



Australian Government

Department of Health and Ageing

Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 107

Limited and controlled release of banana
genetically modified for disease resistance

Applicant: Queensland University of Technology

January 2011

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of application DIR 107 from Queensland University of Technology (QUT). The licence authorises dealings involving the limited and controlled release of genetically modified (GM) banana into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether or not to issue a licence to deal with a genetically modified organism (GMO).

The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public¹.

The application

QUT has applied for a licence for dealings involving the intentional release of GM banana into the environment on a limited scale and under controlled conditions. The GM banana lines have been genetically modified for disease resistance. The field trial is authorised to take place at one site in the local government area (LGA) of Litchfield Municipality, Northern Territory, on a maximum area of 1.5 ha between the date of issue of the licence and November 2014.

The purpose of the trial is to conduct proof of concept experiments to assess the disease response and/or development of the GM banana lines. Material from the GM banana plants will not be used in human food or animal feed.

A total of up to 151 lines² of GM banana are intended for release, comprising 18 GM Cavendish banana lines and 133 GM Lady Finger banana lines. Up to 131 of the GM banana lines contain one or two genes that are expected to provide protection from certain disease-causing microorganisms. The genes are derived from a range of organisms including viruses, bacteria and plants.

The remaining 20 GM banana lines contain a reporter gene that expresses a protein which enables visual identification of plant tissues in which it is expressed. This gene is derived from a common gut bacterium.

In addition, all of the GM banana lines contain an antibiotic resistance gene, also derived from a common gut bacterium, which was used to identify transformed plants during initial development of the GM plants in the laboratory.

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

¹ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

² The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

Risk assessment

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

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology are postulated (risk scenarios) and these scenarios are evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight risk scenarios were postulated, including consideration of whether or not expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM banana; or produce unintended changes in the biochemistry of the GMOs. The opportunity for gene flow to other organisms, and its effects if it were to occur, was also assessed.

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

The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not identify any risks that required further assessment.

Risks to the health and safety of people, or the environment, from the proposed release of the GM banana lines into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

Risk management plan

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through the licence conditions.

As none of the eight risk scenarios characterised in the risk assessment gave rise to an identified risk that required further assessment, the level of risk from the proposed dealings was assessed to be **negligible**. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and to limit the 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 1.5 ha at one site between the date of issue of the licence and November 2014. The **control** measures include containment provisions at the trial site; preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with the Regulator's transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concluded that this limited and controlled release of up to 151 lines of GM banana on a maximum total area of 1.5 ha over four years in the Northern Territory LGA of Litchfield Municipality, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, location and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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Abbreviations

the Act	The <i>Gene Technology Act 2000</i>
<i>AtBag4</i>	<i>Arabidopsis thaliana</i> Bcl-2-associated anthanogene-4 gene
<i>AtBI-1</i>	<i>Arabidopsis thaliana</i> BAX inhibitor 1 gene
<i>Avr</i>	Avirulence gene
<i>Bag</i>	Bcl-2-associated anthanogene gene
BAX	Bcl-2 associated protein X
BI	BAX inhibitor
CaMV	Cauliflower mosaic virus
<i>Cat</i>	Catalase gene
<i>Ced-9</i>	Cell death abnormality gene-9
DBFC	Darwin Banana Farming Company
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
Foc	<i>Fusarium oxysporum f.sp. cubense</i>
Foc TR4	<i>Fusarium oxysporum f.sp. cubense</i> Tropical Race 4
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GUS	β -glucuronidase
ha	Hectare
HGT	Horizontal Gene Transfer
HR	Hypersensitive response
HSP	Heat shock protein
IAP	Inhibitor of apoptosis
LGA	Local Government Area
<i>mCed-9</i>	Modified cell death abnormality gene-9
mRNA	Messenger Ribonucleic Acid
NLRD	Notifiable Low Risk Dealing
<i>nos</i>	Nopaline synthase gene
<i>nptII</i>	Neomycin phosphotransferase type II gene
NT	Northern Territory
OGTR	Office of the Gene Technology Regulator
<i>OsBag4</i>	<i>Oryza sativa</i> Bcl-2-associated anthanogene-4 gene
PC2	Physical Containment level 2
PCD	Programmed cell death
PCR	Polymerase Chain Reaction
QUT	Queensland University of Technology
R	Resistance
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RING	Really interesting new gene
RGC	Resistance gene candidate
RNA	Ribonucleic Acid
<i>SfIAP</i>	<i>Spodoptera frugiperda</i> inhibitor of apoptosis gene
T-DNA	Transfer DNA

TEV	Tobacco etch virus
<i>tra</i>	Transfer
<i>Ubi</i>	Ubiquitin gene
<i>vir</i>	Virulence

Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of application DIR 107 from Queensland University of Technology (QUT). The licence authorises dealings involving the limited and controlled release of genetically modified (GM) banana into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether or not to issue a licence to deal with a genetically modified organism (GMO).

The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public³.

The application

QUT has applied for a licence for dealings involving the intentional release of GM banana into the environment on a limited scale and under controlled conditions. The GM banana lines have been genetically modified for disease resistance. The field trial is authorised to take place at one site in the local government area (LGA) of Litchfield Municipality, Northern Territory, on a maximum area of 1.5 ha between the date of issue of the licence and November 2014.

The purpose of the field trial is to conduct proof of concept experiments to assess the disease response and/or development of the GM banana lines. Material from the GM banana plants will not be used in human food or animal feed.

A total of up to 151 lines⁴ of GM banana are intended for release, comprising 18 GM Cavendish banana lines and 133 GM Lady Finger banana lines. Each line contains one or two genes that are expected to provide protection from certain disease-causing microorganisms, or the *uidA* reporter gene, as described below.

Up to 19 of the GM banana lines contain one of two specific disease resistance gene candidates, which were isolated from a species of non-GM banana that is resistant to the pathogen *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4.

Up to 112 of the GM banana lines contain one or two of nine anti-apoptotic genes derived from a range of organisms including viruses, bacteria and plant species. These genes are expected to confer disease resistance by preventing cells from undergoing programmed cell death (or apoptosis) in response to infection by certain pathogenic microorganisms. Expression of the anti-apoptotic genes may also affect growth and development of the GM banana plants and confer enhanced tolerance to a range of biotic and abiotic stresses.

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/>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) 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 remaining 20 GM banana lines contain the reporter gene *uidA* derived from *Escherichia coli*. The *uidA* gene encodes an enzyme, β -glucuronidase (GUS), which enables visual identification of plant tissues in which it is expressed. GM banana plants containing the *uidA* gene will be used as controls to ascertain if any observed phenotype is a result of the expression of the introduced genes for disease resistance and not the transformation process.

In addition, all of the GM banana lines contain the antibiotic resistance gene *neomycin phosphotransferase type II (nptII)*, which is also derived from *E. coli*. The *nptII* gene encodes the enzyme neomycin phosphotransferase, which confers kanamycin or neomycin resistance on the GM plants. This was used as a selective marker during initial development of GM plants in the laboratory.

The expression of the introduced genes in the GM banana lines is under the control of short regulatory sequences. These are derived from: the plants *Zea mays* (maize), *Ricinus communis* (castor bean) and *Musa acuminata* ssp. *malaccensis* (banana); the soil bacterium *Agrobacterium tumefaciens*; and the plant viruses Cauliflower mosaic virus (CaMV) and Tobacco etch virus (TEV). Although *A. tumefaciens*, CaMV and TEV are plant pathogens, the regulatory sequences comprise only a small part of their respective total genomes, and are not in themselves capable of causing disease.

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

Risk assessment

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

A reference document, *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>>.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology are postulated (risk scenarios) and these scenarios are evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight risk scenarios were postulated, including consideration of whether or not expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM banana lines; or produce unintended changes in the biochemistry of the GMO. The opportunity for gene flow to other organisms, and its effects if it were to occur, was also assessed.

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

The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not identify any risks that required further assessment. The principal 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 spread and persistence of the GM banana plants and their genetic material

- limited ability and opportunity for the GM banana plants to transfer the introduced genes to commercial banana crops or other sexually related species
- effectiveness of removal of GM *A. tumefaciens*, which were used during the genetic modification process, from the GM banana plants prior to field release
- 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 the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

Risks to the health and safety of people, or the environment, from the proposed release of the GM banana into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

Risk management plan

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through the licence conditions.

As none of the eight risk scenarios characterised in the risk assessment gave rise to an identified risk that required further assessment, the level of risk from the proposed dealings was assessed to be **negligible**. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and to limit the 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

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

- limit the release to a maximum total area of 1.5 ha at one site in the Litchfield Municipality LGA between the date of issue of the licence and November 2014
- locate the trial site at least 50 m away from waterways
- maintain a 10 m zone around the GM bananas in which no bananas may be grown
- 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
- harvest the GM banana separately from other crops
- clean all equipment used in connection with the GMOs
- monitor the field site for at least 12 months after harvest and destroy any volunteer banana plants that may grow
- destroy all GM plant material, including fruit, not required for further analysis
- transport and store all GMOs in accordance with the Regulator's guidelines
- not permit any GM banana plant material to be used in human food or animal feed.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. The Regulator is

responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. However, dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority, Therapeutic Goods Administration, National Industrial Chemicals Notification and Assessment Scheme and Australian Quarantine Inspection Service⁵.

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 sold as food.

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

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for a large scale or commercial release of these GM banana lines, or to justify a reduction in containment conditions. This would include:

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

Suitability of the applicant

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

Conclusions of the RARMP

The risk assessment concluded that this limited and controlled release of up to 151 lines of GM banana on a maximum total area of 1.5 ha over four years in the Northern Territory LGA of Litchfield Municipality, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, location and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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

Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed (Figure 1).

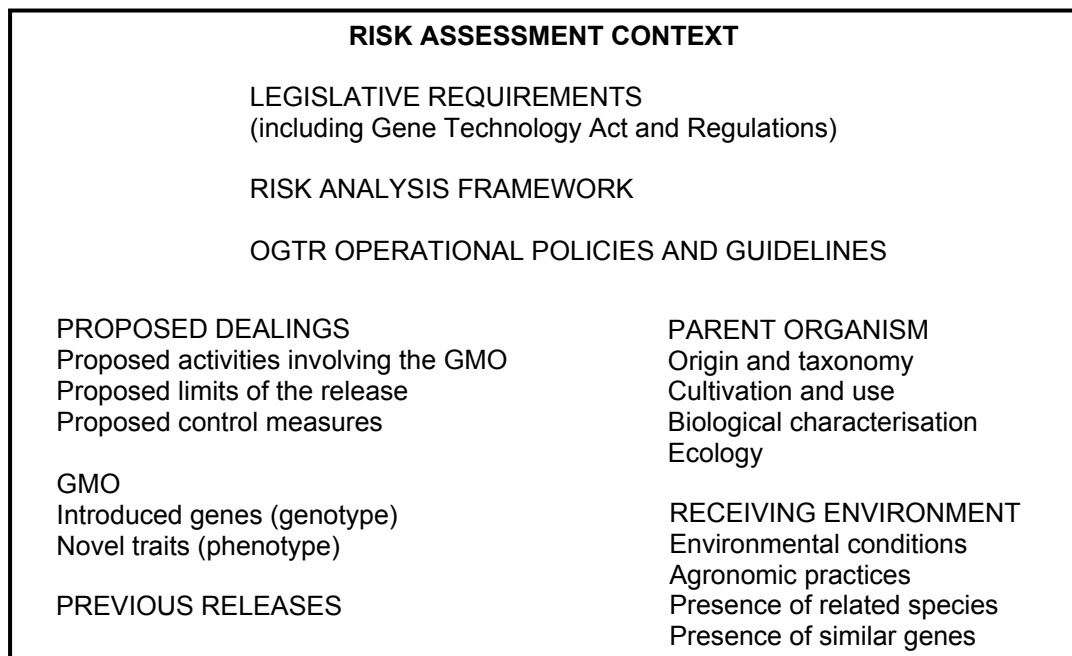


Figure 1. Parameters used to establish the risk assessment context

2. The risk assessment context is developed within the framework of the *Gene Technology Act 2000* (the Act) and Gene Technology Regulations 2001 (the Regulations, Section 2), the *Risk Analysis Framework*, and operational policies and guidelines <<http://www.ogtr.gov.au>>.

