



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 079/2007**

**Limited and controlled release of banana genetically
modified for disease resistance**

Applicant: Queensland University of Technology

July 2008

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Executive Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of up to 17 lines of banana modified for disease resistance into the environment in respect of application DIR 079/2007 from the Queensland University of Technology (QUT).

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

QUT applied for a licence for dealings involving the intentional release of up to 17 GM banana lines on a limited scale and under controlled conditions. The GM banana lines have been modified for disease resistance. The trial is authorised to take place at one site in the local government area of Cassowary Coast, Queensland on a maximum total area of 1.4 hectares between 2008 and 2010.

Up to 16 of the GM banana lines will contain a gene that is expected to provide protection from certain disease-causing micro-organisms. The gene is derived from a nematode.

One GM banana line will contain a reporter gene that expresses a protein which provides a visual indication of where successful transformation of plant tissues has occurred. The gene is derived from a jellyfish.

All of the GM banana lines will contain an antibiotic resistance selectable marker gene, which was used to identify transformed plants during initial development of GM plants in the laboratory.

The purpose of the trial is to conduct proof of concept research involving experiments with the GM banana lines to assess their development and disease response. The GM bananas will not be used for human food or animal feed.

QUT proposed a number of controls that have been considered during the evaluation of the application to restrict the dissemination or persistence of the GM banana lines and the introduced genetic materials in the environment.

Risk assessment

The risk assessment takes into account information in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge, advice

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

received from a wide range of experts, agencies and authorities consulted on the RARMP and submissions 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. Consideration was then given as to whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

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

The characterisation of the 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 banana lines into the environment are estimated 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 proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

The licence conditions require QUT to **limit** the release to a total area of up to 1.4 ha at one site between July 2008 and April 2010. The **control** measures to restrict the spread and persistence of the GMOs include preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with OGTR transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed².

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 17 GM banana lines on a maximum total area of 1.4 ha over two years in the Queensland local government

² The licence for DIR 079/2007 is available on the OGTR website (<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir079-2007>) via the link to DIR 079/2007

area of Cassowary Coast poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

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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
<i>ced-9</i>	Anti-apoptotic gene (<i>ced</i> = cell death abnormal)
cv	cultivar
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
FSANZ	Food Standards Australia New Zealand
<i>gfp</i>	Green fluorescent protein gene
GFP	Green fluorescent protein
GM	Genetically Modified
GMO	Genetically Modified Organism
GTAC	Gene Technology Technical Advisory Committee
ha	hectare(s)
HR	Hypersensitive response
km	kilometre
mRNA	Messenger Ribonucleic Acid
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
<i>nptII</i>	neomycin phosphotransferase type II gene
OGTR	Office of the Gene Technology Regulator
PCD	Programmed Cell Death
PCR	Polymerase Chain Reaction
QDPI&F	Queensland Department of Primary Industries and Fisheries
QUT	Queensland University of Technology
RARMP	Risk Assessment and Management Plan
the Regulations	Gene Technology Regulations 2001
the (Acting) Regulator	The (Acting) Gene Technology Regulator
RNA	Ribonucleic Acid
RT-PCR	Reverse transcriptase
TEV	Tobacco etch 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 079/2007) to the Queensland University of Technology (QUT) for dealings involving the intentional release of genetically modified (GM) banana 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

QUT applied for a licence for dealings involving the intentional release of up to 17 lines⁴ of banana (*Musa acuminata* cv. Grande Naine) that have been genetically modified for disease resistance. The trial is authorised to take place at one site in the local government area of Cassowary Coast, Queensland on a maximum total area of 1.4 hectares between 2008 and 2010.

Up to 16 of the GM banana lines contain the *ced-9* gene, derived from the nematode *Caenorhabditis elegans*, which encodes a protein that is expected to confer disease resistance by preventing cells from undergoing programmed cell death (or apoptosis) in response to infection by certain pathogenic micro-organisms. The gene may also affect growth and development of the GM banana plants and confer enhanced tolerance to a range of biotic and abiotic stresses.

One GM banana line contains the *gfp* gene derived from the jellyfish *Aequorea victoria*. It encodes a green fluorescent protein that enables visual identification of plant tissues in which the gene is expressed. GM banana plants containing the *gfp* gene will be used as controls to ascertain whether any of the observed disease response is as a result of the expression of the *ced-9* gene.

In addition, all of the GM banana 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. The *nptII* gene was used as a selective marker to identify transformed plants during initial development of GM plants in the laboratory.

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 (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>> and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

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

The purpose of the trial is to conduct proof of concept research involving experiments with the GM banana lines to assess their development and disease response. The GM bananas will not be used for human food or animal feed.

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

Risk assessment

The risk assessment considered information contained in the application, relevant previous approvals, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received from consultation 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. However, feedback on the consideration of previously raised issues enabled their clarification in the final RARMP.

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

A reference document on the parent organism, *The Biology of Musa L. (banana)*, was produced to inform the risk assessment process for licence applications involving GM banana plants. The document is available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

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

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

The characterisation of the 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, location and duration of the release proposed by QUT
- ◆ suitability of controls proposed by QUT to restrict the dissemination or persistence of the GM banana plants and their genetic material
- ◆ limited capacity of the GM banana lines to spread and persist outside the areas proposed for release
- ◆ limited ability and opportunity for the GM banana lines to transfer the introduced genes to commercial banana 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 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 banana lines into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment⁵.

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 estimated as **negligible**.

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

Licence conditions to manage this limited and controlled release

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

- ♦ conduct the release on a total area of up to 1.4 hectares per year at one site in the local government area of Cassowary Coast (Queensland) between July 2008 and April 2010
- ♦ remove and destroy all male/hermaphrodite flowers on the inflorescences unless they are required for experimental analysis
- ♦ cover any male/hermaphrodite flowers left on the inflorescences
- ♦ cover fruit bunches
- ♦ remove and destroy all fruit not required for experimental analysis
- ♦ destroy any plant waste containing meristematic tissue
- ♦ clean all equipment used in cultivation practices
- ♦ not permit any materials from the release to be used in human food or animal feed
- ♦ at the end of the trial, destroy all plant materials not required for further analysis.

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

⁵ As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the *Gene Technology Act 2000* mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator allowed 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standard Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)⁶.

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 banana lines proposed for release to be used in human food. Accordingly, the applicant has not applied to FSANZ to evaluate any of the GM banana lines. FSANZ approval would need to be obtained before they could be used in 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 banana lines that may be selected for further development or to justify a reduction in containment conditions. This would include:

- ◆ characterisation of the introduced genetic material in the plants, including genotypic stability
- ◆ additional data on the potential toxicity of plant materials from the GM banana lines
- ◆ additional data on the allergenicity of the protein encoded by the introduced gene for disease resistance
- ◆ characteristics indicative of weediness including measurement of altered reproductive capacity; tolerance to environmental stresses; and disease susceptibility.

Suitability of the applicant

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

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 17 GM banana lines on a maximum total area of 1.4 ha over two years in the Queensland local government area of Cassowary Coast poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to

⁶ 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>>.

limit the proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people and the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation⁷, details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

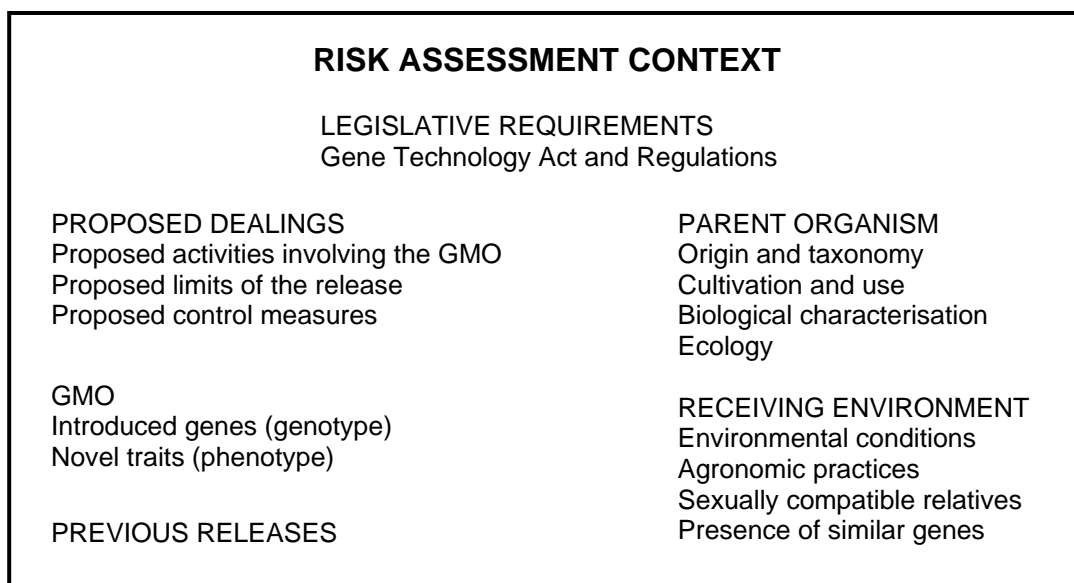


Figure 1 Components of the context considered during the preparation of the risk assessment

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

⁷ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <http://www.ogtr.gov.au/ir/process.htm> and in the *Risk Analysis Framework* (OGTR 2007) <http://www.ogtr.gov.au/pubform/riskassessments.htm>.

Section 2 The legislative requirements

3. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Regulator must take into account, and 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 have been proposed 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 Regulator has prepared a RARMP for this application.

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

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

Section 3 The proposed dealings

7. Queensland University of Technology (QUT) proposes to release up to 17 banana lines⁸ that have been genetically modified for disease resistance into the environment under limited and controlled conditions.

3.1 The proposed activities

8. The applicant has stated that the principal purpose of the proposed release is to conduct proof of concept research involving experiments with the GM banana lines to assess their development and disease response. The GM bananas will not be used for human food or animal feed.

