proposed trial of GM torenia. 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.

139. These events were considered in the context of the scale of the proposed release (a maximum total area of 20 m² over eight months (October 2008 – May 2009) on a single site in the Victorian local government area of Darebin), the containment measures (Chapter 1, Section 3), and the receiving environment (see Chapter 1, Section 6).

Section 4 General risk management

140. 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 the environment are negligible. The conditions are detailed in the licence¹⁷ and summarised in Sections 4.1.2 and 4.1.3 of this chapter.

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by Florigene

141. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls of the release and reference is made to these in the discussion of the events characterised for the release. The following comments are made on the suitability of these limits and controls.

142. The release would be confined to one site, within the fenced perimeter of existing Florigene greenhouse infrastructure at La Trobe University, Bundoora, Victoria. The site is at the same location where GM torenia plants with altered flower colour are currently being grown under Licence DIR 068/2006. The site is surrounded by a 2.1 m fence, which has lockable gates. Only trained and authorised staff have access to the site and handle the GMOs. This minimises the potential for GM torenia plants to disperse into the environment (Event 4) or come into contact with the public (Event 1). Limiting the duration of the release to an eight month period (and growing the plants during a single 12 week period) also restricts the opportunity for GM plants to establish outside the site (Events 2 and 3).

143. Growing the plants hydroponically at ground level in 1000 litre plastic tubs containing only 300 litres of medium minimises the potential for spread and persistence in the environment because plants will not be in contact with soil (Events 2 and 3). In addition, soil organisms will not be exposed to GM plant material (Event 1). Visually monitoring the trial site twice per week during the 12-week growing period and completing full written inspections (on a monthly basis during the 12-week growing period; and after severe weather;

¹⁷ The licence for DIR 084/2008 is available on the OGTR website via the link to DIR 084/2008 (<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir084-2008>)

and if any non-compliances are detected during a bi-weekly visual monitoring) assists in ensuring that plants or plant material are not dispersed into the environment (Event 3).

144. The applicant intends destroying all plant material not required for analysis by placing it in plastic bags then storing it in a lidded waste skip for at least a month on site. Non-viable plant material would then be disposed of via standard methods for non-GM plants (eg landfill). Placing all GM torenia plant material, in plastic bags for at least a month within a lidded waste skip would kill it. This is mainly due to the adverse conditions within the plastic bags and waste skip (high temperatures, insufficient light, lack of nutrients and water) that would prevent survival. A number of other factors would also limit the potential survival of GM torenia plant materials:

- ♦ As outlined in the RARMP for DIR 068/2006, GM torenia do not produce seed or long-term survival structures (such as stolons, runners, rhizomes) and detached stems do not have the propensity to form roots (and possibly new plants) except through deliberate horticultural intervention
- ♦ The applicant has stated that *T. x hybrida*, cv. Summer Wave[®] Blue (the parent organism) does not regenerate from roots and cannot be propagated by root cuttings
- ♦ Results from a preliminary experiment on GM torenia with altered flower colour indicated that leaves and stem pieces were desiccated after 23 days when left on the ground or under mulch.

145. Licence DIR 068/2006 permits the destruction of GM *T. x hybrida* with altered flower colour by the above method with the additional condition that roots be separated from stems prior to the storage of plant material in plastic bags. Given that this method of plant destruction has already been approved by the Regulator, it is considered appropriate that the licence for DIR 084/2008 also requires that roots should also be separated from their stems as part of the destruction process.

146. Dispersal of GM plant material prior to its complete destruction would be unlikely as it would be securely stored within the confines of the existing trial site, which is fenced and only accessible to Florigene staff. Even if plant material were to be inadvertently dispersed it would be unlikely to be a source of potential harm as discussed in Events 1 and 2.

147. Licence DIR 068/2006 permits the destruction of plant material by incineration (in addition to the destruction method described above) and therefore it is appropriate that this method of destruction be included in the current licence.

148. The applicant proposed to monitor the release site after the trial for any volunteers. However, as the three GM torenia lines cannot set seed and as detached stem pieces cannot produce roots and form new plants without horticultural intervention, it is considered unnecessary to monitor the release site post-harvest once the site has been cleaned. This is consistent with Licence DIR 068/2006.

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

149. A number of licence conditions have been imposed by the Acting Regulator to limit and control the release including requirements to:

- ♦ conduct the release on a total maximum area of 20 m² at one site in the local government area of Darebin (Victoria) between October 2008 and May 2009
- ♦ locate the trial site within the perimeter of existing Florigene greenhouse infrastructure, which is surrounded by a 2.1 metre fence and lockable gates

- ♦ grow the plants hydroponically at ground level in 1000 litre plastic tubs
- ♦ visually monitor the site twice per week during the 12-week growing period
- ♦ complete full written inspections of the site: on a monthly basis during the 12-week growing period; and as soon as possible after severe weather; and if any non-compliances are detected during a bi-weekly visual inspection
- ♦ clean all equipment used in cultivation practices
- ♦ not permit any materials from the release to be used in human food or animal feed, and
- ♦ at the end of the trial, destroy all plant materials not required for further analysis.