3. In addition, establishing the risk assessment context for this application includes consideration of:

- the proposed dealings (Section 3)
- the parent organism (Section 4)
- the genetically modified organisms (GMOs), nature and effect of the genetic modification (Section 5)
- the receiving environment (Section 6)
- previous releases of these or other GMOs relevant to this application (Section 7)

Section 2 The legislative requirements

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom he must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of his decisions on licence applications. In addition, the Regulations outline matters the Regulator must consider when preparing a RARMP.

5. In accordance with section 50A of the Act, the Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits have been proposed on the size, location and duration of the release and controls have been proposed by the applicant to restrict the spread and persistence of the GMOs

and their genetic material in the environment. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application is considered to be a limited and controlled release and the Regulator has prepared a RARMP for this application.

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

7. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a ‘significant risk’ to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d). The decision is provided in Section 3 of Chapter 2.

Section 3 The proposed dealings

8. The Queensland University of Technology (QUT) proposes to release up to 151 lines⁶ of GM banana, which have been genetically modified (GM) for disease resistance, into the environment under limited and controlled conditions.

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

- conducting experiments with the GMOs
- propagating, growing, raising or culturing the GMOs
- transporting the GMOs
- disposing of the GMOs
- possession, supply or use of the GMOs for the purposes of any of the above.

10. These dealings are detailed further throughout the remainder of the current Chapter.

3.1 The proposed activities

11. The applicant has stated that the purpose of the trial is to conduct experiments to assess the disease response and/or development of the GM banana lines.

12. For each GM banana line, 10 clones (replicates) would be released making a total of up to 1510 GM plants. Non-GM bananas would also be planted as controls. Virus indexed GM and non-GM bananas would be transported in tissue culture vessels (flasks) from QUT to the proposed release site, property of the Darwin Banana Farming Company (DBFC), where they would be de-flasked and acclimatised (hardened off) in a shadehouse for 2–4 months. Acclimatised plants would be moved approximately 250 m to the field location where they would be planted and maintained using similar cultural practices to those applied to conventional banana. A description of the relevant agricultural activities proposed for this release can be found in Section 6.3.

13. Plants would be grown for at least two cycles (approximately 2 years); that is, fruit would be obtained from the plant crop and one ratoon crop (see Section 6.3), in order to allow sufficient exposure to the pathogen to determine resistance. The GM banana plants would be progressively released, such that the total time for the proposed trial is four years.

⁶ The term ‘line’ is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

14. The disease status of the GM banana plants would be determined visually at the field location. Ratings would be given to external symptoms (e.g. number of yellow leaves, degree of stem splitting), and to internal symptoms by cutting across the stem and assessing the percentage of discoloration.

15. Plant material not required for further analysis would be decomposed at the trial site. A small number of samples of uninfected plant material may be taken. Any such samples taken from the GM bananas may be transported back to contained facilities at the Gardens Point Campus of QUT for analysis and destruction. Plant material from 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)

16. The release is proposed to take place at one site in the local government area (LGA) of Litchfield Municipality in the Northern Territory (NT). Within the site there is a field location and a shadehouse (to be used for hardening off tissue-culture generated plants before they are transferred to the field location) that together will occupy a maximum total area of 1.5 ha. The release is proposed to occur between 2010 and 2014.

17. Only trained and authorised staff would be permitted access to the proposed site. Training of staff would include standard health and safety induction and also training in practices relevant to DIR licence conditions, including practices in handling and disposal of material on site and sample packaging, labelling and transport. Staff would also be instructed that all plants within GM areas (trial site or shadehouse) are to be regarded as GM.

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

18. The applicant has proposed a number of controls to restrict the spread and persistence of the GM banana lines and the introduced genetic material in the environment including:

- locating the proposed trial site on flat land at least 1 km away from natural waterways
- bordering the field location with a 10 m zone in which no other banana plants are grown
- 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 or bagging the immature male bud (bell) of inflorescences to prevent access by pollinators
- complying with State and Territory Government legislation for banana disease control that would also aid in containment of GM plants
- analysing GM plant materials from the trial in an OGTR-certified PC2 facility and then destroying the materials by autoclaving
- destroying all (GM and non-GM) plant materials from the field trial not required for further analysis
- post harvest monitoring of the trial site for 12 months and destroying any volunteers
- transporting GM plant materials to and from the proposed trial site in accordance with Regulator's guidelines
- not permitting any GM plant material to be used in human food or animal feed.

19. These controls, and the limits outlined in Section 3.2, have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 3, Section 3.1.1.

Section 4 The parent organism

20. The parent organism is banana, *Musa* spp.. 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 NT near Darwin.

21. Most edible bananas are intraspecific or interspecific hybrids of *Musa acuminata* and *Musa balbisiana*. Four cultivars were used to generate the GM bananas proposed for release: ‘Williams’, ‘Grande Naine’, ‘Dwarf Cavendish’ and ‘Lady Finger’, all of which are exotic to Australia. The cultivars Williams, Grande Naine and Dwarf Cavendish are all very closely related and belong in the Cavendish subgroup of the triploid intraspecific hybrid of *M. acuminata* (AAA genome). The Lady Finger cultivar is in the Pome subgroup of the interspecific hybrid of *M. acuminata* and *M. balbisiana* (AAB genome).

22. Cavendish cultivars account for approximately 95% of the bananas on the Australian market. Lady Finger bananas comprise about 4% of the Australian market. Edible bananas have extremely low fertility. 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. Lady Finger bananas also have poor fertility and produce very little or no viable pollen and no seeds. Further detailed information about the parent organism is contained in a reference document, *The Biology of Musa L. (banana)*, which 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

23. The applicant proposes to release up to 151 GM banana lines, comprising 18 GM Cavendish banana lines and 133 GM Lady Finger banana lines. The genes introduced into the GM banana lines proposed for release are listed in Table 1. Details of the constructs used to generate the GM banana lines are provided in Tables 2 and 3.

24. Up to 131 of the GM banana lines contain one or two genes that are expected to confer disease resistance. The remaining 20 GM banana lines contain the *uidA* reported gene. GM banana plants containing the *uidA* gene would be used as controls to ascertain whether any of the observed phenotypic effects are a result of the expression of the introduced genes for disease resistance and not the transformation process.

25. In addition, all of the GM banana lines contain the antibiotic resistance selectable marker gene *neomycin phosphotransferase type II (nptII)* from the common gut bacterium *Escherichia coli*. This gene, encoding the enzyme neomycin phosphotransferase, confers kanamycin or neomycin resistance on GM plant cells. The *nptII* gene was used during initial development of the GM plants in the laboratory to select plant cells containing the introduced genes.

26. Short regulatory sequences would be used to control expression of the introduced genes. These sequences are derived from plants (maize, banana and castor bean), a soil bacterium (*Agrobacterium tumefaciens*) and the plant viruses Cauliflower mosaic virus (CaMV) and Tobacco etch virus (TEV) (see Tables 2 and 3 and Section 5.4).

Table 1. The genes introduced into the GM banana lines proposed for release.

Gene	Gene – full name	Source	Intended function
<i>RGC2</i>	Resistance gene candidate-2	<i>Musa sp.</i>	<i>Fusarium</i> resistance
<i>RGC5</i>	Resistance gene candidate-5	<i>Musa sp.</i>	<i>Fusarium</i> resistance
<i>Ced-9</i>	Cell death abnormality gene-9	<i>Caenorhabditis elegans</i>	Inhibition of apoptosis
<i>mCed-9</i>	Cell death abnormality gene-9, plant codon optimised	<i>C. elegans</i>	Inhibition of apoptosis
<i>p35</i>	<i>p35</i> gene	<i>Autographa californica nucleopolyhedrovirus</i> (Baculovirus)	Inhibition of apoptosis
<i>Sf-IAP</i>	Inhibitor of apoptosis	<i>Spodoptera frugiperda</i>	Inhibition of apoptosis
<i>AtBag4</i>	Bcl-2-associated anthanogene-4	<i>Arabidopsis thaliana</i>	Inhibition of apoptosis
<i>OsBag4</i>	Bcl-2-associated anthanogene-4	<i>Oryza sativa</i>	Inhibition of apoptosis
<i>p65</i>	<i>p65</i> gene (heat shock protein-70 homologue)	<i>Citrus tristeza virus</i>	Inhibition of apoptosis
<i>p61</i>	<i>p61</i> gene (heat shock protein-90 homologue)	<i>Citrus tristeza virus</i>	Inhibition of apoptosis
<i>AtBI-1</i>	Bax inhibitor	<i>A. thaliana</i>	Inhibition of apoptosis
<i>uidA</i>	β -glucuronidase gene	<i>Escherichia coli</i>	Reporter gene
<i>nptII</i>	Neomycin phosphotransferase type II gene	<i>E. coli</i>	Selectable marker

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

Identity of construct	Promoter (source) ^{1,2}	Additional genetic elements ¹	Gene of interest	Terminator ²	Max. lines per construct	Max. total no. of plants
pPTN261	<i>Ubi</i>	<i>Ubi</i> intron and TEV leader sequence	<i>Ced-9</i>	<i>CaMV35S</i>	9	90
pYC35	<i>RGC2</i> (<i>Musa sp.</i>)	-	<i>RGC2</i>	<i>Nos</i>	2	20
pYC16	<i>Nos</i>	-	<i>RGC2</i>	<i>Nos</i>	7	70

Table 3. Gene constructs used to generate the GM Lady Finger banana lines proposed for release.

Identity of construct	Promoter (source) ^{1,2}	Additional genetic elements ¹	Gene(s) of interest	Terminator ²	Max lines per construct	Total no. of plants
pPTN261	<i>Ubi</i>	<i>Ubi</i> intron and TEV leader sequence	<i>Ced-9</i>	<i>CaMV35S</i>	3	30
pYC10	<i>Ubi</i>	<i>Ubi</i> intron	<i>Ced-9</i>	<i>Nos</i>	10	100
pYC11	<i>Ubi</i>	<i>Ubi</i> intron	<i>mCed-9</i>	<i>Nos</i>	10	100
pYC13	<i>Nos</i>	-	<i>RGC5</i>	<i>Nos</i>	10	100
pYC14	<i>Ubi</i>	<i>Ubi</i> and <i>Cat</i> (castor bean) introns	<i>uidA</i>	<i>Nos</i>	10	100
pYC15	<i>Nos</i>	-	<i>uidA</i>	<i>Nos</i>	10	100
pYC17	<i>Ubi</i>	<i>Ubi</i> intron	<i>Sf-IAP</i>	<i>Nos</i>	10	100
pYC18	<i>Ubi</i>	<i>Ubi</i> intron	<i>AtBI-1</i>	<i>Nos</i>	10	100
pYC19	<i>Ubi</i>	<i>Ubi</i> intron	<i>p35</i>	<i>Nos</i>	10	100
pYC20	<i>Ubi</i>	<i>Ubi</i> intron	<i>p61</i> (<i>hsp90h</i>)	<i>Nos</i>	10	100
pYC21	<i>Ubi</i>	<i>Ubi</i> intron	<i>p65</i> (<i>hsp70h</i>)	<i>Nos</i>	10	100
pYC22	<i>Ubi</i>	<i>Ubi</i> intron	<i>OsBag4</i>	<i>Nos</i>	10	100
pYC23	<i>Ubi</i>	<i>Ubi</i> intron	<i>AtBag4</i>	<i>Nos</i>	10	100
pYC24	<i>Ubi</i> <i>Ubi</i>	<i>Ubi</i> intron <i>Ubi</i> intron	<i>AtBag4</i> <i>p65</i>	<i>Nos</i> <i>Nos</i>	10	100

¹ The *Ubi* promoter and intron were obtained from *Zea mays* (maize).

² The *Nos* promoter and terminator were obtained from *Agrobacterium tumefaciens*.

5.2 Introduction to plant-pathogen interactions

27. Plant-pathogen interactions are multi-faceted and complex. For example, plants encounter a multitude of potential pathogens; however, only a few of these will be able to cause disease in any given plant. The plant is said to display non-host resistance towards those pathogens that are unable to colonise it – even under conditions that are favourable for the pathogen.