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

9. The release is proposed to take place at one site within the State Government Research Station (Queensland Department of Primary Industries & Fisheries – QDPI&F) at South Johnstone in the local government area of Cassowary Coast in Queensland. Within the site there is a field location and a shadehouse (to be used for hardening off tissue-cultured plants for approximately 4 months before they are transferred to the field location) that together will

⁸ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

occupy a maximum total area of 1.4 ha. The release was proposed to occur between April 2008 and April 2010.

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

10. The field location does not directly abut any public roads. The shadehouse is a permanent, glass-panelled, lockable structure located some 200 m from the field location. The closest population centre (South Johnstone, population 300) is 1 km from the site; the next largest centre is Innisfail (population 15,000), 12 km from the site. Only trained and authorised staff will be permitted access to the proposed locations.

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

- ♦ locating the proposed field location on flat land 250 m away from, and 18 m above, the nearest natural waterway (South Johnstone River)
- ♦ utilising a parent plant that has been cultivated to the extent that it is essentially female and male sterile
- ♦ applying bunch covers to prevent access to the developing fruit by birds and mammals that may feed on the fruit
- ♦ removing the immature male bud (bell) of inflorescences or bagging these to prevent access by pollinators
- ♦ complying with Queensland State Government legislation for banana disease control that would also aid in containment of GM plants
- ♦ destroying (GM and non-GM) plant materials from the field trial by:
 - herbicide treatment and surface decomposition (material containing vegetative meristems)
 - surface decomposition (non-propagative vegetative parts)
 - decomposition in covered containers (fruits and male flowers not required for experimental analysis)
- ♦ analysing GM plant materials from the trial in an OGTR-certified PC2 facility and then destroying the materials by autoclaving
- ♦ post harvest monitoring of the field location for 12 months and destroying any volunteer GM banana suckers
- ♦ transporting GM plant materials to and from the proposed site and within the site in accordance with OGTR transportation guidelines.

12. These controls, and the limits on size, location and duration outlined in Chapter 1, Section 3.2, have been taken into account in establishing the risk assessment context, and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.

Section 4 The parent organism

13. The parent organism is sweet banana (*Musa acuminata* Colla cv. Grande Naine) which is exotic to Australia. Bananas are grown commercially on the east coast of Australia from northern New South Wales to far north Queensland. They are also grown in Western Australia around Carnarvon, Kununurra and Broome and in the Northern Territory near Darwin. The 'Grande Naine' cultivar is one of several cultivars in the sub-group Cavendish

that accounts for approximately 95% of the bananas on the Australian market. Members of the Cavendish subgroup set seed so rarely that they can be regarded as female sterile and produce so little viable pollen that they are effectively male sterile. Further detailed information about the parent organism is contained in a reference document, *The Biology of Musa L. (banana)* (the *Musa* Biology document), that was produced to inform the risk assessment process for licence applications involving GM banana plants (OGTR 2008). The document is available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

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

5.1 Introduction to the GMOs

14. The GM banana lines contain either a gene encoding a protein involved in the inhibition of apoptosis or a gene that expresses a protein which provides a visual indication where successful transformation of plant tissue has occurred. In addition, each of the banana lines contains a selectable marker gene (Table 1).

Table 1 The genes used to genetically modify banana

Gene	Accession No (Genbank)	Protein produced	Protein involved in	Source	Intended purpose
<i>ced-9</i>	AAA20080	CED-9	Inhibition of apoptosis	<i>Caenorhabditis elegans</i>	Disease resistance
<i>gfp</i>	P42212	green fluorescent protein		<i>Aequorea victoria</i>	Reporter
<i>nptII</i>	AAF65403	neomycin phosphotransferase	Kanamycin resistance	<i>Escherichia coli</i>	Selectable marker

15. Up to 17 GM banana lines are proposed for release, with each line representing a single transformation event (see Table 2). Up to 16 of the GM lines will be generated using a construct containing the *ced-9* gene. For each of these lines, 20 replicates (ie clones) will be released. The remaining GM line will be generated using a construct containing the green fluorescent protein (*gfp*) gene. Up to 50 replicates of this GM line will be released. Up to 50 non-GM plants would be used as controls.

Table 2 Gene constructs used to generate the GM banana lines proposed for release

Identity of construct(s) associated with each experiment	Promoter	Gene of interest	Terminator	Max lines per construct	Replicates per line	Total no. of plants
pPTN261	Ubi	<i>ced-9</i>	35S	16	20	320
pART-TEST7	35S	<i>GFP</i>	Nos	1	50	50
				17		370

5.2 The introduced genes, encoded proteins and effects

5.2.1 Gene expected to confer disease resistance, and its encoded protein

16. Up to 16 of the GM lines contain the *ced-9* gene, which encodes a protein that protects cells from undergoing programmed cell death (PCD), or apoptosis. The expression of this anti-apoptotic gene is expected to confer disease resistance by preventing cells from undergoing PCD in response to infection by certain pathogenic micro-organisms. The gene may also affect growth and development of the GM banana plants and confer enhanced tolerance to a range of biotic and abiotic stresses.

17. The *ced-9* gene (*ced*, cell-death abnormal) is derived from the nematode *Caenorhabditis elegans*, where it protects cells from undergoing PCD during *C. elegans* development (Conradt & Xue 2005).

18. While the mechanisms of PCD are not well understood in plants, PCD is important to normal plant physiological processes during growth and development, and in response to abiotic and biotic stresses (Pennell & Lamb 1997). These processes include the deletion of cells with temporary functions, removal of unwanted cells, deletion of cells during formation of the plant body and leaves, death of cells during plant specialisation and leaf senescence (Dickman 2004). Some of the hallmarks of animal PCD have been found at the cellular and molecular level in plant cells (Danon et al. 2000). In addition, evidence of inhibition of plant cell death by animal anti-apoptotic genes suggests that the genes involved in the control of PCD are conserved across wide evolutionary distances (Dickman et al. 2001; Li & Dickman 2004; Xu et al. 2004; Dickman 2004; Khanna et al. 2007; Shabala et al. 2007). Some plant homologues of these genes have been found (Danon et al. 2000). It is therefore likely that non-GM Cavendish bananas are likely to contain homologues of the *ced-9* gene, or proteins of similar function.

5.2.2 Toxicity/allergenicity of the protein encoded by the introduced gene for disease resistance

19. Homologues of the CED-9 protein, or proteins with a similar function, occur naturally in a range of organisms, including plants widely consumed by people and animals (see discussion in Section 6.5 of this chapter). On this basis, people and other organisms have a long history of exposure to proteins involved in the inhibition of programmed cell death.

20. No toxicity/allergenicity tests have been performed on the purified encoded protein as the proposed trial is still at proof of concept stage. Such tests would 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).

21. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The predicted amino acid sequence of the protein encoded by the introduced gene was compared to a database of known allergens. The results of this analysis did not indicate that the encoded protein shares any biologically relevant sequence or structural similarities with any known allergens (information supplied by the applicant).

22. A comprehensive search of the scientific literature also yielded no information to suggest that the encoded protein is toxic or allergenic to people, or toxic to other organisms.

5.2.3 The reporter gene (GFP) and the encoded protein

23. One of the GM banana lines contains the marker/reporter gene *gfp*, derived from the jellyfish *Aequorea victoria*. It encodes a green fluorescent protein, which enables visual identification of plant tissues in which this gene is expressed. GM banana plants containing the *gfp* gene will be used as controls to ascertain whether any observed disease response is as a result of the expression of the *ced-9* gene.

24. Plant tissues containing the *gfp* gene can be visualised directly in living plant tissue through the fluorescence of GFP under ultraviolet or blue light. Consequently, non-destructive visualisation is possible, which removes the need to destroy research material and avoids problems of tissue distortion associated with fixing, sectioning and staining tissues

(Chiu et al. 1996; Miki & McHugh 2004). The GFP protein contains an autofluorescent chromophore and does not perform any other biochemical function (Tsien 1998).

25. GFP is generally regarded as non-toxic to plant cells and has been most widely used as a means of monitoring intracellular location and trafficking of selected proteins of interest in plants and animals (Leffel et al. 1997; Andreeva et al. 2000; Kallal & Benovic 2000) and to detect transformed cells (Elliott et al. 1999).

26. A modified version of the *gfp* gene has been used in the GM banana plants. Haseloff and co-authors (1997) found that expression of wildtype *A. victoria* GFP in plant cells was prevented by aberrant mRNA splicing. A cryptic intron was recognised and excised by *Arabidopsis thaliana* cells that led to poor or no fluorescence. A modified version of the *gfp* gene was constructed whereby codon usage was altered to avoid recognition of this cryptic intron, allowing proper expression of the *gfp* gene. This modified *gfp* gene has the same spectral characteristics as the wildtype version of the gene (Haseloff et al. 1997).

27. The *gfp* gene has been used for over 10 years in research settings and is considered a neutral marker gene (Stewart 2006). GFP has been shown to be non-toxic to rats when ingested in purified form or when synthesised in transgenic plants (Richards et al. 2003). This study also reported that GFP was rapidly degraded by gastric juices, although in the rats fed pure GFP, some protein did survive intact in the faeces. Field trials of GM tobacco expressing GFP did not show signs of any measurable fitness costs to the host plants, which suggests that the *gfp* gene is ecologically neutral (Harper et al. 1999). A modified version of the *gfp* gene, similar to that used in the GM bananas in the proposed trial, was used in both studies.

28. The *gfp* gene has been discussed in previous RARMPs, including DIR 052/2004 (GM rice), DIR 050/2004 (recombinant bovine herpesvirus vaccine), DIR 031/2002 (GM grapevines) and DIR 019/2002 (GM sugarcane). More detailed information on the *gfp* gene and the encoded protein can be found in these RARMPs, which are available at <http://www.ogtr.gov.au> or by contacting the OGTR.

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

29. All of the GM banana 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. The *nptII* gene was used as a selective marker to identify transformed plant tissue during initial development of GM plants in the laboratory.

30. 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/ir/dir070.htm>) 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 (EFSA). This report 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.

5.2.5 The effects associated with the introduced gene for disease resistance

31. Multicellular organisms use a genetically controlled cell death pathway, known as programmed cell death (PCD), to remove unwanted, damaged or used cells (Pennell & Lamb

1997). PCD can occur during different stages of growth and development, as well as in response to abiotic or biotic stresses.