4.1.3 Measures to control other activities associated with the trial

150. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, July 2007*¹⁸; *Policy on transport and supply of GMOs, July 2005*¹⁹). 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.

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

4.2 Other risk management considerations

152. 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 site by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

153. 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

¹⁸ Guidelines for the transport of GMOs

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

¹⁹ Policy on transport and supply of GMOs

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

- ♦ the capacity of the applicant to meet the conditions of the licence.

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

155. 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.

156. Florigene 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

157. Prior to planting the GM torenia lines, Florigene is required to submit a plan detailing how it intends to ensure compliance with the licence conditions and document that compliance. This plan is required before the planting of the GM torenia lines occurs.

158. Florigene 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 torenia lines outside of the permitted areas.

159. Florigene 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

160. 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

161. 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.

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

163. 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 harvest
- ♦ expected and actual dates of destruction and cleaning after harvest.

4.2.5 Monitoring for Compliance

164. 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 site.

165. 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.

166. 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

167. 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 torenia lines that may be selected for further development, or to justify a reduction in containment conditions. This includes:

- ♦ characterisation of the genetic material inserted into the plants, including genetic stability
- ♦ characteristics indicative of weediness including altered sexual and asexual reproductive capacity, tolerance to environmental stresses and disease, altered plant growth in soil, and
- ♦ additional data on the potential toxicity and allergenicity of plant materials from the GM torenia lines.

Section 6 Conclusions of the RARMP

168. The risk assessment concludes that this limited and controlled release of three GM torenia lines on a maximum total area of 20 m² over eight months in the Victorian local government area of Darebin poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

169. 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 materials 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.

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

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

Consequence

outcome or impact of an adverse event

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

Event*

occurrence of a particular set of circumstances

Hazard*

source of potential harm

Hazard identification

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

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

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

Risk

the chance of something happening that will have an undesired impact

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

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

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

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

Risk analysis

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

Risk analysis framework

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

Risk assessment

the overall process of hazard identification and risk estimation

Risk communication

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

Risk Context

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

Risk estimate

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

Risk evaluation

the process of determining risks that require treatment

Risk management

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

Risk management plan

integrates risk evaluation and risk treatment with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

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

States

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

Uncertainty

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

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities²⁰ on the consultation RARMP for DIR 084/2008

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 or issues raised	Comment
Consideration should be given to disposing of GM plant material by autoclaving or a destruction treatment other than storing in plastic bags. This would reduce risk of live dissemination.	Disposal of GM torenia plant material (including that in which roots must have been detached from any intact plants) by storage in plastic bags in a lidded skip for at least 1 month, was a condition of a previous GM torenia release (DIR 068/2006). The appropriateness of this method of destruction is discussed in Section 4.1.1 of the DIR 084/2008 RARMP and the imposed destruction method is considered to be adequate to restrict the spread and persistence of the GM torenia lines in the environment.
<i>Torenia fournieri</i> might be able to pollinate with the GM <i>Torenia x hybrida</i> to produce viable offspring.	In <i>T. x hybrida</i> plants there is no natural mechanism for crossing with other species because the plants do not produce viable pollen or seeds. This is addressed in chapter 1 of the RARMP.
The tubs containing the GM torenia might overflow following heavy rainfall, or the tubs may crack or leak; plant material may be dispersed into drains.	Event 3 in Chapter 2 of the RARMP addresses the likelihood of this possibility occurring and concludes that because 700 litres of rain would be required to cause overflow, and because the tubs are constructed from heavy duty plastic that is unlikely to crack, the scenario is highly unlikely. Section 6.1 in Chapter 1 notes that the tubs are located on level ground approximately 7 m from two concrete-edged, grated, storm water drains that are raised above the gravel and that in the unlikely event of any solution bursting or overflowing from tubs it would be likely to seep into the gravel before reaching the drains. Plant material from the tubs would therefore be unlikely to reach the drains and would remain on the surface of the ground.
Possible dissemination of plant material by birds	Event 3 in Chapter 2 of the RARMP addresses the likelihood of this possibility occurring and concludes that the potential for dispersal of viable GM materials is not an identified risk.

²⁰ GTTAC, State and Territory Governments, Australian Government agencies, the Minister for the Environment, Heritage & the Arts and the Local Council(s) where the release may occur.