28. Plant pathogens can be grouped on the basis of feeding style: necrotrophs feed on dead or dying plant material; biotrophs require live host cells for their establishment and survival; and hemibiotrophs feed off live cells in the early stages of interaction and, as cells are dying, are able to switch to a necrotrophic life style.

29. Resistance against diverse pathogen strains commonly involves the production of a number of plant defence compounds that lead to an incompatible plant-pathogen interaction, whereas resistance that is highly specific to particular pathogen strains commonly involves the expression of a specific resistance (R) gene (Flor 1971). The presence and expression of a specific R gene in a plant leads to recognition of a gene product that is present in the pathogen and encoded by a specific avirulence (*Avr*) gene. Only in plant-pathogen interactions where both the specific R gene product and the corresponding *Avr* gene product are present, is the plant resistant to the pathogen. Where gene-for-gene interaction is the foundation of resistance, the plant will be susceptible to disease if the host variety lacks the R gene product or if the pathogen strain lacks the *Avr* gene product.

30. Host cell death occurs during many interactions between plants and pathogens. In animal systems, the concept of programmed cell death (PCD) has long been established. Three different types of cell death are found: apoptosis, autophagic cell death and necrosis, with apoptosis being the most studied (see review by Reape et al. 2008 and references therein). Each type of animal cell death is characterised by certain distinguishing features. Apoptotic cells undergo a number of biochemical and morphological changes resulting in DNA laddering, cell shrinkage, membrane blebbing and disassembly into apoptotic bodies (Pennell & Lamb 1997). Apoptosis is an active process requiring a coordinated cell death mechanism. In living cells, pro- and anti-apoptotic regulators are present. When pro-apoptotic stimuli override anti-apoptotic suppression of cell death in animals, a caspase cascade is activated and cell death occurs (Salvesen 1999; Bossy-Wetzell & Green 1999).

31. In plants, PCD is important for normal growth and development, as well as during pathogen and stress responses (see review by Reape et al. 2008 and references therein). Some cases of PCD in plants display characteristics found in animal cell apoptosis, such as DNA fragmentation and cleaving of caspase substrates, while at the same time there are differences, such as the lack of classical caspases. This type of PCD in plants is known as apoptosis-like PCD. 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).

32. In plant-pathogen interactions, PCD occurs when the pathogen unsuccessfully parasitises the host, as well as during susceptible reactions in which the pathogen successfully causes disease (Greenberg & Yao 2004), suggesting common biochemical pathways during both interactions. The disease resistance 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 defence genes and the induction of PCD (Tadege et al. 1998; Dickman 2004).

33. HR cell death is effective at restricting biotrophic infections, but can provide a food source for invading necrotrophs (Dickman 2004; Glazebrook 2005). Thus, biotrophs actively suppress HR while necrotrophs promote HR-like cell death (Laluk & Mengiste 2010). The expression of anti-apoptotic genes in plants can therefore confer resistance to necrotrophic pathogens but can also increase susceptibility to biotrophic pathogens (Babaeizad et al. 2009).

5.3 The introduced genes and their encoded proteins

5.3.1 The introduced genes for disease resistance and their encoded proteins

34. The genes and their encoded proteins are described in brief to illustrate their potential function within the GM banana lines. They have been grouped according to the trait associated with the

introduced genes: specific disease resistance genes and genes conferring disease resistance through inhibition of apoptosis.

Specific disease resistance genes – *RGC2* and *RGC5*

35. R gene candidates 2 and 5 (*RGC2* and *RGC5*) were isolated from a banana species, *Musa acuminata* ssp. *malaccensis*, which is resistant to *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4 (Foc TR4). *RGC2* and *RGC5* show sequence similarity to known R genes that encode proteins with a nucleotide binding site (NBS) and a leucine-rich repeat (LRR) (Peraza-Echeverria et al. 2008; Peraza-Echeverria et al. 2009).

36. Plants expressing disease resistance genes have been used extensively in conventional breeding programs. More recently, molecular techniques have been used to transfer R genes to more distantly related species. Expression of R genes in several GM plants has been demonstrated to confer resistance to pathogens carrying the appropriate *Avr* gene (see review by Hulbert et al. 2001). For example, the *Pto* resistance gene from tomato (*Lycopersicon esculentum*) has been shown to function in tobacco (*Nicotiana tabacum*) and *N. benthamiana* (Rommens et al. 1995; Thilmony et al. 1995). Other than disease resistance, no other phenotypic changes were reported by the authors.

Genes conferring disease resistance through inhibition of apoptosis

37. Most of the introduced genes in the GM banana lines are expected to confer disease resistance by preventing cells from undergoing PCD in response to infection by necrotrophic pathogens. It has been shown that Lady Finger banana plants transformed with anti-apoptotic genes are resistant to *Fusarium oxysporum* f.sp. *cubense* (Foc) Race 1 in glasshouse trials (Paul 2009).

The *Ced-9* and *mCed-9* genes

38. The *ced-9* (cell death abnormality) 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 (summarised in Conradt & Xue 2005). The GM Grande Naine banana lines containing the *ced-9* gene have been trialled in the field under licence DIR 079/2007. The applicant states that most plants in this field trial were phenotypically normal (see Section 5.6.2).

39. 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* and plays a similar role by inhibiting apoptosis in mammals. Both genes encode members of a protein family that has an important role in regulating programmed cell death in a range of organisms (reviewed in Conradt & Xue 2005).

40. 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 (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). 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).

41. The *mCed-9* gene is a synthetic version of *ced-9* with the nucleotide sequence optimised for plant codon usage. The predicted amino acid sequence of *mCed-9* is identical to *ced-9* (information provided by the applicant).

The SfiAP gene

42. The *Sf-iap* (inhibitor of apoptosis) gene was derived from the lepidopteran *Spodoptera frugiperda* (Huang et al. 2000). SfiAP has been demonstrated to inhibit apoptosis in human cells (Huang et al. 2000).

43. IAP homologues have been identified in mammals, insects, nematodes and yeasts (reviewed in Deveraux & Reed 1999). IAPs contain one to three motifs termed baculovirus IAP repeats, approximately 70 amino acids in length, which bind caspases, thereby inhibiting caspase activity and preventing cell death (Deveraux & Reed 1999). Some IAPs, including SfiAP (Huang et al. 2000), also contain a carboxy-terminal really interesting new gene (RING) finger domain. RING domains have a primary function in targeted protein degradation (reviewed in Kabbage et al. 2010). In SfiAP, the RING domain is important for the E3 ubiquitin ligase activity of the protein (Kabbage et al. 2010).

44. GM tobacco and tomato plants expressing SfiAP constitutively were tolerant to heat and salt stress and showed no features associated with apoptosis-like cell death, such as DNA laddering (Li et al. 2010). When the GM tomato seedlings were exposed to the mycotoxin fumonisin B1, no phenotypic changes or apoptotic bodies were observed, whereas non-GM tomato seedlings developed systemic lesions and apoptotic-like bodies. Inoculation of tomato leaves with the necrotrophic fungus *Alternaria alata* resulted in tolerance of the GM tomatoes to infection with the fungus, whereas non-GM tomatoes were susceptible to the pathogen. In addition, it was found that expression of SfiAP resulted in the delayed fruit ripening in GM tomato plants. It was suggested that SfiAP influences ethylene signalling in the GM tomatoes as application of an ethylene analogue, ethephon, restored normal fruit ripening.

The AtBag4 and OsBag4 genes

45. The *AtBag4* (*Arabidopsis thaliana Bcl-2 associated athanogene 4*) gene was derived from thale cress (*A. thaliana*) (Doukhanina et al. 2006), and its homologue *OsBag4* was derived from rice (*Oryza sativa*); information supplied by the applicant).

46. *Bag*-like genes have been identified in a variety of organisms, including yeasts, plants, animals and humans (Kabbage & Dickman 2008). BAG-like proteins are characterised by the presence of a BAG domain near their carboxy-terminus that is involved in direct interaction with the ATPase domain of 70-kDa heat shock proteins (HSP70s) and the constitutively expressed cytosolic isoform of HSP70 (HSC70). Plant *Bag* expressed sequence tags were found both in plants exposed to biotic or abiotic stresses as well as in specific tissues, indicating a role in environmental and developmental responses. For example, in thale cress *AtBAG-4* was detectable 20 min after cold treatment, whereas *AtBAG-6* was only detectable after 90 min. *AtBAG-6* (but not *AtBAG-4*) was also detectable in response to heat stress. The involvement of *AtBAG-4* in thale cress plant growth and development was indicated by heterozygous knock-out mutants exhibiting earlier flowering, and shorter vegetative and reproductive phases with more branched roots and inflorescences compared to wild-type plants (Doukhanina et al. 2006).

47. GM tobacco plants expressing *AtBAG-4* were found to have greater tolerance to abiotic stresses. These tobacco plants were categorised into low, intermediate and high level expressing lines. As a result of inhibition of PCD, GM tobacco plants expressing low levels of *AtBAG-4*, and in some cases also those expressing intermediate levels, showed higher tolerance to abiotic stresses, such as cold, drought, salinity, menadion, paraquat, hydrogen peroxide and acifluorfen (Doukhanina et al. 2006).

The p61 and p65 genes

48. The *p61* and *p65* genes are also known as *hsp90h* and *hsp70h*, respectively (reviewed in Satyanarayana et al. 2000). These genes were derived from an Australian Citrus tristeza virus isolate and show sequence similarities to genes encoding heat shock proteins (HSPs). HSPs are present in all types of cellular organisms (reviewed in Peremyslov et al. 1999).

49. HSPs are implicated in virion assembly (Satyanarayana et al. 2000). In addition, HSP70H is involved in cell-to-cell movement of another plant virus, beet yellows closterovirus, for which the ATPase function of the HSP70H is needed (Peremyslov et al. 1999).

50. HSP70 proteins are central components of the cellular network of molecular chaperones, which assist in protein folding, disaggregation, and membrane translocation. The biological activity of a number of regulatory proteins involved in important cell functions, including cell death, is under the control of HSP70. The specific action of HSP70 proteins is broadened by cooperation with other chaperones, including HSP90. In some cases it has been demonstrated that regulatory proteins can be kept inactive through interaction with HSP70 and HSP90 until the appropriate signal transduction pathway is activated (reviewed in Mayer & Bukau 2005). For example, in mammalian cells, HSP70 and HSP90 have been demonstrated to inhibit apoptosis. HSP70 inhibits apoptosis by preventing activation of the caspase cascade by caspase-9 (Beere et al. 2000). HSP90 inhibits the oligomerisation of APAF-1, a factor that is also involved in the activation of caspase-9 (reviewed in Dias et al. 2002).

51. In thale cress, HSP70 is implicated in heat tolerance and the autoregulation of the heat stress response (Lee & Schoffl 1996). GM *Nicotiana benthamiana* plants, in which either *hsp70* or *hsp90* expression was silenced, displayed: a stunted phenotype; no hypersensitive response after either inoculation with a bacterial non-host pathogen, *Pseudomonas cichorii*, or infiltration with a protein known to cause hypersensitive response in non-GM *N. benthamiana*; compromised non-host resistance to *P. cichorii*; and decreased transcription levels of genes encoding pathogenesis related proteins (Kanzaki et al. 2003).

The p35 gene

52. The *p35* gene was derived from *Autographa californica nucleopolyhedrovirus*, an insect baculovirus. The protein P35, encoded by the *p35* gene, has been identified as a regulator in apoptosis. In virus-infected animal cells, P35 has been shown to inhibit the action of certain caspases, thereby blocking cell death until viral replication is completed (reviewed in Lincoln et al. 2002).

53. In maize callus, expression of *p35* reduced the rapid, hypersensitive type of cell death triggered by *A. tumefaciens*. However, recovery of transformed plants was not significantly improved, reportedly because of the low efficiency of T-DNA transfer from *A. tumefaciens* to maize (Hansen 2000).