32. In animals, the most common form of PCD is apoptosis. Apoptosis depends on the active participation of the dying cells, and is characterised by morphological and biochemical changes in cells that result in the organised disassembly of the cell (Khurana et al. 2005). Three genetically distinct phases occur: the specification phase, where the cell is instructed to undergo apoptosis; the killing phase, where the cell death program is activated in the cell instructed to die; and the execution phase, where the cells are dismantled and engulfed by surrounding cells (Conradt & Xue 2005). These three phases are associated with cell shrinkage, membrane blebbing, nuclear and cytoplasmic condensation, and DNA fragmentation. In animals, the DNA fragments form apoptotic bodies that are rapidly digested by macrophages (Dickman 2004).

33. The genes that control PCD are conserved across wide evolutionary distances and encode either anti-apoptotic or pro-apoptotic proteins. Overexpression of some anti-apoptotic genes can enable animal and plant cells to resist a wide range of cell death stimuli (Navarre & Wolpert 1999; Dickman 2004).

34. The *ced-9* gene, derived from the nematode *Caenorhabditis elegans*, is an anti-apoptotic gene that is active during the killing phase of PCD, where it protects cells from undergoing death. Loss of function of the *ced-9* gene can lead to the death of cells that normally live (Hengartner et al. 1992). The human proto-oncogene *bcl-2* encodes a protein similar to that encoded by *ced-9*. The *bcl-2* gene plays a similar role in inhibiting apoptosis in mammals. Both genes are members of a family that plays an important role in regulating apoptosis in diverse organisms (Conradt & Xue 2005).

35. PCD is important in a wide variety of normal physiological processes (Danon et al. 2000; Dickman 2004). For example, PCD eliminates cells that are unwanted or in inappropriate positions, such as cells between developing digits in animals or stamen primordia cells in unisexual flowers; removes cells that have served temporary functions, such as tadpole tail cells at metamorphosis or aleurone in plants seeds; or causes death of cells during cell specialisation, such as keratinocytes at the surface of the skin in animals or tracheary element cells in plants (Pennell & Lamb 1997; Dickman 2004).

36. Disruption of the normal cell death pathway in humans can lead to diseases where there is excessive cell accumulation (eg cancer) or inappropriate cell death (eg Alzheimer's, Parkinson's, AIDS). PCD is also a feature of some infectious diseases as most viruses and intracellular bacteria can control the cell death pathway in hosts (Dickman 2004). As a result of their involvement in these diseases, the genes involved in apoptosis are being used for therapeutic manipulation of degenerative and proliferative diseases in animals and humans (Khurana et al. 2005).

37. While the regulatory mechanisms of PCD in animals are well known, the genes and pathways of PCD in plants remain unclear (Shabala et al. 2007). However, there is evidence to suggest that PCD plays an important role in plant development (Dickman et al. 2001; Xu et al. 2004), in interactions of plants with a variety of pathogens, including bacteria, fungi and viruses; as well as abiotic stresses, such as salinity, cold stress, waterlogging and hypoxia (Khurana et al. 2005; Shabala et al. 2007). Some of the hallmarks of animal PCD have been found at the cellular and molecular level in plant cells (Danon et al. 2000). Also, the inhibition of plant cell death by animal anti-apoptotic genes suggests that the genes involved in the control of PCD are conserved across wide evolutionary distances (Dickman et al. 2001; Li & Dickman 2004; Xu et al. 2004; Dickman 2004; Khanna et al. 2007; Shabala et al. 2007). Some plant homologues of these genes have been found (Danon et al. 2000).

38. Expression of the *ced-9* gene in plants has shown a range of effects, including improved plant survival under abiotic and biotic stresses, and developmental abnormalities. The *ced-9* gene has been previously expressed in banana embryonic cell suspensions where it was found to suppress cell death in *Agrobacterium tumefaciens* transformed plant cells, resulting in high transformation frequency (Khanna et al. 2007). In GM tobacco, the *ced-9* gene was shown to confer protective advantages against necrotrophic fungal pathogens (Dickman et al. 2001) as well as a range of abiotic stresses including heat, cold, menadione and hydrogen peroxide (Li & Dickman 2004), salt and oxidative stress (Shabala et al. 2007). In the same study, GM tobacco plants expressing high levels of the *ced-9* gene were extremely resistant to the pathogens tested but also showed altered growth patterns, such as variegated leaf pigmentation. Moderately expressing GM tobacco plants did not show any developmental abnormalities but retained pathogen resistance (Dickman et al. 2001). Expression of the *ced-9* gene in tomato plants enhanced plant survival by inhibiting PCD induced by virus infection and exposure to cold temperatures (Xu et al. 2004). Developmental abnormalities were also observed in the GM tomato plants expressing high levels of *ced-9* gene. These abnormalities included stunted growth and the formation of none or few viable seeds (Xu et al. 2004).

39. There is also the potential for the expression of the *ced-9* gene in plants to impact on pathogens. In plant-pathogen interactions, PCD occurs when the pathogen unsuccessfully parasitises the host, as well as when the pathogen successfully causes disease. Plant-pathogen interactions depend on a gene-for-gene resistance model, where there is an avirulence gene in the pathogen and a corresponding resistance gene in the plant. This results in an incompatibility reaction, leading to successful disease resistance (Khurana et al. 2005). A compatibility reaction due to the loss of the plant resistance gene or changes to the pathogen avirulence gene leads to disease.

40. The resistance disease response in plants is characterised by a hypersensitive response (HR) at the infection site. HR causes rapid, localised cell death which kills the cells near the site of infection, thereby limiting the spread of pathogens and providing the plant with disease resistance (Khurana et al. 2005). HR is associated with the expression of a variety of plant defense genes and the induction of PCD (Tadege et al. 1998; Dickman 2004). Further evidence suggests that the HR may also activate specific defences in neighbouring tissue. PCD also occurs during susceptible host-pathogen interactions in which pathogens can replicate in their hosts (Greenberg & Yao 2004). This suggests that common biochemical pathways exist between resistant and susceptible plant-pathogen interactions. The expression of anti-apoptotic genes in plants may therefore confer resistance during susceptible plant-pathogen interactions (ie necrotrophic diseases), but could also increase susceptibility in resistant plant-pathogen interactions (ie biotrophic diseases) (Dickman 2004).

5.3 The regulatory sequences

5.3.1 Regulatory sequences for the *ced-9* and *gfp* genes

41. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. The promoter for the *ced-9* gene is derived from the ubiquitin gene of maize (*Zea mays*, accession #S94466)(Christensen et al. 1992). The promoter for the *gfp* gene is derived from the 35S RNA promoter of the Cauliflower mosaic virus (CaMV, accession# AF234316)(Odell et al. 1985; Ohta et al. 1990). Both promoters are constitutive promoters, which means that genes that are linked to these promoters are generally expressed at relatively high levels throughout the growing season and in most tissues of the plant.

42. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA termination region for the *ced-9* gene is derived from the nopaline synthase gene (*nos*) of *Agrobacterium tumefaciens*. This sequence is widely used in constructs for plant genetic modification (Reiting et al. 2007). The mRNA termination region for the *gfp* gene is derived from the CaMV 35S terminator.

43. The 5' end of the *ced-9* gene has been linked to a leader sequence from the Tobacco etch virus (TEV) to enhance translation. The genomic RNA of TEV is a polyadenylated mRNA that naturally lacks a 5' cap structure but that is nevertheless efficiently translated. The TEV 5' leader is sufficient to confer cap-independent translation to an mRNA and is functionally analogous to a cap in that it interacts with the poly(A) tail to promote efficient translation. The TEV leader itself is not translated (Carrington & Freed 1990).

44. The *gfp* gene has been linked to the first intron of the castor bean (*Ricinus communis*) catalase gene (CAT-1), which prevents expression in *Agrobacterium*. This ensures that any expression of the green fluorescent protein in the GMOs is occurring in eukaryotic cells rather than in residual *Agrobacterium* (Ohta et al. 1990). A small synthetic exon from GUS has been placed before the intron to facilitate splicing.

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

5.3.2 Regulatory sequences for the expression of the *nptII* gene

46. In the pPTN261 vector, the bacterial *nptII* gene has been modified by the addition of the 35S RNA transcription initiation region and the 35S RNA transcription termination region from CaMV. The regulatory sequences comprise only a small part of the total genome, and have not been reported to cause any adverse effects.

47. In the pART-TEST7 vector, the bacterial *nptII* gene has been modified by the addition of the *nos* transcription initiation region and the *nos* transcription termination region from *A. tumefaciens*. Although *A. tumefaciens* is a plant pathogen, the regulatory sequences comprise only a small part of the total genome, and have not been reported to cause any adverse effects.

5.4 Method of genetic modification

48. All of the GM banana lines will be generated by *Agrobacterium tumefaciens*-mediated transformation. 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/ir/dir060.htm>> or by contacting the OGTR)].

49. Disarmed binary plasmid vectors containing each of the constructs (see Table 2) will be introduced into *Agrobacterium tumefaciens* strain AGL1 (ATCC® Number: BAA-101™) through electroporation. Strain AGL1 carries the hypervirulent, attenuated tumor-inducing plasmid pTiBo542 (Lazo et al. 1991) and shows high rates of T-DNA transfer when used with banana suspension cultures (Khanna et al. 2004).

50. For each transformation, embryogenic cell suspensions that have been derived from immature male flowers of *Musa acuminata* cv. Grande Naine are co-cultivated with the *A. tumefaciens* using a centrifugation-assisted procedure (Khanna et al. 2004). Following transformation, the banana cells are cultured on medium containing kanamycin to select for

those that have been transformed. Somatic embryos are developed on a regeneration medium and are then grown on into plantlets that are transferred to soil after hardening off in a shadehouse (see paragraph 67). PCR testing by the applicant, using primers specific to regions outside the T-DNA has demonstrated that the *A. tumefaciens* does not persist in GM banana plants for longer than approximately 3 weeks after removal from the *in vitro* environment. The applicant intends to confirm this by testing samples of plants prior to release.

51. The 17 GM banana lines would be generated from independent transformation events.

5.5 Characterisation of the GMOs

5.5.1 Stability and molecular characterisation

52. All genes, promoters and terminators have been sequenced to confirm the nucleotide sequence of each segment to be used for vector construction.

53. The GM banana lines are at a very early development stage and have not been tested for genotypic stability.

54. The presence of the *ced-9* gene has been verified by PCR and Southern blot analysis. RT-PCR was used to confirm expression of this gene at the mRNA level. However, the *ced-9* gene could not be detected by Western blot analysis due to the lack of a suitable antibody. Levels of expression of the *gfp* gene have not been determined.

55. The exact location of the inserted genes within the banana genome of the lines is not known. *Agrobacterium tumefaciens* inserts introduced genes into plant genomic DNA via illegitimate recombination⁹ and the banana genome is poorly characterised. The combination of these two factors suggests that meaningful data on the location of transgenes cannot be determined at this stage.

56. The 16 GM banana lines containing the *ced-9* gene have between 1 and 8 copies of the gene. The GM banana line containing the *gfp* gene has one copy of this gene.

5.5.2 Characterisation of the phenotype of the GMOs

57. The purpose of the proposed trial is to conduct proof of concept research involving experiments with the GM banana lines to assess their development and disease response. To date, no GM banana plants of sufficient size have been obtained from any of the lines to provide any meaningful phenotypic data. Phenotypic data will be collected during the proposed trial.

Section 6 The receiving environment

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

⁹ Illegitimate recombination is a term used to describe the recombination that occurs between DNA sequences that contain no or very little homology. It results in the random insertion of foreign DNA into the host genome.

6.1 Relevant abiotic factors

59. The cultivation of bananas occurs throughout the tropics and sub-tropics of Asia, America, Africa and Australia.

60. The abiotic factors relevant to the growth and distribution of commercial bananas in Australia are discussed in the *Musa* Biology document (OGTR 2008).

61. The release is proposed to take place in the north Queensland local government area of Cassowary Coast. This region is in the tropical rainforest climatic type (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology) in which 70% of Australia's banana production occurs (Biosecurity Australia 2007). The rainfall and temperature statistics for South Johnstone, in which the proposed site is located, are given in Table 3.

Table 3 Monthly temperature and rainfall statistics for South Johnstone – Northern Queensland (Tropical rainforest)*

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Mean max temp (°C)	31.1	30.6	29.8	28.1	26.2	24.3	23.8	25.1	27.0	29.0	30.5	31.2	28.1
Mean min temp (°C)	22.5	22.7	21.9	20.4	18.3	15.8	15.0	15.4	16.6	18.8	20.5	21.8	19.1
Mean rainfall (mm)	522.8	580.6	615.4	393.8	270.9	154.9	109.4	90.1	85.3	89.1	143.0	252.0	3305.5

*data taken from the Australian Bureau of Meteorology website (<http://www.bom.gov.au/index.shtml>) Temperature data are an average of 54 years of records; rainfall data are an average of 78 years of records

62. The proposed field location is 7 m above the highest flood level recorded for the nearest waterway (South Johnstone River) on flat land that is not prone to heavy runoff or landslip and is approximately 250 m from the nearest waterway. The applicant is not aware of any recorded incident of a storm or cyclone uprooting mature banana plants in the immediate area and dispersing vegetative propagative material.

6.2 Relevant biotic factors

63. The biotic factors pertaining to the growth and distribution of commercial bananas in Australia are discussed in the *Musa* Biology document (OGTR 2008). Of relevance to this proposed release are the following points:

- ♦ the Research Station, in which the proposed site is located, occurs within a major banana growing region.
- ♦ the applicant states that the nearest observed population of native bananas is approximately 10 km from the site (see paragraph 74).
- ♦ there are some 200 *Musa* spp. accessions growing as part of a germplasm collection at the Research Station in which the proposed site is located. Under Queensland State legislation these are not permitted to fruit or produce male flowers.
- ♦ invertebrates, vertebrates and microorganisms would all be exposed to the introduced genes, their encoded proteins and end products. In particular:
 - native vertebrates including nectar feeding birds, nectar feeding marsupials and flying foxes that are attracted to the flowers or fruit of banana plants may visit the field location.
 - feral pigs have access to the site but, while they have been observed knocking down and eating small plants, there is no evidence that they uproot plants,

detach suckers or eat fruit that is still on the plant. The applicant states that trapping of the pigs commenced at the Research Station in January 2008, as a control measure. There are no domestic livestock at the Research Station on which the proposed site is located.

64. One of the major fungal diseases of bananas, yellow Sigatoka (*Mycosphaerella musicola*) is known to occur in the proposed trial site. Another form of the disease, black Sigatoka (*M. fijiensis*), is a major threat to the banana industry. The early stages of infection of banana plants by Sigatoka leaf spot diseases are believed to be biotrophic (Hoss et al. 2000). Biotrophic fungi cause a hypersensitive response (HR) at the plant infection site. HR causes rapid, localised cell death which kills the cells near the site of infection, thereby limiting the spread of pathogens and providing the plant with disease resistance (Khurana et al. 2005). However, in the later stages of infection, the fungal pathogens becomes necrotrophic. Necrotrophic fungi trigger host cell death pathways because they require host cell death to grow, colonise and reproduce (Dickman 2004). Further details of these fungal diseases, and their methods of control, are discussed in the *Musa* Biology document (OGTR 2008).

65. Another major fungal disease of bananas known to occur in the proposed trial site is Panama disease or Fusarium wilt (*Fusarium oxysporum*). The relationship of this disease to its host is unclear. Again, further details of these fungal diseases, and their methods of control, are discussed in the *Musa* Biology document (OGTR 2008).

6.3 Relevant agricultural practices

66. The location of the proposed limited and controlled release of the GM banana lines are outlined in Chapter 1, Section 3.2.

67. The applicant proposes to transport the planting material (tissue cultured plants) to the site from a PC2 facility and to harden off the plants in a shadehouse for up to 4 months before transferring them to the field location. The cultivation and movement of banana planting material is regulated by the Queensland Department of Primary Industries and Fisheries (Qld DPI&F) and the Banana Industry Protection Board (BIPB). The applicant will consult with both of these organisations regarding the movement and planting of the GM and non-GM banana material for this trial.

68. Planting of bananas in north Queensland is possible all year round. The cultivation practices used for planting and managing the proposed trial will follow the standard practices used for commercial (non-GM) banana. These are outlined in Section 2.3.3 of the *Musa* Biology document (OGTR 2008) and detailed in Broadley et al. (2004) and include compliance with Queensland State Government legislation for banana disease control (Queensland Government 1999). These practices are the same as those described in the previous assessment for the RARMP for DIR 076/2007 which is available from the OGTR or from the website.

69. The GM banana plants and the non-GM controls will not be sprayed with fungicide. They will be monitored for disease, and plants showing signs of disease will be treated according to disease-specific control measures required under Queensland State Government legislation (Queensland Government 1999). The control measures vary depending on the disease, but can include a controlled spray program; removing leaves that have more than the maximum allowable level of leaf disease (eg. yellow Sigatoka); destruction of affected plants by burning, burying, or herbicide treatment (eg black Sigatoka); fencing off the infected site from other banana plants and the prevention of further planting of bananas on the infected site for at least 5 years (eg Fusarium wilt) (Queensland Government 1999). Quarantine

regulations further limit the spread of disease by restricting the planting and cultivation of bananas in pest quarantine areas, and preventing the movement of soil, water or plant materials from infected to non-infected areas (eg Fusarium wilt) (Queensland Government 1999).

70. It is standard practice to remove the male bell from inflorescences as this allows transport of assimilates to the developing fruit (Broadley et al. 2004). The applicant proposes to remove the male bells from most inflorescences but wishes to observe phenotypes in some bells. In this case, the bells will be bagged.

71. Fruit will be obtained from the plant crop¹⁰, which will then be ratooned and grown to fruiting before the proposed trial is concluded. In a commercial situation, bunches from new plantings are usually harvested about 16 to 18 months after planting, but this can be as early as 12 months. Subsequent (ratoon) crops are harvested 6 -12 months after sucker set (Morton 1987). Under the conditions at the proposed field location it is expected that the initial plant crop will start to produce bunches after 12 months and the ratoon crop will be harvested after 9 – 11 months. Fruit will be harvested while still green (standard commercial practice).

72. Non-propagative detached plant material at the field location (generated, for example, by desuckering, leaf thinning, cutting down of pseudostems) would be left on the ground to decompose. Any excess material potentially containing meristems would be treated with distillate or kerosene or other methods approved in writing by the Regulator. Excess fruit (not required for experimental analysis) would be decomposed in a secure container to prevent access by fruit-eating animals before being returned to the site. Plant waste from the shadehouse would also be decomposed on the ground at the field location, with meristematic tissue firstly receiving appropriate herbicide treatment.

6.4 Presence of related plants in the receiving environment

73. Commercial sweet banana cultivars are grown in the vicinity of the proposed field location. These all show parthenocarpy and male sterility.

74. There are two recognised *Musa* species that are native to Australia (Ross 1987):

- ♦ *M. acuminata* subsp. *banksii*, a fertile diploid, is the most common and can be found along the tip of Cape York and northern Queensland. The applicant states that the nearest observed naturally occurring population of this species is approximately 10 km from the proposed site at South Johnstone but that native vegetation consistent with the type of community in which *M. acuminata* subsp. *banksii* is found occurs much closer to the site. Plantings of the species are also present in a field collection at the Research Station on which the site is located but under compliance with State legislation for banana disease control (Queensland Government 1999), are not permitted to fruit or produce male flowers. *M. acuminata* subsp. *banksii* has the potential to cross with cultivated triploid and tetraploid cultivars with a *M. acuminata* background (such as the cultivar 'Grande Naine'). However, the applicant has stated that seed has not been found in commercial banana plantations growing in northern Queensland where the native species is in close proximity.
- ♦ *M. jackeyi* has been found in Bellenden Ker (approximately 50 km north of South Johnstone) and Cooktown (approximately 350 km north of South Johnstone).