54. The fungus *Alternaria alata* f.sp. *lycopersici* (Aal) produces mycotoxins (AAL) that trigger cell death in susceptible tomato lines. This toxin-dependant cell death is a prerequisite for colonisation by the fungus, eventually resulting in *Alternaria* stem canker disease. Expression of baculoviral P35 in GM tomatoes resulted in resistance to the fungi Aal, *A. alternata*, *Colletotrichum coccodes* and the bacterium *Pseudomonas syringae* pv. tomato (Lincoln et al. 2002).

The AtBI-1 gene

55. The *AtBI-1* gene was derived from thale cress (*Arabidopsis thaliana*) (Sanchez et al. 2000). Another report in the literature also describes the isolation of the *AtBI-1* gene as well as the rice (*Oryza sativa*) homolog *OsBI-1* (Kawai et al. 1999).

56. BAX inhibitor (BI) proteins are able to counter the effect of Bcl-2 associated protein X (BAX), a pro-apoptotic protein. The predicted sequence of the AtBI-1 protein shows high homology to human derived BI-1 protein. In thale cress, AtBI-1 expression is upregulated upon

pathogen challenge or wounding, indicating involvement of the protein in both biotic and abiotic stress responses (Kawai-Yamada et al. 2004).

57. When constitutively expressed in GM yeast (*Saccharomyces cerevisiae*), AtBI-1 suppressed murine BAX-induced cell death (Kawai et al. 1999; Sanchez et al. 2000). Similarly, GM *Arabidopsis* plants co-expressing BAX and AtBI-1 showed a reduced rate of cell death compared to plants expressing BAX only. This result indicates that a plant gene is able to complement a component of the mammalian mechanism with regard to apoptosis. GM tobacco BY2 cells transformed with AtBI-1 showed resistance to levels of hydrogen peroxide or salicylic acid that leads to cell death in non-GM tobacco cells (Kawai-Yamada et al. 2004). An intact carboxy-terminus has been found necessary for the functioning of the protein (Kawai-Yamada et al. 2004).

5.3.2 Toxicity/allergenicity of the proteins associated with the introduced genes for disease resistance

58. Homologues of the introduced proteins, and proteins with a similar function, occur naturally in a range of organisms, including animals or plants widely consumed by people and animals (see Section 6.5). On this basis, people and other organisms have a long history of exposure to the proteins expressed by the introduced genes for disease resistance.

59. No toxicity/allergenicity tests have been performed on the purified encoded proteins or on the GM banana lines 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 or their products to be used for human consumption in Australia (see discussion in Section 7.1.2).

60. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein based on similarity to known toxins and allergens. The results of such analyses are not definitive and should be used to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The predicted amino acid sequences of the proteins encoded by each of the introduced genes for disease resistance were compared to a database of known allergens, the Food Allergy Research and Resource Program allergen protein database <<http://www.allergenonline.com>>. According to this website, the most predictive search is the overall FASTA alignment, with identity matches greater than 50% indicating possible cross-reactivity. An additional precautionary search is a sliding window of 80 amino acid searches, looking for identities greater than 35%, which is a threshold often used to highlight an allergenicity concern (Fiers et al. 2004).

61. The only positive result of these analyses was for the p65 protein, which showed up to 40.7–42.5% sequence homology over 80 amino acids with three known allergens. The allergens were all proteins from the HSP70 family isolated from *Corylus avellana* (hazelnut) and the fungi *Davidiella tassiana* and *Penicillium citrinum*. However, the overall homology between p65 and each of these proteins was low (<26%). In addition, HSP70s are present in all cellular organisms, including plants that are widely consumed by people and animals.

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

5.3.3 The reporter gene (uidA) and its encoded protein (GUS)

63. Up to 20 of the GM Lady Finger banana lines contain the *uidA* gene, which would be used to verify that any effects on disease resistance observed are due to the introduced disease resistance genes and not the transformation process itself.

64. The *uidA* gene encodes the enzyme β -glucuronidase (GUS), which is derived from the common gut bacterium *E. coli*. The GUS protein is a monomer with a molecular weight of 68 kDa, and the GUS enzyme is active as a tetramer. GUS catalyses the hydrolysis of β -glucuronides and, less efficiently, some β -galacturonides. *E. coli* lives in the digestive tract of vertebrates, including

humans (Jefferson et al. 1986), and the GUS enzyme enables it to metabolise β -glucuronides as a main source of carbon and energy.

65. The *uidA* gene is the most widely used reporter gene in GM plants (Miki & McHugh 2004) as it allows GM tissues to be identified using a simple visual assay. A number of GM crops containing the *uidA* gene have been approved for limited and controlled release in Australia, including GM banana in DIR 076/2007, GM maize in DIR 086 and GM sugarcane in DIR 095. In addition, the *uidA* gene is present in commercially approved Bollgard II® cotton (DIR 066/2006). No adverse effects on humans, animals or the environment have been reported from these releases. The US EPA does not consider the GUS protein to be toxic and has approved its exemption from the requirements to establish tolerance levels (EPA 2001). FSANZ has approved the use of food derived from GM plants containing the *uidA* gene (for example see FSANZ 2002; FSANZ 2003).

5.3.4 The antibiotic resistance marker gene (*nptII*) and its encoded protein

66. All of the GM banana lines contain the *nptII* gene. 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, and in more detail in the RARMPs for DIR 070/2006 and DIR 074/2007 (available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir070-2006>> and <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir074-2007>> or by contacting the OGTR), regulatory agencies in Australia and in other countries have assessed the use of the *nptII* gene in GM plants as not posing a risk to human or animal health or to the environment. The most recent detailed international evaluation of *nptII* in terms of human safety was by the European Food Safety Authority, which concluded that the use of the *nptII* gene as a selectable marker in GM plants (and derived food or feed) does not pose a risk to human or animal health or to the environment (EFSA 2009).

5.4 The regulatory sequences

67. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. Other sequences, such as introns, may contribute to the expression pattern of a given gene. The regulatory sequences used in the GM banana lines are listed in 0 and 0, Section 5.1, and detailed below.

68. Although some of regulatory sequences are derived from plant pathogens (CaMV, TEV and *A. tumefaciens*), the regulatory sequences comprise only a small part of the total genome, and are not in themselves capable of causing disease. Similarly, those regulatory sequences derived from plants that are associated with allergenic or toxic responses in humans (*Zea mays* and *Ricinus communis*) are not in themselves toxic or allergenic.

5.4.1 Regulatory sequences for expression of the introduced genes for disease resistance

69. Expression of all of the introduced genes for inhibition of apoptosis is controlled by the *Ubiquitin* gene (*Ubi*) promoter from *Z. mays* (maize), including an intron of approximately one kilobase. Expression of *RGC2* is controlled either by the native *RGC2* promoter or by the *Nopaline synthase* gene (*Nos*) promoter from *A. tumefaciens*. Expression of *RGC5* is also controlled by the *Nos* promoter.

70. The *Ubi* and *Nos* promoters are constitutive, that is they are expected to direct the genes to be expressed in most plant tissues and throughout the plant life cycle (Ebert et al. 1987; Christensen et al. 1992). The *RGC2* gene and promoter were isolated from Foc TR4 resistant wild banana, and in these plants *RGC2* transcript has been detected in leaves and roots (Peraza-Echeverria et al. 2008). The applicant has stated that R genes are currently thought to be expressed constitutively at low levels and induced to higher levels on infection by the pathogen, but that expression levels are unlikely to be as high as those driven by the *Ubi* promoter.

71. One of the constructs for expression of the *ced-9* gene (pPTN261) contains the 35S gene termination region from CaMV. This construct also includes a TEV leader sequence, which can promote efficient translation (Gallie et al. 1995). The TEV sequence itself is not translated.

72. The mRNA terminator for the remaining introduced genes for disease resistance in the GM banana lines is derived from the *Nos* gene. The *Nos* terminator has been used in a wide variety of constructs for plant genetic modification (Reiting et al. 2007).

5.4.2 Regulatory sequences for expression of the *uidA* reporter gene

73. Two constructs have been used for expression of the *uidA* reporter gene in the GM bananas. In GM bananas generated using the construct pYC14, the *uidA* gene is under the control of the *Ubi* promoter and intron, an intron from the Castor Bean (*R. communis*) *catalase* gene (*Cat*), and the *Nos* gene mRNA termination sequence. The *Cat* intron prevents expression in *A. tumefaciens*, ensuring that any expression of the reporter gene in the GMOs is occurring in eukaryotic cells rather than in residual *Agrobacterium*. The *Cat* intron can also enhance expression of introduced genes in plants.

74. In GM bananas generated using the construct pYC15, expression of the *uidA* gene is controlled by the promoter and mRNA termination region of the *Nos* gene from *A. tumefaciens*.

5.4.3 Regulatory sequences for expression of the *nptII* marker gene

75. All of the GM bananas also contain the *nptII* gene under the control of the promoter and mRNA termination region of the CaMV 35S gene.

5.5 Method of genetic modification

76. *Agrobacterium tumefaciens*-mediated transformation is being used to generate the GM banana lines in the proposed release. *A. tumefaciens* is a soil bacterium that causes gall formation on a wide range of plant species. The gall is induced by transfer of hormone-producing genes from the bacterial cell into the plant genome. The genes are carried on an extrachromosomal, circular DNA molecule found within the bacterial cell called a Tumour-inducing (Ti) plasmid. During the infection process, only a section of the Ti plasmid known as the Transfer DNA (T-DNA) is transferred to the plant. Molecular biologists have studied the infection and T-DNA transfer process of *A. tumefaciens* for many years and have harnessed this natural process to facilitate genetic modification of plants. Well-characterised *A. tumefaciens* Ti plasmids have been produced that lack the genes responsible for tumour formation (disarmed plasmids) and instead enable genes of interest to be inserted between the T-DNA border sequences. When used to infect plants, *A. tumefaciens* cells carrying such plasmids cannot produce a tumour but will transfer the T-DNA sequence carrying the genes of interest into the plant cell where they stably integrate into the plant genome (Bevan 1984; Klee & Rogers 1989).

77. In addition to transfer of the T-DNA sequence, recent publications have shown that small segments of flanking Ti plasmid sequence and *A. tumefaciens* chromosomal sequence may be transferred into the plant genome at a low frequency during the transformation process (Smith 1998; Ulker et al. 2008). However, *A. tumefaciens*-mediated plant transformation has been used extensively in Australia and overseas and is not known to adversely affect human health and safety or the environment.

78. To genetically modify the banana lines in this application, *A. tumefaciens* strain AGL1 (ATCC[®] Number: BAA-101[™]) is being used (Lazo et al. 1991), which shows high rates of T-DNA transfer when used with banana suspension cultures (Khanna et al. 2004).

79. For each transformation, embryonic cell suspensions of banana are co-cultivated with *A. tumefaciens* AGL1 carrying one of the gene constructs listed in Tables 2 and 3, using a centrifugation assisted protocol (Khanna et al. 2004). Following transformation, the banana cells are cultured on medium containing both the antibiotics timentin (to remove *A. tumefaciens*) and kanamycin (to select for banana cells expressing the introduced *nptII* gene). Transformed embryos

are developed on a regeneration medium and then grown into plantlets, which are hardened off in a shadehouse before being transferred to soil.

5.6 Characterisation of the GMOs

5.6.1 Stability and molecular characterisation

80. Not all of the GM banana lines proposed for release have been generated prior to submission of this application. The applicant states that all gene constructs are being fully sequenced prior to transformation.

81. As the project is at an early stage, full molecular characterisation of the GM banana lines has not been carried out. The presence of the *Ced-9* gene in GM Cavendish and Lady Finger banana lines, and *RGC2* in GM Cavendish banana lines, has been verified using polymerase chain reaction (PCR). These lines would be the first ones planted in the field in the proposed release. As the remaining GM banana lines are progressively generated, they will also be screened for the presence of the introduced genes using PCR. Plants are also tested for the presence of *Agrobacterium* by PCR using primers specific to non-T-DNA regions, and no plants which show any remaining *Agrobacterium* will be released.

82. The exact location of the inserted genes within the banana genome of the lines is not known. *A. tumefaciens* inserts introduced genes into plant genomic DNA via illegitimate recombination, which can potentially result in insertion of foreign DNA anywhere in the host genome. Furthermore, the banana genome is poorly characterised, so that it would be difficult to generate meaningful data on the locations of introduced genes at this stage.