¹⁰ The term 'plant crop' is routinely used in the banana industry to designate the fruit-bearing plant that develops from the propagative material first planted in the ground. Subsequent fruit develops from a 'ratoon crop'.

75. The Regulator has approved the limited and controlled release of banana genetically modified for enhanced nutrition (licence DIR 076/2007). GM bananas from DIR 076/2007 will be grown on the same site, in the same plots of land as the GM bananas from this proposed trial. The applicant has proposed the same containment measures for both trials.

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

76. All of the introduced genes are isolated from naturally occurring organisms that are already present in the environment.

77. Programmed cell death (PCD) is an integral part of plant and animal tissue development (see Section 5.2.5 of this chapter). Consequently, multicellular organisms in which apoptosis is a normal function already contain anti-apoptotic genes. Therefore, it is expected that humans, herbivores/omnivores and microorganisms routinely encounter the *ced-9* gene, homologues or proteins with a similar function, through contact with plants and food derived from plants. This information forms the baseline data for assessing the risks from exposure to the CED-9 protein as a result of the trial of GM banana lines.

78. GFP is a jellyfish protein and sources of GFP in the terrestrial environment would be minimal and confined to other GMOs.

79. 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.

Section 7 Australian and international approvals

7.1 Australian approvals of the GM banana lines

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

80. There has been no release of these GM banana lines in Australia. However, the Regulator has approved the limited and controlled release of banana genetically modified for enhanced nutrition (DIR 076/2007) for trials to be conducted on up to 1.4 ha between 2008 and 2010.

7.1.2 Approvals by other Australian government agencies

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

82. 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 banana lines in human food, accordingly an application to FSANZ has not been submitted. FSANZ approval would need to be obtained before materials from these GM banana lines could be used in food.

7.2 International approvals

83. There has been no release of these GM banana lines in any other country. The applicant states that at least three GM banana field trials using other introduced genes have been conducted overseas by private companies. Detailed data have not been published but the genetic modifications have been concerned with nematode and disease resistance.

Chapter 2 Risk assessment

Section 1 Introduction

84. 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.

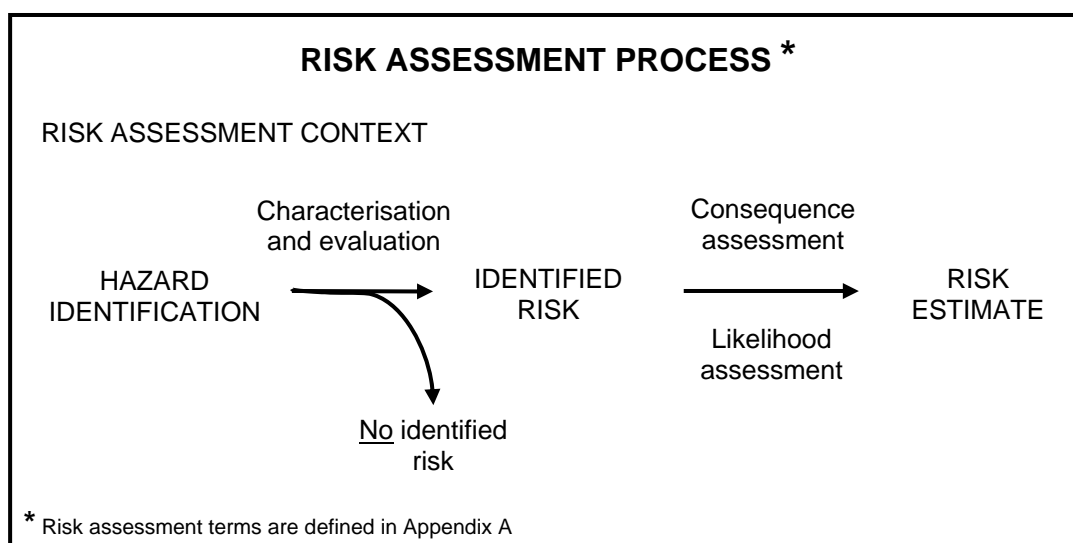


Figure 4 The risk assessment process

85. 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.

86. 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).

87. 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.

88. 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.

89. 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

90. 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.

91. 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.

92. 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 GMOs¹¹
- ♦ 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(s) of release
- ♦ agronomic management practices for the GMOs.

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

¹¹ As discussed in Sections 5.2.4, the *nptII* gene and its product has already been considered in detail in previous RARMPs and by other regulators. They have not been found to pose risks to either people or the environment and will not be considered further.

Table 5 Summary of events that may give rise to an adverse outcome through the expression of the introduced gene for disease resistance.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Ingestion of, contact with, or inhalation of GM plant material containing proteins encoded by the introduced genes, or their end products.	Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The encoded proteins and their end products are found in the environment and are unlikely to be toxic/allergenic to people or toxic to other organisms. The proposed release is limited and controlled, further reducing exposure of people and other organisms to products of the introduced genes.
Section 2.2 Spread and persistence (weediness) of the GM banana lines in the environment	2. Expression of the introduced genes improving the survival of GM banana plants.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> Non-GM, commercial banana does not possess weedy characteristics. The genetic modifications are not expected to affect the survival of the GM lines. The limits and controls proposed for the release would minimise persistence.
	3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> Opportunities for dispersal are limited by the very low level of seed production and lack of easily dislodged vegetative propagules. The proposed limits and controls would minimise dispersal.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced genes or regulatory sequences in commercial, non-GM sweet banana plants or in other sexually compatible plants.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The very low fertility of non-GM, commercial banana is not expected to be altered by the introduced genes. Thus it is highly unlikely that crossing with sexually compatible plants would occur. The applicant proposes to remove or cover male flowers, further reducing the likelihood of gene flow, which would limit the potential for vertical gene flow.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence of the introduced genes, 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 genes 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 did not identify any risks to people or the environment associated with expression of the introduced genes.
Section 2.5 Unintended changes in biochemistry, physiology or ecology	6. Changes to biochemistry (including innate toxic or allergenic compounds), physiology or ecology of the GM banana lines resulting from altered expression or random insertion of the introduced genes.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> Adverse effects as a result of unintended changes, if any, would be minimised by the proposed limits and controls. Unexpected alterations are likely to be detected and eliminated during the selection process. One of the purposes of the trial is to identify any unintended phenotypic effects

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>Agrobacterium tumefaciens</i> containing the introduced genes	7. Transfer of the introduced genes from <i>Agrobacterium</i> to other organisms	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> There is very low likelihood that the <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 and 5 did not identify any risks to people or the environment associated with expression of the introduced genes
Section 2.6 Unauthorised activities	8. Use of the GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	<ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

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

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

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

96. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced gene for disease resistance. Workers cultivating the bananas would be exposed to all plant parts. Organisms may be exposed directly to the proteins through biotic interactions with GM banana plants (vertebrates, insects, symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include organisms that feed on organisms that feed on GM banana plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

Event 1: Ingestion of, contact with, or inhalation of GM plant materials containing proteins encoded by the introduced genes, or their end products.

97. Expression of the introduced genes could potentially result in the production of novel toxic or allergenic compounds in the GM banana lines, or alter the expression of endogenous banana proteins. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these humans or other organisms.

98. Non-GM banana is not known to be toxic to humans or other organisms (OGTR 2008). Allergic reactions as a result of ingesting the fruit can take the form of either fruit-latex allergy or oral allergy syndrome (OGTR 2008).

99. Although no toxicity studies on the GM banana plant material or CED-9 protein have been performed (Chapter 1, Section 5.2.2), the introduced gene for disease resistance is isolated from a naturally occurring organism (Chapter 1, Sections 5.2.5 and 6.5). Furthermore,

the *ced-9* gene may be homologous to similar genes in other plants, or express a protein with a similar function to endogenous banana proteins (Chapter 1, Sections 5.2.1, 5.2.5 and 6.5).

100. GFP is a jellyfish protein and sources of GFP in the terrestrial environment would be minimal and confined to other GMOs. GFP has been shown to be non-toxic to rats when ingested in purified form or when synthesised in transgenic plants (Chapter 1, Section 5.2.3). The GFP protein contains an autofluorescent chromophore and does not perform any other biochemical function (Tsien 1998), so it is not expected that any novel products would be produced as a result of the expression of this introduced gene.

101. No information was found to suggest that the proteins encoded by the introduced genes are toxic or allergenic to people or to other organisms (Chapter 1, Section 5.2.2 and 5.2.3). Therefore exposure to the GM plant materials is not expected to adversely affect the health of humans or other organisms.

102. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. Public exposure to GM plant materials via ingestion, skin contact or inhalation would be minimal as no plant material will be used as food, animal feed and public access to the trial site is restricted. Fruit from the trial will be decomposed in a waste bin so as to avoid consumption by frugivores. Human exposure to the GM plant materials would be limited to trained and authorised staff associated with the field trial.

103. **Conclusion:** The potential for allergic reaction in people, or toxicity in people and other organisms as a result of consumption of, contact with, or inhalation of, GM plant materials containing proteins encoded by the introduced genes as a result of the genetic modification is **not an identified risk** and will not be assessed further.

2.2 Spread and persistence of the GM banana lines in the environment

104. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM banana plants in particular, is provided in the *Musa* Biology document (OGTR 2008). In summary, commercial banana cultivars do not possess characteristics that are usually associated with weediness and such cultivars do not pose a weed problem in Australia because their low fertility limits sexual dissemination and the integrity of the underground plant structure limits vegetative spread. Additionally, State legislation ensures that there are obligations for growers to destroy volunteers that may arise.

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

Event 2: Expression of the introduced genes improving the survival of the GM banana plants

106. If the GM banana lines were to establish or persist in the environment they could increase the exposure of humans and other organisms to the GM plant material. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials, the encoded proteins or end products has been considered in Event 1 and was not considered an identified risk.

107. If the expression of the introduced gene for disease resistance were to provide GM banana plants with a significant selective advantage over non-GM banana plants and they were able to establish and persist in favourable non-agricultural environments this may give

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

108. The impact of the genetic modifications on survival of the GM banana lines is uncharacterised under field conditions. However, the applicant states the introduced anti-apoptotic gene (*ced-9*) may confer disease resistance by preventing cells from undergoing PCD in response to infection by Sigatoka leaf spot fungal pathogens (*Mycosphaerella musicola* and *M. fijiensis*). Inhibition of apoptosis may also increase plant susceptibility to certain pathogens (Chapter 1, Section 5.2.5). In an environment in which disease was the main factor limiting the spread and persistence of banana, expression of the anti-apoptotic gene could result in increased weediness of the GM banana lines relative to non-GM banana.

109. PCD plays an important role in mediating the adaptive response of plants to changes in the environment. Studies have shown that PCD occurs when plants are exposed to salinity, cold stress, waterlogging and hypoxia (Pennell & Lamb 1997; Huh et al. 2002; Gladish et al. 2006). A number of studies have also shown that the expression of animal anti-apoptotic genes (including the *ced-9* gene) in plants confer protective advantages against a range of biotic and abiotic stresses. For example, when expressed in tobacco, the *ced-9* gene has been shown to confer protective advantages against necrotrophic fungal pathogens (Dickman et al. 2001) as well as a range of abiotic stresses including heat, cold, menadione and hydrogen peroxide (Li & Dickman 2004), salt and oxidative stress (Shabala et al. 2007). Expression of the *ced-9* gene in tomato plants enhanced plant survival by inhibiting PCD induced by virus infection and exposure to cold temperatures (Xu et al. 2004). Therefore, it is possible that GM banana plants expressing the *ced-9* gene will be able to survive other biotic and abiotic stresses compared to non-GM banana plants.

110. Additionally, PCD is integral to plant growth and development (Chapter 1, Section 5.2.5). High expression of animal anti-apoptotic genes, including the *ced-9* gene, in GM tobacco and tomato plants led to developmental abnormalities. In GM tobacco, these abnormalities included stunted growth, male sterility, stem bleaching, flower deformation and altered leaf pigmentation (Dickman et al. 2001). In GM tomato plants, developmental abnormalities also included stunted growth, the production of none or few viable seeds, and abnormal flower structure (Xu et al. 2004). GM tobacco and tomato plants expressing moderate levels of these anti-apoptotic genes did not show evidence of growth abnormalities. Therefore, it is unlikely that GM banana plants expressing the *ced-9* gene will have improved growth and developmental characteristics compared to non-GM banana plants. The applicant will monitor the GM banana plants for aberrant phenotypes.

111. As discussed in paragraph 104, commercial banana cultivars do not possess characteristics that are usually associated with weediness. Furthermore, such cultivars do not pose a weed problem in Australia because their low fertility limits sexual dissemination. Expression of the introduced genes are not expected to alter these characteristics.

112. As described in Chapter 1 (Section 5), one of the GM banana lines contains the *gfp* gene, derived from the jellyfish *Aequorea victoria*. It encodes a green fluorescent protein that enables visual identification of plant tissues in which the gene is expressed. The GFP protein contains an autofluorescent chromophore and does not perform any other biochemical function (Tsien 1998). Expression of the *gfp* gene is therefore unlikely to confer any selective advantage to the GM banana plants over non-GM banana plants.

113. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM banana lines proposed for release. The release would be of limited size and short duration and the applicant proposes a

number of control measures to restrict dissemination or persistence of the GM banana plants, including monitoring for and destroying any volunteer banana plants (Chapter 1, Section 3.3).

114. The purpose of the proposed release is to conduct proof of concept experiments with the GM banana lines to assess their development and disease resistance. Thus, any characteristics that may impact on the survivability of the GM plants will be closely monitored during the proposed trial.

115. **Conclusion:** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced genes for disease resistance improving the survival of the GM banana lines is **not an identified risk** and will not be assessed further.

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

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

117. The lack of seed production that is a characteristic of commercial non-GM banana cultivars is not expected to be altered in the GM lines.

118. The limits and controls on the proposed release (outlined in Chapter 1, Sections 3.2 and 3.3) will restrict dispersal of GM plant material. In addition, the fruits will be harvested while still green (a standard practice in the cultivation of commercial banana) so that they are less appealing to frugivores than fully ripe fruit and will be less likely to detach from a bunch and be inadvertently dispersed into the environment.

119. A number of native and feral animals such as kangaroos, flying foxes and feral pigs would have access to the proposed field location. They would be unable to reach the fruit either because of its height above ground or the fact that the applicant has proposed to place bunch covers over the fruit. Furthermore, even if fruit were dispersed by animals, it is highly unlikely to contain viable seed. Feral pigs, in particular could damage plants at ground level, but the applicant states that they have not been recorded uprooting plants or detaching suckers on the land at the site.

120. Information in paragraph 62 indicates that extremes of weather are unlikely to cause dispersal of plant parts that are capable of reproduction.

121. All GM plant material will be transported in accordance with the OGTR transport guidelines which will minimise the opportunity to disperse the GM material.

122. In the unlikely event that material is dispersed away from the proposed locations, Queensland State legislation requires the destruction of any volunteers that may arise as a result. This would also limit the persistence of any dispersed materials in the environment.

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

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

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

125. Baseline information on vertical gene transfer associated with non-GM banana plants is provided in the *Musa* Biology document (OGTR 2008). In summary, commercial banana cultivars are effectively sterile and therefore the chances of natural hybridisation either within the same species or between different *Musa* species are remote where a cultivated variety is one of the parents.

Event 4: Expression of the introduced genes and regulatory sequences in commercial, non-GM sweet banana plants or in other sexually compatible plants

126. Transfer and expression of the introduced genes in other banana or sexually compatible plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

127. However, as discussed in Event 2, the survival of GM banana plants would be limited as commercial banana cultivars do not possess characteristics that are usually associated with weediness. Furthermore, such cultivars do not pose a weed problem in Australia because their low fertility limits sexual dissemination. Expression of the introduced genes is not expected to alter these characteristics.

128. As discussed in Event 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM banana plants by the introduced genes or regulatory sequences. This will be the same if the introduced genes are expressed in other commercial, non-sweet banana plants. Similarly, if the introduced genes are expressed in other sexually compatible species, allergenicity and toxicity are not expected to be altered.

129. All of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to banana 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.

130. Cavendish bananas are effectively male sterile and the introduced genes are not expected to alter this trait. Therefore, gene transfer occurring through pollination is highly unlikely.

131. The commercial, non-GM sweet banana varieties growing in proximity to the GM lines from this proposed release are effectively female sterile so even if pollen were to be produced by the GM lines from this proposed release, fertilization of other non-GM plants would be highly unlikely. Other *Musa* species growing as part of a germplasm collection at the site are not permitted to flower.

132. The applicant proposes to remove most male flowers from the GM plants. Those flowers that would remain (for experimental analysis) will be bagged to prevent loss of pollen (in the unlikely event that pollen is produced).

133. While it is unlikely that the GMOs 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 banana plants during the proposed trial. In addition, the control measures proposed to restrict size, location and duration of the trial would minimise the likelihood of vertical gene transfer occurring.

134. The limited and controlled release of banana genetically modified for enhanced nutrition has been approved by the Regulator (licence DIR 076/2007) (Chapter 1, Section 7.1.1). The parent organism for the GM bananas in DIR 076/2007 is another cultivar ('Williams) from the Cavendish sub-group. The GM bananas from DIR 076/2007 will be grown on the same site, in the same plots of land as the GM bananas from this proposed trial. The transfer and expression of the introduced genes from both GM banana trials could lead to stacking of introduced genes, which could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting banana plants. However, as discussed in paragraph 130, Cavendish bananas are male sterile and the introduced genes from both trials are not expected to alter this trait. Furthermore, the applicant has proposed the same containment measures for each trial (see Chapter 1, Section 3.3).

135. The nearest sexually compatible native *Musa* species is 10 km from the proposed release. It should be noted that blossom foraging bats of *Pteropus* spp., which could feed on the flowers of *Musa* spp., could carry pollen that becomes trapped in their head fur as far as 50 km (Eby 1995). However, the likelihood of this occurrence posing a risk with regard to gene flow involving pollen from GM banana flowers is very low because of the low viability of the pollen produced by Cavendish flowers (Fortescue & Turner 2004) and because of the proposal by the applicant to remove or bag any male flowers produced on the inflorescences.

136. A number of regulatory sequences are derived from plant or pathogenic species that are associated with mild allergic reactions in susceptible individuals, a toxic response in humans and animals (in the case of *Ricinus communis*), or disease in plants or humans. However, the regulatory elements represent only a small proportion of the parent organism genetic material and have not been reported to cause any adverse effects.

137. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for pollen flow and gene transfer to sexually compatible plants. In particular, the applicant proposes to remove or bag any male flowers produced on the inflorescences to prevent access by pollinators. The applicant also proposes to perform post harvest monitoring of the site for at least 12 months and destroy any volunteer plants found in the site. This would ensure that any remaining GM banana plants in these areas would be destroyed prior to flowering.

138. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genes and regulatory sequences in other GM banana lines, or commercial, non-GM sweet banana plants or other sexually compatible plant species as a result of gene transfer is **not an identified risk** and will not be assessed further.

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

139. Horizontal gene transfer is the movement of genetic information (DNA) between sexually unrelated organisms (Thomson 2001). 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/ir/dir057.htm>> 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 the introduced regulatory sequences, in unrelated organisms as a result of gene transfer

140. The probability of transferring introduced genes contained in the GM banana plants is no greater than that of transferring any of the native genes. Non-GM banana is expected to contain homologues of the *ced-9* gene, or proteins of similar function to that expressed by the *ced-9* gene (Chapter 1, Section 5.2.1 and 5.2.5), and therefore these genes are already available for transfer via demonstrated natural mechanisms (Chapter 1, Section 6.5). In addition, homologues of anti-apoptotic genes, or proteins with similar function, occur in all multicellular animals, and are likely to occur in all plant species, and thus are widespread in the environment. The *gfp* gene is derived from a jellyfish, and is therefore not widespread in the terrestrial environment. However, it is widely used for experimental purposes (Chapter 1, Section 5.2.3).

141. The introduced regulatory sequences are already present in the environment and are available for horizontal transfer.