83. The number of copies of the introduced genes in the GM banana lines has not been characterised. However, the applicant states that *A. tumefaciens*-mediated transformation of banana routinely results in the integration of between one and three copies of the transgene into the banana genome, and a representative sample of the GM banana lines proposed for release will be characterised by Southern blot to confirm this.

5.6.2 Characterisation of the phenotype of the GM banana lines

84. The purpose of the proposed trial is to conduct proof of concept experiments to assess the disease response and/or development of the GM banana lines. The applicant states that it is not possible to assess the response of banana to all pathogens in a glasshouse. Nor is it possible to grow numerous large banana plants for assessment of fruit characteristics in a glasshouse. Such phenotypic data will be collected during the proposed trial.

85. Most of the GM banana lines proposed for release have not been phenotypically characterised. Some characterisation of the GM Lady Finger lines containing *ced-9* (genetically modified using the pPTN261 gene construct) has been carried out under glasshouse conditions (Paul 2009). Most of the lines were phenotypically normal. Leaf bleaching was observed in some of the lines, while other abnormalities observed were conventional “off-types”. In addition, it was demonstrated that some lines were resistant to Foc Race 1, and some lines, when treated with the herbicide paraquat, suffered slightly less damage than treated non-GM control bananas.

86. A field trial of the GM Grande Naine banana lines containing the *ced-9* gene construct has recently been completed by the applicant under licence DIR 079/2007. The applicant states that most plants in this field trial were phenotypically normal. One line had a slightly lower bunch weight, but the applicant considers this to be a tissue-culture off type unrelated to the introduced gene. Results of this trial indicate that there are no adverse effects of the introduced *ced-9* gene on plant development or increased susceptibility to disease.

87. The applicant states that the GM banana plants will be monitored for aberrant phenotypes during the proposed trial.

Section 6 The receiving environment

88. The receiving environment forms part of the context in which the risks associated with dealings involving the GMO are assessed. This includes: any relevant biotic/abiotic properties of the geographic regions where the release would occur; intended agricultural practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2009).

6.1 Relevant abiotic factors

89. The abiotic factors relevant to the growth and distribution of bananas in Australia are discussed in *The Biology of Musa L. (banana)* (OGTR 2008).

90. The release is proposed to take place at one site in the Litchfield Municipality LGA (NT) on the property of the Darwin Banana Farming Company Pty Ltd (DBFC). The acclimatisation of the tissue cultured GM banana lines would occur in a separate, lockable area within the DBFC shadehouse, which is located approximately 250 m from the proposed field location. The field location is bordered on the west by a large shed and permanent dirt road, and on the east by a deep drainage channel.

91. The proposed release site is 500 m from a public road which runs alongside the DBFC property. The property adjoins a melon farm on one side and native bushland on the remaining sides. The proposed site is approximately 1 km from the boundary of Fogg Dam Conservation Reserve and 25km from Djukbinj National Park.

92. The closest population centre to the proposed release site is Humpty Doo (8 km), which has a population of approximately 3000 people. The town of Palmerston is approximately 22 km from the release site and has an estimated population of 28,000 people. The major agricultural activities around the proposed release site are the cultivation of melons, mangoes, Asian vegetables and cut flowers.

93. The applicant proposes to locate the GM banana trial site at least 1 km from the Adelaide River, the nearest natural waterway. The proposed release site is 15 m above sea level and the DBFC property owners state that the site has never been known to flood. The property was laser levelled five years ago and drainage channels were dug. The proposed release site is not adjacent to sloping ground or on a hill such that it would be prone to heavy run off or landslides.

6.2 Relevant biotic factors

94. The biotic factors pertaining to the growth and distribution of commercial bananas in Australia are discussed in *The Biology of Musa L. (banana)* (OGTR 2008). In addition, the following points are of particular relevance to the proposed release:

- ♦ The proposed release site is on the property of a commercial banana farm, and non-GM bananas may be grown commercially on the property during the proposed release.
- ♦ The area under cultivation to bananas in the NT is relatively small; the next closest commercial banana farm is approximately 60 km south of the proposed release site.
- ♦ Native *Musa* species are not present in the NT.
- ♦ Invertebrates, vertebrates and microorganisms could 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.

95. One of the purposes of the proposed release is to assess the disease response of the GM banana lines, particularly to the fungal diseases Yellow Sigatoka (*Mycosphaerella musicola*) and Panama disease or Fusarium wilt (*Fusarium oxysporum f.sp. cubense*; Foc). Yellow Sigatoka is

widespread in tropical growing regions and is currently controlled by deleafing and/or fungicide application.

96. There are three broad forms (Races) of Foc of significance in Australia: Race 1 infects Lady Finger bananas and is found in most banana growing regions; Subtropical Race 4 infects stressed Cavendish and Lady Finger plants and its distribution is sporadic within southern Queensland and northern New South Wales; Tropical Race 4 (TR4) infects Cavendish and Lady Finger cultivars and to date has only been found in the NT. Currently there is no chemical control available for Foc TR4 and there is concern that the movement of this disease into northern Queensland could severely impact the commercial banana industry.

97. Both Yellow Sigatoka and Foc TR4 are known to occur at the proposed release site, although the incidence of Yellow Sigatoka is currently low. The applicant states that proposed release site was selected because the soil contains Foc TR4 at levels that would be expected to provide high, evenly distributed disease pressure. Further details of these fungal diseases and their methods of control are discussed in *The Biology of Musa L. (banana)* (OGTR 2008).

6.3 Relevant agricultural practices

98. The applicant proposes to transport the planting material (tissue cultured plants) to the site from QUT and to acclimatise (harden off) the plants in a shadehouse for 2-4 months before transferring them to the field location.

99. The cultivation and movement of banana planting material is regulated in the NT and Queensland by the NT Department of Resources and the Queensland Department of Primary Industries and Fisheries, respectively. Foc TR4 is a notifiable disease under the NT *Plant Diseases Control Act 1979* and is currently under strict quarantine management in the NT (see http://www.nt.gov.au/d/Primary_Industry/index.cfm?newscat1=Quarantine&newscat2=&header=N%20Quarantine). The NT Department of Resources is aware that the property on which the release is proposed to occur is infested with *Fusarium*. The property has been gazetted and is subject to quarantine conditions restricting the movement of plants, soil and water. The applicant will consult with Queensland and NT authorities regarding the movement and planting of GM and non-GM banana material for this trial and adhere to all relevant regulations.

100. It is anticipated that the cultivation practices used for planting and managing the proposed trial will not differ significantly from the standard practices used for commercial (non-GM) banana. These are outlined in *The Biology of Musa L. (banana)* (OGTR 2008).

101. It is standard practice to remove the male bell from inflorescences to increase bunch weight and remove feeding sites for pests (Broadley et al. 2004). The applicant proposes to remove the male bells from most inflorescences but wishes to observe the phenotype of some bells. In this case, the male inflorescence would be bagged rather than removed when it emerges. When removed, male inflorescences would be collected and placed in a container to prevent access by birds and bats. Once decomposed they would be left on the ground at the field location.

102. During commercial cultivation of banana plants it is necessary to undertake desuckering and removal of dead leaves for both disease management and to encourage plant vigour. Desuckering would occur by cutting the sucker off at ground level and pouring a solvent (distillate or kerosene) down the centre of the pseudostem. The removed pseudostem and any detached leaf material is non-propagative and would be left on the ground at the field location to decompose. Waste plant material from the shadehouse would also be placed on the ground at the field location for decomposition.

103. It is intended that the GM bananas be grown through to fruiting to allow assessment of fruit characteristics. Fruit would be obtained from the plant crop⁷, which would then be ratooned and grown to fruiting before the proposed trial is concluded. Bunches would be bagged, as is done in commercial non-GM banana cultivation to protect developing fruit from being eaten or damaged by frugivores and to optimise ripening conditions (Broadley et al. 2004). Fruit would be harvested while still green (standard commercial practice). After assessment, fruit would be shredded and placed on the ground at the field location.

104. At the end of the trial, any remaining bunches would also be shredded and placed on the ground at the field location. Plants would then be injected with herbicide (2,4-D or glyphosate) and monitored for volunteer banana plants for a period of 12 months.

6.4 Presence of related plants in the receiving environment

105. The proposed release site is in a plantation where commercial bananas are grown. These all show parthenocarpy and male sterility.

106. There are two recognised *Musa* species that are native to Australia, *M. acuminata* subsp. *banksii* and *M. jackeyi* (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. *M. jackeyi* is rare and has only been reported at two locations in Queensland: Bellenden Ker and Cooktown. Neither of these species is known to be present in the NT.

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

107. The introduced genes and regulatory sequences were originally isolated from naturally occurring organisms that are already widespread and prevalent in the environment.

108. Programmed cell death (PCD) is an integral part of plant and animal tissue development (see Section 5.2 of this chapter). Consequently, multicellular organisms in which apoptosis is a normal function already contain anti-apoptotic genes. Therefore, it is expected that humans, animals and microorganisms routinely encounter the introduced genes for inhibition of apoptosis, homologues of these genes, or proteins with a similar function, through contact with plants and food derived from plants.

109. The source organism of the two R genes is the wild diploid banana *Musa acuminata* ssp. *malaccensis*. *M. acuminata* is widely distributed in Asia and considered one of the ancestors of modern eating banana (OGTR 2008). Homologues of the R genes are widely distributed in plants, including many other species that are consumed by humans and animals.

110. 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.

111. The *uidA* reporter gene is also derived from *E. coli*. GUS enzyme activity has been detected in numerous microbial, plant and animal species (Flavell et al. 1992; Gilissen et al. 1998) and is recognised as commonly present on fresh food. As such, it is expected humans and animals routinely encounter the encoded protein through contact with plants and food.

⁷ 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’.

Section 7 Australian and international approvals

7.1 Australian approvals of GM banana

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

112. Nine of the GM banana lines proposed for release have been previously approved for limited and controlled release under licence DIR 079/2007. DIR 079/2007 was issued to QUT for GM Grande Naine banana lines genetically modified with the pPTN261 gene construct, in which the *ced-9* gene is expressed under the control of the maize *Ubi* promoter and the CaMV 35S terminator. The release occurred at one site in Queensland on up to 1.4 ha between July 2008 and April 2010, and is currently in the post harvest monitoring phase.

113. There has been no release of the remaining GM banana lines in Australia.

114. The Regulator has also issued a licence (DIR 076/2007) to QUT for the limited and controlled release of banana genetically modified for enhanced nutrition (DIR 076/2007) on up to 1.4 ha between 2008 and 2012.

7.1.2 Approvals by other government agencies

115. Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. However, dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority, Therapeutic Goods Administration, National Industrial Chemicals Notification and Assessment Scheme and Australian Quarantine Inspection Service⁸.

116. 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 sold as food.

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

7.2 International approvals of GM banana

118. There has been no release of these GM banana lines internationally. However, there have been some international field trials of other GM banana lines modified for traits including disease resistance⁹. The applicant states that at least three GM banana field trials have been conducted overseas by private companies, but that detailed data on these trials have not been published.

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

⁹ Source: <http://www.isb.vt.edu/cfdocs/fieldtests1.cfm> - accessed 23 August 2010.

Chapter 2 Risk assessment

Section 1 Introduction

119. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 2). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

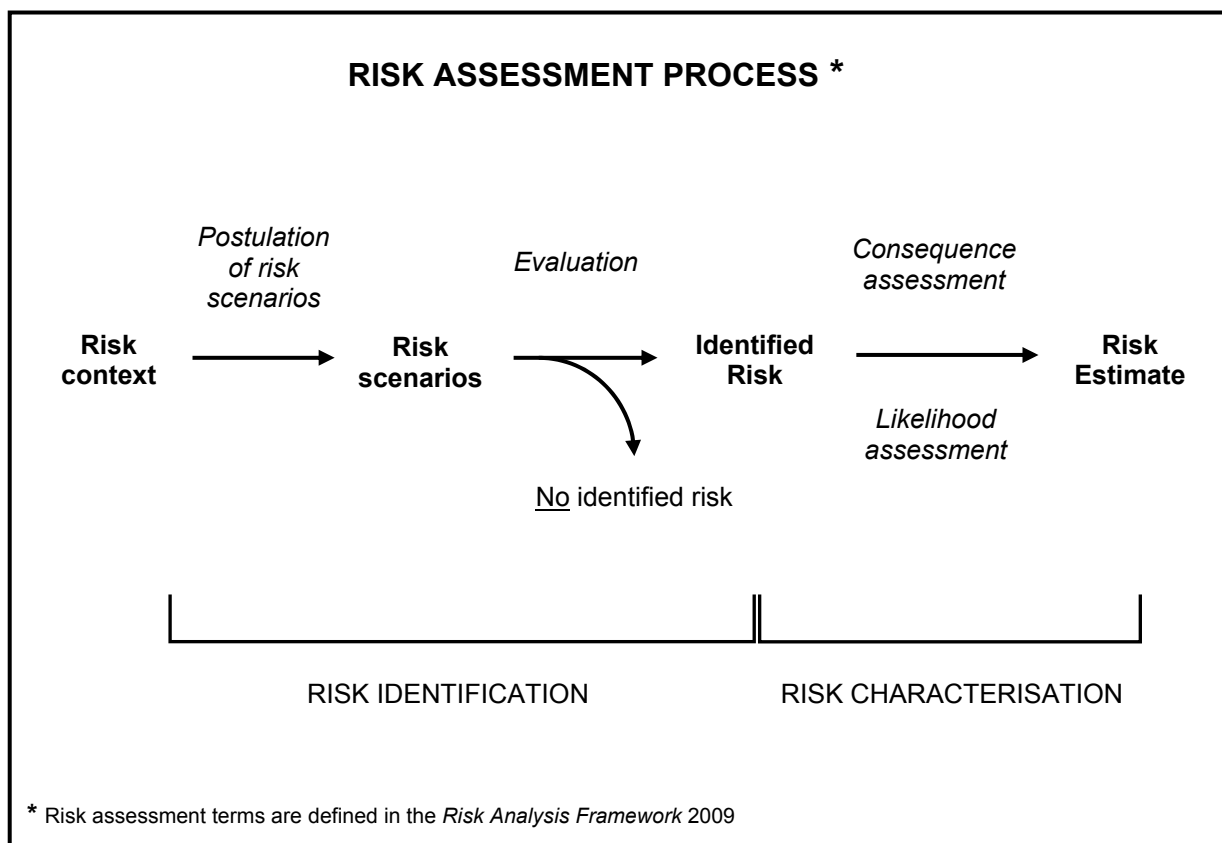


Figure 2. The risk assessment process.

120. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios).

121. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

122. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2009). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

123. Identified risks (*i.e.* those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments.

Section 2 Risk Identification

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

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

125. Eight risk scenarios were identified and evaluated. These are summarised in Table 4, where circumstances that share a number of common features are grouped together in broader risk categories. None of the risk scenarios were considered to lead to an identified risk that required further assessment. More detail of the evaluation of these scenarios is provided later in this Section.

126. As discussed in Chapter 1, Sections 5.3.3 and 5.3.4, some of the GM banana lines contain the reporter gene *uidA*, and all of the lines contain the antibiotic resistance selectable marker gene *nptII*. The *uidA* and *nptII* genes and their products have already been considered in detail in previous RARMPs (for example see DIRs 066/2006, 070/2006 and 086/2008) and by other regulators (EFSA 2007). Since neither of these genes has been found to pose risks to either people or the environment, their potential effects will not be further assessed for this application.

Table 4. Summary of risk scenarios from dealings with GM banana genetically modified for disease resistance

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 Production of a toxic or allergenic substance	1. Exposure to GM plant material containing the proteins encoded by the introduced genes.	Allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> • The proteins encoded by the introduced genes occur naturally in the environment and are unlikely to be toxic or allergenic to people or toxic to other organisms. • None of the GM banana material would be used for human food or animal feed. • The limited scale, short duration and other proposed limits and controls minimise exposure of people and other organisms to the GM plant material.
Section 2.2 Spread and persistence (weediness) of the GM banana plants in the environment	2. Expression of the introduced genes improving the survival of the GM banana plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> • Non-GM, commercial banana does not possess weedy characteristics and the genetic modifications are not expected to change the weediness characteristic of the GMOs. • The limits and controls proposed for the release would restrict spread and persistence of the GM banana plants.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
	3. Dispersal of reproductive GM plant materials through various means, including animals and extreme weather conditions	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> • Opportunities for dispersal are limited since the banana cultivars used are effectively sterile and vegetative propagules are not easily dislodged. • Dispersal would be minimised by the proposed limits and controls, which include locating the trial site away from waterways and transporting material according to the Regulator's guidelines.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced genes or regulatory sequences in commercial, non-GM banana plants or in other sexually compatible plants	Weediness; allergic reactions in people or toxicity in people and 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. • Risk scenarios 1 – 3 associated with expression of the introduced genes did not constitute identified risks for people or the environment. • The proposed limits and controls would restrict gene flow between the GM lines and other banana plants.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> • The introduced genes or similar genes and regulatory sequences are already present in the environment and are thus already available for horizontal gene transfer from those sources. • Risk scenarios 1 – 4 associated with expression of the introduced genes did not constitute identified risks for people or the environment.
Section 2.5 Unintended changes in biochemistry, physiology or ecology	6. Changes to biochemistry, physiology or ecology of the GM banana plants resulting from expression, or random insertion, of the introduced genes	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> • Obvious unexpected alterations are likely to have been detected and eliminated during production of the GM banana lines. • Unintended adverse effects, if any, would be minimised by the proposed limits and controls.
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 and other organisms	No	<ul style="list-style-type: none"> • The GM <i>A. tumefaciens</i> are effectively removed during propagation of the GM banana plants in the laboratory. • The GM banana plants are screened for the presence of <i>Agrobacterium</i>, and any found to contain <i>Agrobacterium</i> will not be released. • The introduced genes or similar genes and regulatory sequences are already present in the environment. • Risk Scenarios 1–3 and 5 were not considered to give rise to identified risks.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.7 Unauthorised activities	8. Use of the GMOs outside the proposed licence conditions (non-compliance)	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 toxic or allergenic substance

127. 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).

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

129. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced genes for disease resistance. Workers cultivating the GM banana would be exposed to all plant parts. Organisms may be exposed directly to the proteins through biotic interactions with GM banana plants (vertebrates, invertebrates, 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 tissues or degrade them (vertebrates, invertebrates, fungi and/or bacteria).

Risk Scenario 1. Exposure to GM plant material containing the proteins encoded by the introduced genes

130. Expression of the introduced genes for disease resistance could potentially result in the production of novel toxic or allergenic compounds in the GM banana plants, 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 people or other organisms.

131. Non-GM banana is not known to be toxic to humans or other organisms. Allergic reactions as a result of ingesting the fruit can take the form of either fruit-latex allergy or oral allergy syndrome (OGTR 2008). These properties are not expected to be altered in the GM banana lines proposed for release.

132. Although no toxicity or allergenicity studies have been performed on the GM banana plant material, the introduced genes for disease resistance were isolated from naturally occurring organisms that are already widespread and prevalent in the environment, including common food plants and soil organisms (see Chapter 1, Section 6.5). Therefore, people and animals are exposed to the same or similar proteins through their diet and the environment.

133. As discussed in Chapter 1, Section 5.3.2, the predicted protein sequence for the p65 gene showed some similarity to three allergens from the HSP70 protein family. However, the overall homology was low, and HSP70s are present in all cellular organisms, including plants that are widely consumed by people and animals. No further information was found to suggest that any of the introduced genes or their encoded proteins are toxic or allergenic to people or other organisms. Some uncertainty exists in this area due to data gaps. Further information on potential allergenicity of the P65 protein may be required to assess an application for a large scale or commercial release of GM bananas containing the introduced *p65* gene.

134. The proposed limits and controls of the trial (see Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. Exposure of frugivores and flower feeding animals such as insects, birds and bats would be minimised by the

applicants proposal to bag fruit and to either bag or remove male inflorescences. The proposed trial site is within a property that is surrounded by a fence with ring lock pig mesh, double barb wire on top and lockable gates that are kept locked out of hours. This will reduce inadvertent access by humans and prevent animals such as feral pigs and water buffalo from entering the site, which minimises exposure of the public and animals to the GM plant material.

135. Contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff. There is little potential for exposure of the public to GM plant material via ingestion, skin contact or inhalation as no GM plant material would be used for human food. Animals would not be intentionally exposed as the GM plant material will not be used as feed.

136. At the end of the trial, the applicant proposes to destroy GM banana materials produced, apart from retaining some plant materials for research purposes. These measures would minimise exposure to the GM plant material. The short duration (2010-2014) and small size (up to 1.5 ha) of the proposed trial would also limit the potential for exposure to the GM plant material.

137. **Conclusion:** The potential for allergic reactions in people, or toxicity in people and other organisms as a result of exposure to GM plant materials containing proteins encoded by the introduced genes is not identified as a risk that warrants further assessment.

2.2 Spread and persistence (weediness) of the GM banana plants in the environment

138. 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 given in *The Biology of Musa L. (banana)* (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.

139. Scenarios that could lead to increased spread and persistence of the GM banana plants include expression of the introduced gene conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials outside the release site. These risk scenarios could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins or end products.

Risk Scenario 2. Expression of the introduced genes improving the survival of the GM banana plants

140. If the GM banana plants were to establish or persist in the environment the exposure of humans and other organisms to the GM plant material could be increased. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials has been considered in Risk scenario 1 and was not considered an identified risk.

141. If the expression of the introduced genes for diseases resistance were to provide the GM banana plants with a significant selective advantage over non-GM banana plants and if 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 banana.

142. The impact of the genetic modification on survival of the GM banana plants is uncharacterised. However, the applicant states that expression of the R genes in the GM banana plants may confer resistance to Foc or another pathogen. The introduced anti-apoptotic genes may also confer disease resistance by preventing cells from undergoing PCD in response to infection by pathogens which live at least part of their lives as necrotrophs, including both Foc TR4 and *M. musicola*. Inhibition of apoptosis may also increase plant susceptibility to biotrophic pathogens

(see Chapter 1 Section 5.2). In an environment in which disease was the main factor limiting the spread and persistence of banana, expression of the anti-apoptotic and R genes could result in increased weediness of the GM banana lines relative to non-GM banana.

143. PCD plays an important role in mediating the adaptive response of plants to changes in the environment. A number of studies have also shown that the expression of anti-apoptotic genes (including the *ced-9*, *SfIAP*, *AtBAG*, *p35* and *AtBI-1* genes) in plants confers protective advantages against a range of biotic and abiotic stresses, including necrotrophic fungal pathogens, viral infection, heat, cold, drought, salt, oxidative stress, hydrogen peroxide and other chemicals (see Chapter 1, Section 5.3.1). In glasshouse trials, some lines of GM Lady Finger bananas expressing anti-apoptotic genes, when treated with the herbicide paraquat, suffered slightly less damage than treated non-GM control bananas (Paul 2009). Therefore, it is possible that GM banana plants expressing the anti-apoptotic genes will show enhanced tolerance to biotic and abiotic stresses compared to non-GM banana plants, which could impact on their spread and persistence.

144. Additionally, PCD is integral to plant growth and development (Chapter 1, Section 5.2). High expression of some anti-apoptotic genes, including the *ced-9* gene, in GM tobacco and tomato plants led to developmental abnormalities. These abnormalities included stunted growth, male sterility, the production of none or few viable seeds, stem bleaching, flower deformation and altered leaf pigmentation (Dickman et al. 2001; Xu et al. 2004). GM tobacco and tomato plants expressing moderate levels of these anti-apoptotic genes did not show evidence of growth abnormalities. Under glasshouse conditions, some of the lines of GM Lady Finger bananas expressing anti-apoptotic genes showed leaf bleaching, but most lines were phenotypically normal (Paul 2009). Therefore, it is unlikely that GM banana plants expressing the introduced anti-apoptotic genes will have improved growth and developmental characteristics compared to non-GM banana plants. The applicant will monitor the GM banana plants for aberrant phenotypes.