142. 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).

143. A key consideration in the risk assessment process should be the safety of the protein product(s) resulting from the expression of the introduced gene(s) rather than horizontal gene transfer *per se* (Thomson 2001). If the protein products are not associated with any risk then even in the unlikely event of horizontal transfer occurring, it should not pose any risk to humans, animals or the environment. Events 1-4 associated with the expression of the introduced genes or end products did not represent an identified risk.

144. **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

145. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy¹² (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

¹² Pleiotropy is the effect of one particular gene on the expression of other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

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

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

Event 6: Changes to biochemistry, physiology or ecology of the GM banana lines resulting from expression or random insertion of the introduced genes

147. Considerations relevant to altered biochemistry, physiology and ecology in relation to expression of the introduced genes have already been discussed in Events 1 to 3, and were not considered identified risks.

148. The biochemical function of the *ced-9* gene in banana and other plants is unknown. Indeed, it is not fully understood how the *ced-9* gene inhibits cell death and promotes cell survival in animal cells (Xu et al. 2004). Therefore, it is possible that the biological activities of the introduced gene may interrupt and inhibit different regulation pathways, including PCD, in the GM banana plants.

149. Expression of the *ced-9* gene in banana plants may lead to changes in growth and development (Chapter 1, Section 5.2.5). As discussed in Event 2, it is possible that high levels of *ced-9* expression in the GM banana plants may result in morphological and physiological abnormalities. The GM bananas will be monitored for aberrant phenotypes by the applicant.

150. As discussed in Chapter 1, Section 5.2.5, the expression of the *ced-9* gene in the GM banana plants may confer resistance to necrotrophic diseases, but increase susceptibility to biotrophic diseases. This may result in the GM banana plants having higher rates of infection of biotrophic diseases than their non-GM counterparts, which may spread to other non-GM banana plants at, or nearby, the trial site. Under Queensland State Government legislation for banana disease control (Queensland Government 1999), banana plants (GM or non-GM) must be monitored for any diseases. Any banana plants showing signs of infection are subject to strict control measures, including eradication and destruction, as outlined in the legislation. Therefore, in the unlikely event that increased susceptibility of the GM banana plants to disease were to occur as a result of an unintended change, any possible related outbreak of disease in other bananas would be effectively restricted by the mandatory measures already in place under Queensland legislation.

151. The GFP protein contains an autofluorescent chromophore and does not perform any other biochemical function (Tsien 1998). Expression of GFP is not expected to impact on biochemical pathways (Chapter 1, Section 5.2.3).

152. The *ced-9* and *gfp* genes have been expressed in banana embryonic cell suspensions (Khanna et al. 2004; Khanna et al. 2007). However, because of the large size, growing space and soil requirements of mature banana plants it has not been possible to conduct preliminary characterisation of the effects of the introduced genes under contained conditions (such as found in a glasshouse). The proposed field trial thus represents the first opportunity for such characterisation.

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

154. The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2 and 3.3. In particular, the scale and duration of the trial would limit the potential for adverse effects.

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

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

156. *Agrobacterium tumefaciens* is a soil-borne, Gram-negative bacterium that, in nature, causes crown gall on plants. The bacterium enters wounded somatic cells of the host and causes surrounding host cells to proliferate irregularly and form a gall. The bacterium is confined to the cells of the gall. Eventually, degradation of the gall releases the *A. tumefaciens* back into the soil where it can live saprophytically for several years (Krimi et al. 2002; Escobar & Dandekar 2003).

157. 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. *A. 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).

158. 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 banana) since there would be no opportunity for elimination of the *A. tumefaciens* in sexually produced generations.

159. The possibility of persistence of *A. tumefaciens* in GM plant tissue has led to suggestions for *Agrobacterium*-mediated transformation to include protocols for the detection and elimination of the bacterium (Cubero & López 2004). Suggested detection methods include PCR-based methods employing primers that amplify parts of the bacterial genome present in the Ti plasmid but are not transferred to the plant genome after transformation (Cubero & López 2004).

160. The transfer of GM banana 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 a previous RARMP concerning GM rose (see DIR 060/2005 - available at <<http://www.ogtr.gov.au/ir/dir060.htm>> or by contacting the OGTR). In this instance, the GM rose plants 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 genes from *A. tumefaciens* to other organisms

161. The GM banana lines proposed for release were generated by *Agrobacterium tumefaciens*-mediated genetic modification (Section 5.4, Chapter 1). The applicant has shown, through PCR testing of GM banana plant material, using primers specific to regions outside the T-DNA (see paragraph 50), that the *A. tumefaciens* does not persist in the GM banana plant material for longer than approximately 3 weeks after removal from the *in vitro* environment. As plants will be hardened off for several months in a shadehouse (see paragraph 67) it is unlikely that plants transferred to the field would be carrying residual *A. tumefaciens*.

162. If *A. tumefaciens* containing an introduced gene construct were present in the cells of GM banana plants it could transfer the introduced genes via conjugation with a wild type strain or other bacteria and yeast naturally present in the soil at the site (Hammerschlag et al. 2000). This general possibility of horizontal gene transfer has already been discussed in Event 5 and was not considered to be an identified risk.

163. If the *A. tumefaciens* were present in GM banana tissue it could also genetically modify cells of other plants. Although the conditions for *A. tumefaciens* infection and gene transfer to plants would exist in nature, the creation of a GM plant would be highly unlikely because: (1) it would be unlikely that the *A. tumefaciens* would genetically modify a cell or cells that would give rise to a new organism, (2) it is unlikely that conditions in nature would exist that would select for the survival of the infected GM plant cells, and (3) not all the GM plant cells would have expression of the introduced genes (eg due to position effect, genetic re-arrangements, silencing due to multiple copies). Should a new GM plant arise it is unlikely that it would have a selective advantage. In addition, homologues of the introduced *ced-9* gene, or proteins with similar function, are already widespread in plants.

164. **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)

165. 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 banana 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.

166. **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

167. 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.

168. 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 or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

169. 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.

170. 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, location and duration of the release proposed by QUT
- ♦ suitability of controls proposed by QUT to restrict the dissemination or persistence of the GM banana plants and their genetic material
- ♦ limited capacity of the GM banana lines to spread and persist outside the areas proposed for release
- ♦ limited ability and opportunity for the GM banana lines to transfer the introduced genes to commercial banana 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 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 banana lines into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment¹³.

Section 4 Uncertainty

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

¹³ As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

172. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹⁴. For field trials some knowledge gaps are inevitable because they involve the conduct of research. 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.

173. For DIR 079/2007 which involves proof of concept research, uncertainty exists in relation to the characterisation of:

- ◆ Event 1, regarding potential increases in allergenicity or toxicity through contact with plant material containing proteins encoded by the introduced gene for disease resistance
- ◆ Event 2, associated with a potential for increased survival of the GMOs
- ◆ Event 6, regarding unintended changes as a result of the expression or insertion of the introduced genes.

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

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

¹⁴ A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at <<http://www.ogtr.gov.au/pubform/riskassessments.htm>> or via Free call 1800 181 030.

Chapter 3 Risk management

176. 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

177. 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.

178. 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.

179. 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

180. 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, Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹⁵.

181. 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.

¹⁵ 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/pubform/riskassessments.htm>>

182. 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 banana lines to be used in human food. Accordingly the applicant has not applied to FSANZ for evaluation of any of the GM banana lines for use in human food. FSANZ approval would need to be obtained before they could be used in food.

183. No other approvals are required.

Section 3 Risk treatment measures for identified risks

184. 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 banana. 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.

185. These events were considered in the context of the scale of the proposed release [a maximum total area of 1.4 hectares over two years (July 2008 – April 2010) on a single site in the Queensland local government area of Cassowary Coast], the containment measures (Chapter 1, Section 3), and the receiving environment (see Chapter 1, Section 6).

Section 4 General risk management

186. Licence conditions have been proposed to control the dissemination and persistence of the GMOs and their genetic material in the environment and limit the release to the size, location and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are summarised in Sections 4.1.2 and 4.1.3.

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by QUT

187. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls proposed by QUT in their application, and discussed in the characterisation of events in Chapter 2. The appropriateness of these limits and controls was considered in detail in the previous assessment for the RARMP for DIR 076/2007 which is available at <http://www.ogtr.gov.au> or from the OGTR. A summary of these comments is provided below.

188. The potential for the GM banana plants to disperse into the environment (Event 3) or come into contact with the public (Event 1) is minimised because the proposed release would be confined to one site, staffed by appropriately trained personnel, over a two year period. In addition, the site is located in an area that is not vulnerable to landslip, flooding or erosion.

189. The applicant's proposal to remove and dispose of male flowers, or bag any male flowers remaining on the plant, will minimise the chances of pollen dispersing in the unlikely event that pollen is produced (Event 4). Since Cavendish bananas also produce hermaphrodite flowers that can contain pollen, it would be appropriate to also remove or bag hermaphrodite flowers.

190. The covering of bunches of fruit on GM plants would minimise the likelihood of adverse effects on frugivores (e.g. toxicity) (Event 1) and fruit/seed being dispersed, in the unlikely event that any seed is produced (Event 3).

191. The applicant proposes to desucker plants at the field location using the kerosene/distillate method and to leave all waste on the ground at the field location to decompose. It is proposed that waste from the shadehouse used for hardening-off would be transported to the field location and left to decompose on the ground. Whole plants (that would not be more than approximately 4 months old and therefore still immature) would additionally be sprayed with herbicide, so as to destroy the corm, before their transfer to the ground at the field location. As waste from the site will be non-propagative it is unlikely to result in dispersal of GM banana plants (Event 3) and it is unlikely to contain products that are harmful to any organisms that may eat it (Event 1). Therefore, the proposed method for disposal is considered to be appropriate.

192. The applicant proposes destroying fruit that is not required for experimental analysis by removing it from the plant and allowing it to decompose in lockable commercial waste bins located at the field location. The addition of agricultural lime would hasten decomposition. Once the material has fully decomposed (i.e. is odourless and would not be of interest to frugivores) it will be removed from the bins and left on the ground at the field location. This practice would ensure that the fruit will not be consumed by frugivores (Events 1 & 3).