145. As discussed in paragraph 138, 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. Even if an increase in resistance or tolerance was conferred, the GM plants will most likely be less fit than other commercially available banana varieties because of the potential metabolic/physiological burdens associated with expression of the introduced genes (Pretty 2001; Burdon & Thrall 2003; Denby & Gehring 2005). However, some uncertainty remains in this area due to data gaps. Further information on the tolerance of the GM bananas to environmental stresses and their susceptibility to diseases may be required to assess an application for a large scale or commercial release of these GM banana lines.

146. The proposed release is for early stage research and the proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the spread and persistence of the GM banana plants proposed for release. The release would be of limited size and short duration. The applicant proposes a number of control measures, including destruction of all plant materials not required for further analysis and post harvest monitoring of the proposed release site for 12 months and destruction of any volunteer banana plants.

147. **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 plants is not identified as a risk that warrants further assessment.

Risk Scenario 3. Dispersal of reproductive GM plant materials through various means, including animals and extreme weather conditions

148. If the GM banana plants were to be dispersed from the release site the exposure of humans and other organisms to the GM plant material could be increased, and/or they could establish and persist in the environment. The effects of contact, inhalation or ingestion of the GM banana plants have been assessed in Risk scenario 1 and were not an identified risk. The potential for the

introduced genes to result in improved survival of the GM banana plants in the environment was assessed in Risk scenario 2 and was not an identified risk.

149. 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.

150. Dispersal of other plant material could occur via the activity of frugivores or other animals, or through extremes of weather such as flooding. Banana plants can propagate vegetatively from sections of the corm containing buds from which suckers are produced. However, bananas are large plants with a fibrous root system, and suckers are firmly attached to the corm. Therefore, dispersal of propagative plant material is unlikely.

151. Fruit 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.

152. A number of native and feral animals, such as kangaroos and flying foxes, may 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.

153. Feral pigs and water buffalo can damage plants at ground level. However, the proposed trial site is within a property surrounded by a fence that excludes pigs and buffalo, limiting the possibility of dispersal by large animals or by unauthorised people accessing the site. Dispersal by authorised people entering the proposed trial site would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing.

154. Extremes of weather, such as flooding or strong winds, can cause dispersal of plant parts. The applicant has stated that the proposed trial site has never been known to flood. The trial site is within a property that was laser levelled and had drainage channels dug five years ago. In the past five years, erosion has never washed plants into the drainage channels. The proposed release site is not prone to heavy run-off or landslips. The applicant has stated that there is no recorded incident of storm or cyclone uprooting propagative material at the proposed release site and dispersing it.

155. In addition, control measures have been proposed by the applicant to minimise dispersal outside the trial site (Chapter 1, Section 3.3). These include locating the proposed release site away from waterways to prevent dispersal in the event of flooding, and transporting the GM plant materials in accordance with the Regulator's transportation guidelines.

156. **Conclusion:** The potential for allergenicity, toxicity or increased weediness due to the dispersal of reproductive GM plant materials through various means including animals and extreme weather conditions is not identified as a risk that warrants further assessment.

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

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

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

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

159. Baseline information on vertical gene transfer associated with non-GM banana plants can be found in *The Biology of Musa L. (banana)* (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.

Risk Scenario 4. Expression of the introduced genes or regulatory sequences in commercial, non-GM banana plants or other sexually compatible plants

160. Transfer and expression of the introduced genes for disease resistance to other banana or sexually compatible plants could increase the weediness potential, or alter the potential allergenicity and/or toxicity of the resulting plants.

161. All of the introduced regulatory sequences are expected to operate in the same manner as regulatory elements endogenous to the banana plants. While the transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects, the impacts from the introduced regulatory elements are likely to be equivalent to, and no greater than, those from endogenous regulatory elements.

162. As discussed in Risk Scenario 1, the GM banana plants are unlikely to be allergenic to people or toxic to people and other organisms. This will also be the case if the introduced genes are expressed in other non-GM banana plants.

163. As discussed in Risk Scenario 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.

164. Both Cavendish and Lady Finger bananas produce very little or no viable pollen and are effectively male sterile, and expression of the introduced genes is not expected to alter this trait. Therefore, gene transfer occurring through pollination is highly unlikely.

165. Commercial edible bananas are inherently female sterile. Therefore, even if the GM banana lines proposed for release were to produce viable pollen, it is highly unlikely that they could fertilise any non-GM banana plants being cultivated in proximity to the GM bananas.

166. As discussed in Chapter 1, Section 6.4, there are two *Musa* species native to Australia, but neither is known to be present in the NT.

167. While it is unlikely that the GM banana plants will differ in their sexual reproduction characteristics from the parent organism, the potential for pollen flow and gene transfer would be further restricted by the close monitoring of the GM banana plants during the proposed trial and the proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3). In particular, the applicant proposes to remove most male flowers from the GM plants and to place them into a secure container for decomposition. Those flowers that would remain (for experimental analysis) would be bagged prior to bracts opening to prevent access by pollinators. The applicant also proposes to perform post harvest monitoring of the site for 12 months and to destroy any volunteer plants found at the site.

168. **Conclusion:** The potential for allergenicity in people, toxicity in people and other organisms or increased weediness due to the expression of the introduced genes and regulatory sequences in commercial, non-GM banana plants or other sexually compatible plants as a result of gene transfer is not identified as a risk that warrants further assessment.

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

169. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). Data are accumulating to show that HGT is more widespread than previously believed and has been a significant force in the evolution of eukaryotic genomes (Bock 2010). In general, HGT between multicellular eukaryotes appears to be rare, occurring only on an evolutionary timescale, but has occurred between plants as well as between plants and less complex organisms (Bock 2010). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but could be part of a scenario potentially leading to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or by altering the expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

170. Risks that might arise from horizontal gene transfer have been reviewed (Keese 2008) and considered in previous RARMPs (for example see DIR 057/2004 and DIR 085/2008), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. From the current scientific evidence, HGT from GM plants to other organisms presents negligible risks to human health and safety or the environment. This is due to the rarity of such events, relative to those HGT events that already occur in nature, and the limited chance of providing a selective advantage to the recipient organism that would promote the spread and persistence of the transferred material.

171. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. All of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

Risk Scenario 5. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer

172. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of potential recipient organisms and the nature of the introduced genetic material.

173. HGT could result in the presence of the introduced genes for disease resistance in bacteria, plants, animals or other eukaryotes. However, the introduced genes were isolated from organisms that are already widespread in the environment and have homologues in a range of other organisms (See Chapter 1, Section 6.5), and are thus already available for HGT from those sources.

174. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced gene rather than horizontal gene transfer *per se* (Thomson 2000). If the introduced genes, the encoded proteins or their end products are not associated with any risk then even in the unlikely event of HGT occurring, they should not pose any risk to humans, animals or the environment. Conclusions reached for Risk Scenarios 1 - 4 associated with the expression of the introduced genes did not represent an identified risk.

175. **Conclusion:** The potential for an adverse outcome as a result of horizontal gene transfer is not identified as a risk that warrants further assessment.

2.5 Unintended changes in biochemistry, physiology or ecology

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

- altered expression of an unrelated gene at the site of insertion
- altered expression of an unrelated gene distant to the site of insertion, for example, due to the protein encoded by the introduced gene changing chromatin structure, affecting methylation patterns or modulating/influencing signal transduction and transcription
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions between the protein encoded by the introduced gene and 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.

177. 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).

Risk Scenario 6. Changes to biochemistry, physiology or ecology of the GM banana plants resulting from expression, or random insertion, of the introduced genes

178. Limited data is available on the phenotype of the GM banana plants as the project is in early stages. Phenotypic data is available for some of the GM banana lines containing the *ced-9* gene, either from glass house experiments (Lady Finger bananas) or from the field trial authorised under DIR 079/2007 (Cavendish bananas). The applicant states that, in both cases, most plants were phenotypically normal.

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

180. Expression of the anti-apoptotic genes in banana plants may lead to changes in growth and development (see Chapter 1, Section 5.3.1). As discussed in Risk Scenario 2, it is possible that expression of these genes in the GM banana plants may result in morphological and physiological abnormalities. The GM banana plants will be monitored for aberrant phenotypes during the proposed trial.

181. As discussed in Chapter 1, Section 5.2, expression of the anti-apoptotic genes in the GM banana plants may confer resistance to necrotrophic diseases, but increase susceptibility to biotrophic diseases (Babaeizad et al. 2009). This may result in the GM banana plants having higher rates of infection by biotrophic diseases than their non-GM counterparts. The applicant does not intend to remove any infected plant material from the trial site. Therefore, the spread of any pathogen would be limited to other, non-GM banana plants at or near the trial site. However, the only commercial banana plantation in proximity to the proposed trial site is that of the property

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

owner, DBFC. DBFC is familiar with the proposed trial and have made the property available to QUT. The applicant has stated that, in the event of disease build up, they would discuss management options with DBFC staff.

182. If the GM banana plants provided a susceptible host for biotrophic infection, this could allow a more virulent pathogen to develop, thereby potentially adversely affecting commercial banana crops, native bananas or other species in the host range of the pathogen. Increased virulence has been observed in pathogens that are serially passaged through susceptible host plants (e.g. Wang et al. 2008). However, the scale of the proposed trial would minimise the likelihood of this occurring. Only 10 plants of each GM banana line are proposed for release. The maximum number of GM banana plants proposed for released that contain an introduced anti-apoptotic gene is 1120. The planting of the GM banana lines will be staggered over four years, so that for most of the duration of the trial, less than the maximum number of plants would be in the ground. Therefore, the opportunity for biotrophic pathogens to proliferate and develop hypervirulence would be limited. The applicant has stated that the GM Grande Naine bananas trialled under licence DIR 079/2007, which contained the introduced anti-apoptotic gene *ced-9*, did not show increased susceptibility to disease.

183. In addition to the above considerations, quarantine conditions including movement restrictions on some plant materials are in place within the NT to prevent the spread of pests and diseases. These restrictions would apply to the GM bananas proposed for release. Under the NT *Plant Diseases Control Act 1979*, banana plants affected by a notifiable disease must immediately be reported to inspectors, who can then carry out wide ranging control and/or eradication measures. The NT Department of Resources also undertakes surveillance for diseases. If an unknown disease is detected, urgent diagnostic work is conducted to identify the disease, and appropriate quarantine and control measures can be imposed. Therefore, even if an unintended change led to increased disease susceptibility of the GM banana plants, the spread of the disease would be further restricted by the measures already in place under NT legislation.

184. 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, the majority of unexpected alterations that occur as a result of the genetic modification process are likely to be detected and eliminated during development of the GM lines (Bradford et al. 2005). Additionally, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant and are therefore unlikely to be perpetuated in the genome (Kurland et al. 2003). During this limited and controlled release the applicant proposes to assess the disease response and/or development of the GM banana lines, and any substantial unintended effects are likely be detected during the trial.

185. The likelihood of any unintended 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 (only 10 plants of each GM banana line are proposed for release). Humans and livestock would not be intentionally exposed as the GM plant material will not be used as food or animal feed.

186. **Conclusion:** The potential for an adverse outcome as a result of altered biochemistry, physiology or ecology is not identified as a risk that warrants further assessment.

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

187. *Agrobacterium tumefaciens* is a soil-borne, Gram-negative bacterium that, in nature, causes crown gall on plants. For genetic modification, ‘disarmed’ strains of *A. tumefaciens* that cannot cause crown gall are used to transfer DNA to plant cells under controlled, optimized laboratory conditions.

188. *A. tumefaciens* has been shown to be persistent in plant tissues and shoots in *in vitro* cultures. 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; Björklöf et al. 2006). However, studies have shown that persistence of *A. tumefaciens* occurs at a very low frequency or not at all in some GM plants (Khanna et al. 2004; Charity & Klimaszewska 2005).

189. The efficacy of antibiotics in eliminating *A. tumefaciens* from GM plants depends on interactions between several factors including the type and concentration of the antibiotic used, the strain of *A. tumefaciens*, and the plant species (Shackelford & Chlan 1996; Cheng et al. 1998; Ogawa & Mii 2005; Björklöf et al. 2006). It is also relevant to note that live cells of *A. tumefaciens* can enter a transient, non-culturable state in response to environmental stress (Manahan & Steck 1997; Alexander et al. 1999) and therefore may pass undetected in an assay system employing growth of colonies on agar plates.