193. Similarly, it has been proposed that male flowers not required for analysis will be removed from the plants prior to the opening of the floral bracts which enclose the flowers, and allowed to decompose in waste bins before the decomposed material is left on the ground at the field location. This practice would ensure that any pollen that may be produced will not be viable or dispersed into the environment.

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

195. The applicant's proposal to monitor the field location for a period of 12 months at the completion of the trial (and destroy any volunteer suckers) would prevent spread and dissemination (Event 3).

196. In addition to the above points, Queensland State Government legislation targeted to the restriction of non-GM banana growing and disease control (see Chapter 1, Section 6.3 and Event 6) will also apply to the release of GM bananas and will act as an effective adjunct to the proposed containment measures.

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

197. 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 area of up to 1.4 ha per year at one site in the local government area of Cassowary Coast (Queensland) between July 2008 and April 2010
- ♦ remove and destroy all male/hermaphrodite flowers on the inflorescences unless they are required for experimental analysis
- ♦ cover any male/hermaphrodite flowers left on the inflorescences
- ♦ cover fruit bunches

- ◆ remove and destroy all fruit not required for experimental analysis
- ◆ destroy any plant waste containing meristematic tissue
- ◆ clean all equipment used in cultivation practices
- ◆ not permit any materials from the release to be used in human food or animal feed
- ◆ 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

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

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

4.2 Other risk management considerations

200. 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

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

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

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

203. 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.

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

4.2.2 Compliance and contingency plans

205. Prior to planting the GM banana lines, QUT is required to submit a plan detailing how it intended to ensure compliance with the licence conditions and document that compliance. This plan would be required before the planting of the GM banana lines could occur.

206. QUT 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 banana lines outside of the permitted areas.

207. QUT 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

208. 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

209. 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.

210. 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.

211. 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 final harvest
- ◆ expected and actual dates of destruction and cleaning after final harvest.

4.2.5 Monitoring for Compliance

212. 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.

213. 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.

214. 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

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

- ♦ characterisation of the introduced genetic material in the plants, including genotypic stability
- ♦ additional data on the potential toxicity of plant materials from the GM banana lines
- ♦ additional data on the allergenicity of protein encoded by the introduced genes for disease resistance
- ♦ characteristics indicative of weediness including measurement of altered reproductive capacity; tolerance to environmental stresses; and disease susceptibility.

Section 6 Conclusions of the RARMP

216. The risk assessment concludes that this limited and controlled release of up to 17 GM banana lines on a maximum total area of 1.4 ha over two years in the Queensland local government area of Cassowary Coast poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

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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 authorities¹⁶ on the consultation RARMP for DIR 079/2007

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

Summary of issues raised	Comment
<p>Notes that there is low probability that the intentional release of viable <i>Agrobacterium</i> in the GM banana plants could lead to harm to the environment.</p> <p>Recommends that consideration be given to the possibility that residual <i>Agrobacterium</i> may be persistent in the GM banana plants and whether any risk treatment measures are warranted.</p>	<p>Noted.</p> <p>The unintended presence in the environment of <i>Agrobacterium</i> containing the introduced genes is addressed in Event 7 and was not considered to be an identified risk. The applicant has shown, through PCR testing of GM banana plant material, using primers specific to regions outside the T-DNA (Chapter 1, Section 5), that the <i>Agrobacterium</i> does not persist in the GM banana plant material for longer than approximately 3 weeks after removal from the <i>in vitro</i> environment.</p>
<p>Notes that the GM banana plants will not be used for human food or animal feed. However, if QUT envisages a future application for permission for use of food derived from these GM banana lines, they should contact the relevant regulatory agency to discuss specific data requirements to support a safety assessment.</p>	<p>Noted.</p> <p>FSANZ is responsible for human food safety assessment, including GM food.</p>
<p>Notes that no risks have been identified for this trial. However, strongly recommends that given the nature of the release and the relatively small amount of data available to predict levels of risk, risks relating to possible toxicity, allergenicity and an increased sensitivity to some pathogens be identified for Section 2.5 ('Unintended changes in biochemistry, physiology or ecology'). If appropriate monitoring and control options are incorporated in the conduct of the trial, then the risks posed by the trial will be low and manageable.</p> <p>The two risks identified are discussed below.</p>	<p>Toxicity and allergenicity are addressed in Event 1 and were not considered to be an identified risk. A discussion on the potential for increased sensitivity to some pathogens and clarification of Queensland State Government legislation for banana disease control has now been included in Event 6. This event was not considered to be an identified risk.</p> <p>The RARMP concludes that this limited and controlled release poses negligible risks which do not require specific risk treatment measures (see discussion below).</p>

¹⁶ GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment, Heritage & the Arts and the Local council(s) where the release may occur.

Summary of issues raised	Comment
<p>1) The potential for the CED-9 protein to have a negative effect on organisms that come into contact with the expressed protein.</p> <p>Agrees that the potential for toxic or allergenic reactions in humans and other organisms may be low. However, there is little information, such as toxicity testing, on which to base the risk assessment, which depends largely on assumed homology between the nematode <i>ced-9</i> gene and plant genes, and the ability to restrict access of organisms to the GM plants. Also, the similarity of plant and animal PCD genes is so low that the effect of expressing the nematode gene in plants is not comparable to the effect of the <i>ced-9</i> gene in its native context.</p> <p>While it is unlikely that there would be harm to organisms coming into contact with the GM plants, it is suggested that impacts on invertebrates should be monitored during the trial. A significant difference in species composition and abundance may indicate a possible toxic, allergenic, or other effect which could be further investigated before commercial release is considered.</p>	<p>Agreement noted to conclusions of Event 2 which considers that harm to organisms coming into contact with GM plant material would be unlikely and the potential for toxicity or allergenicity to people or other organisms would be low.</p> <p>Uncertainty associated with toxicity and allergenicity of these GM banana lines was identified in the RARMP (Chapter 2, Section 4). Hence, additional information relating to toxicity and allergenicity may be required to assess an application for a large scale or commercial release of any of these GM banana lines (Chapter 3, Section 5) and this could include possible impacts on invertebrates.</p>
<p>2) The potential for an increase in sensitivity to pathogens from altering how bananas respond to infection.</p> <p>Agrees with the statement in the RARMP that the 'expression of anti-apoptotic genes in plants may therefore confer resistance during susceptible plant-pathogen interactions (ie necrotrophic diseases), but could also increase susceptibility in resistant plant-pathogen interactions (ie biotrophic diseases)', but feels it warrants further examination. Increased susceptibility to infection from biotrophic pathogens could potentially lead to disease outbreak or selection of a more severe pathogen. This may harm nearby breeding and commercial plantations, and native banana species.</p> <p>Acknowledges that such an event is unlikely. Nonetheless, the risk posed by severely infected plants can be managed by removing the infected plant and treating it as biohazardous waste using established procedures, and by not leaving plant waste on the site.</p>	<p>Under Queensland State Government legislation for banana disease control, the GM banana plants would be required to be monitored for any diseases and subject to strict control measures, including eradication and destruction. As a result, in the unlikely event there is increased disease susceptibility of the GM banana plants, any possible adverse effects will be minimised.</p>

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 079/2007

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

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

Issues raised: **A:** Allergenicity, **DR:** Data requirements; **EN:** Environmental risks, **H:** Human health and safety, **P:** pleiotropic effects, **RA:** Risk Assessment process; **Res:** further research, **T:** Toxicity

Other abbreviations: **Ch:** Chapter; **FSANZ:** Food Standards Australia New Zealand; **GM:** Genetically Modified; **GMO:** Genetically Modified Organism; **GTR:** the Gene Technology Regulator; **OGTR:** Office of the Gene Technology Regulator; **RARMP:** Risk Assessment and Risk Management Plan

Type: **A:** Agricultural/industry organisation; **IG:** interest group; **I:** individual

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	x	none	Objects to the development and release of GM bananas.	Noted.
			EN, H	The finding that the GM bananas do not pose a risk is meaningless in the absence of ecological studies and evidence that GM bananas do not affect human health.	The analysis of risks to human health and safety and the environment that may arise as a result of the proposed release of GM bananas is based on a thorough assessment of currently available scientific information including information on the known functions of the introduced genes and effects observed in other GM plants with similar or the same introduced genes. The small scale and short duration of the proposed trial, as well as the control measures imposed in the licence, reduces the chance of any adverse effects. Further research relating to human health and the environment has been identified as a requirement for the assessment of future releases involving larger scale or reduced containment measures (Chapter 3, Section 5).
2	I	x	DR, H, Res	Considers that it is an assumption to argue that the safety of a certain protein in use in one genome implies safety in another related, or unrelated, genome. Calls for it to be a	QUT's purpose in conducting this limited and controlled release is to conduct proof of concept research involving experiments with the GM banana lines to assess their development and disease response.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
				requirement that animal feeding and breeding trials, using whole GM food product, be used to test the validity of this statement and to determine the level of risk for use of the GM product in the food chain.	Feeding trials are not necessary for the assessment of this application as no GM plant material is permitted to be used in human food or animal feed (see Section 3, Chapter 1 of the RARMP and licence conditions).
			A, T, P	Considers it unacceptable to argue that GM bananas are substantially equivalent to their non-GM counterparts (eg toxicity, allergenicity and pleiotropic effects).	The information available on toxicity, allergenicity and pleiotropic effects was considered to be sufficient for an assessment of the risks that may be associated with the dealings proposed in this limited and controlled release. However, the risk assessment identified additional information that may be required to assess an application for a larger scale trial or reduced containment conditions, including data on toxicity and allergenicity (Chapter 3, Section 5).
			RA	States that the OGTR and FSANZ will eventually approve these GM bananas for human use because neither agency requires animal feeding trials to test the safety of GM food crop products in the food chain. Asks when this situation will change. Is Federal legislation needed in order to make this a requirement?	FSANZ is responsible for the evaluation of GMOs for use in human food. Whole food animal feeding studies may be informative in some limited circumstances but the use of comparative assessment of the GM food with its non-GM counterpart can identify potential adverse effects.