190. During *Agrobacterium*-mediated transformation of plant cells, the *A. tumefaciens* attaches to plant cell walls and a virulence (*vir*) 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, although it has been detected in seed probably as a result of systemic movement (Weller et al. 2002). Systemic movement of *A. tumefaciens* has been described in several plants including fruit trees (Cubero et al. 2006). *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.

191. 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), particularly bacteria. In addition to the *vir* system, the Ti-plasmid in wild type *A. tumefaciens* encodes a transfer system (*tra*) that is responsible for the conjugal transfer of the entire Ti plasmid from one bacterium to another (Farrand 1993; Farrand et al. 1996; Cook et al. 1997). Thus, if the Ti-vector plasmid used in *Agrobacterium*-mediated transformation still contains the *tra* region (*i.e.* is conjugative) or if there are other conjugative plasmids (that could facilitate transfer) in the *A. tumefaciens* strain then the Ti-vector plasmid could be transferred to other bacteria, even interspecifically, and could result in the unintended establishment of the Ti-plasmid in the environment (National Research Council of the National Academies 2004; Björklöf et al. 2006).

Risk Scenario 7. Transfer of the introduced genes from *A. tumefaciens* to other organisms

192. The GM banana lines proposed for release were generated by *Agrobacterium*-mediated genetic modification following the method of Khanna et al. (2004) (see Chapter 1, Section 5.5). The use of the antibiotic timentin was found to be very effective in controlling *Agrobacterium* in this study. Using non-T-DNA region specific primers, Khanna et al. (2004) showed that after a period of cultivation on medium containing timentin there was no *Agrobacterium* remaining in the GM banana plants generated by this method.

193. The applicant states that the GM banana plants are all screened for the presence of *Agrobacterium* by PCR while still in tissue culture. In general, only a small proportion (1-2%) of *Agrobacterium*-positive plants are found during this screening. The applicant states that any plants found to contain *Agrobacterium* will not be sent to the field.

194. If *A. tumefaciens* cells containing the binary vector (plasmid) were present in the GM banana plants they could transfer the introduced genes via conjugation with a wild type strain of

Agrobacterium or other bacteria and yeast naturally present in the soil at the site (Hammerschlag et al. 2000). However, as discussed in Risk Scenario 5, the introduced genes were isolated from organisms that are already widespread in the environment and have homologues in a range of other organisms, and are thus already available for transfer from these sources.

195. Even if GM *A. tumefaciens* were present and could infect other bananas or other plants, without the controlled laboratory conditions associated with deliberate use of *A. tumefaciens* to transfer genes, the impact would be minimal. Since the GM *Agrobacterium* used can no longer lead to the formation of crown galls on plants, any infection would be limited to individual cells and there would be no production/regeneration of multiple GM plants.

196. Furthermore, even if the introduced genes were transferred to another organism(s) they are unlikely to be a source of potential harm (see Risk Scenarios 1-6). Homologues of the introduced genes, or proteins with similar function, are already widespread in plants and other organisms.

197. **Conclusion:** The potential for an adverse outcome resulting from the persistence in the environment of *A. tumefaciens* containing the introduced genes is not identified as a risk that warrants further assessment.

2.7 Unauthorised activities

Risk Scenario 8. Use of the GMOs outside the proposed licence conditions (non-compliance)

198. 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 plants outside of the proposed release areas and/or increased exposure of people and other organisms to GM material. The adverse outcomes that this risk scenario could cause are the same as those discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

199. **Conclusion:** The potential for an adverse outcome as a result of unauthorised activities is not identified as a risk that warrants further assessment.

Section 3 Risk estimate process and assessment of significant risk

200. The risk assessment begins with postulation of potential pathways that might lead to harm to the health and safety of people or the environment during the proposed release of GMOs due to gene technology, and how it could happen, in comparison to the parent organism and within the context of the receiving environment.

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

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

203. The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood 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 principal reasons for this include:

- limits on the size, location and duration of the release proposed by QUT

- controls proposed by QUT to restrict the spread and persistence of the GM banana plants and their genetic material
- limited ability and opportunity for the GM banana plants to transfer the introduced genes to commercial banana crops or other sexually related species
- effectiveness of removal of GM *A. tumefaciens*, which were used during the genetic modification process, from the GM banana plants prior to field release
- none of the GM plant materials or products will be used for human food or animal feed
- widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

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

Section 4 Uncertainty

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

206. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹¹. For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily to treat an identified risk.

207. For DIR 107, the primary purpose of which is to undertake research, uncertainty is noted particularly in relation to the characterisation of:

- Risk scenario 1, regarding potential increases in toxicity or allergenicity as a result of the introduced genes
- Risk scenario 2, associated with the potential for increased survival of the GMOs
- Risk scenario 6, associated with the potential for any unintended effects as a result of changes to biochemistry, physiology or ecology of the GM banana plants.

208. 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 these GM banana lines if they are selected for further development.

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

¹¹ A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

Chapter 3 Risk management plan

Section 1 Background

210. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

211. 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.

212. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

213. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Risk treatment measures for identified risks

214. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed trial of GM banana. The *Risk Analysis Framework* (OGTR 2009), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

215. These risk scenarios were considered in the context of the scale of the proposed release (a maximum area of 1.5 ha on one site between 2010 and 2014), the proposed containment measures (Chapter 1, Section 3), and the receiving environment (Chapter 1, Section 6).

Section 3 General risk management

216. Licence conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and limit the release to the size, location and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are detailed in the licence and summarised in Section 3.1.2 this Chapter.

3.1 Licence conditions

3.1.1 Consideration of limits and controls proposed by QUT

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

218. The release will be limited to a maximum of 1.5 ha on one site in the Litchfield Municipality LGA (NT) and the duration of the release will be limited to four years. Only staff with appropriate training will be allowed access to the trial site. The applicant does not intend to use any of the GM

plant material for human food or animal feed. These measures will limit the potential exposure of humans, vertebrates and other organisms to the GMOs (Risk Scenario 1) and the potential for the GM banana lines to disperse and establish outside the proposed release site (Risk Scenario 3).

219. The proposed trial site is located within a property that is surrounded by a fence with lockable gates. This will reduce access by unauthorised people or by animals, and has been imposed as a licence condition.

220. The field location is bordered on one side by a deep (approximately 1 m) drainage channel which is not connected to any waterway. The applicant has stated that the proposed trial site is at least 1 km from the nearest waterways and that the site is level and not prone to flooding, heavy runoff or landslips, which reduces the likelihood of plant material being washed away from the site (Risk Scenario 3). It is a standard DIR licence condition that trial sites must be located at least 50 metres from a waterway to limit the dispersal of viable GM plant material in the event of flooding. In addition, a licence condition has been imposed requiring immediate notification of any extreme weather conditions affecting the site during the proposed release.

221. The applicant has stated that the proposed field location is bordered on the western edge by a large shed and permanent dirt road, and on the eastern edge by a drainage channel. The applicant has proposed to border the north and south edges of the field trial with a 10 m zone in which no other bananas are grown, which would provide a clear boundary to the trial site on all sides. As discussed in Risk Scenario 4, commercial banana cultivars are effectively sterile and the chances of natural hybridisation are remote, even between adjacent plants. However, for the purpose of segregating the GM bananas from non-GM bananas being grown near the proposed release site, the use of a 10 m isolation zone surrounding the GMOs, in which no bananas can be grown, has been imposed as a licence condition. To further ensure segregation of the GM and non-GM bananas, an additional licence condition has been imposed requiring signs be placed around the field location that indicate that the bananas being grown are for research purposes only, that only authorised persons may access the field location, and that plant material is not to be removed from the site except as authorised by the licence.

222. In addition, the applicant has proposed a number of other measures to ensure segregation. Specifically, only staff with appropriate training will be permitted to enter the trial site. All plants grown within the proposed trial site will be treated as GMOs. Most fruit and other plant material will not be removed from the trial site. Any samples taken will be packaged and labelled before being removed from the trial site.

223. The applicant proposes to remove and dispose of male flowers, or bag any male flowers that are left on the plant for analysis. Removing or bagging of male flowers would occur prior to the opening of the floral bracts that enclose the flowers. Removed flowers would be placed in a secure container and allowed to decompose. Decomposed material would be removed from the container and left on the ground at the field location. Although sexual reproduction is unlikely, these measures would further reduce the chances of any pollen that may be produced remaining viable or being dispersed into the environment (Risk Scenario 4). They would also limit the potential exposure of nectar feeding animals including birds, marsupials and bats (Risk Scenario 1). Since bananas sometimes produce hermaphrodite flowers that can contain small amounts of pollen, it is considered appropriate to also remove or bag hermaphrodite flowers.

224. The covering of bunches is a standard practice used in commercial, non-GM banana cultivation. This practice as applied to GM fruit would limit the potential exposure of frugivores to the GMOs (Risk Scenario 1) as well as limit the potential for the GM banana fruit/seed to disperse, in the unlikely event that any seed is produced (Risk Scenario 3), and has been imposed as a licence condition.

225. The applicant proposes to harvest fruit while it is still hard and green (unripe) and firmly attached to the plant, as is standard commercial practice. Unripe fruit is less appealing to frugivores

such as bats and birds than fully ripe fruit. Fruit that is not required for experimental analysis would be destroyed by shredding and placing it on the ground at the field location to decompose. In tropical environments, shredded fruit is expected to rapidly decompose and become unpalatable to vertebrates. This method of destroying fruit is currently in place at other banana field trials in northern Queensland (DIR 076/2007 and DIR 079/2007) and the applicant states that no vertebrates have been observed consuming shredded fruit from these trials. These practices would further limit the potential for exposure of animals to, or dispersal of, the GMOs (Risk Scenarios 1 and 3).

226. The applicant proposes to desucker plants at the field location by cutting off suckers at ground level, gouging out the centre of the pseudostem and pouring in kerosene or distillate. This method is commonly used in commercial banana cultivation (Broadley et al. 2004) and kills the growing point of the sucker while it is still firmly attached to the corm. Removed pseudostems and leaf material would be left on the ground at the field location to decompose. The applicant also proposes to transport plant waste from the shadehouse to the field location to be left on the ground to decompose. It is considered that any whole plants (which would not be more than approximately 4 months old and therefore still immature) should additionally be sprayed with herbicide, so as to destroy the corm, before being transferred to the field location. As all waste material left on the ground to decompose would therefore be non-propagative, this destruction method is considered to be appropriate for preventing dispersal (Risk Scenario 3).

227. The applicant proposes to destroy plants at the end of the trial by injection with herbicide (2,4-D or glyphosate). The systemic nature of these herbicides means that the whole plant, including the corm, starts to die and decay rapidly and virtually no regrowth occurs. Plants treated in this way and left over the summer and wet season period are in an advanced state of decay by the end of the wet season (Lindsay et al. 2003). Therefore, the applicant's proposal to monitor the field site for 12 months for volunteer banana plants, and to destroy by herbicide treatment any volunteers found, would minimize the persistence of the GMOs in the environment (Risk Scenario 2). In addition to this, it is considered that post harvest monitoring of the release sites should continue until no volunteers are detected for at least six continuous months.

228. The applicant has stated that any plant material taken to or from the site will be transported according to the Regulator's *Guidelines for the Transport of GMOs* (<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/transport-guide-1>). The guidelines are standard protocols for the handling of GMOs to minimise exposure of the GMOs to human and other organisms (Risk Scenario 1) and dispersal into the environment (Risk Scenario 3).

229. In addition to the above points, NT Government legislation targeted to the control of plant diseases (see Chapter 1, Section 6.3) would also apply to the proposed release of GM bananas and would act as an effective adjunct to the proposed control measures.

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

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

- limit the release to a maximum total area of 1.5 ha at one site in the Litchfield Municipality LGA between the date of issue of the licence and November 2014
- locate the trial site at least 50 m away from waterways
- maintain a 10 m zone around the GM bananas in which no bananas may be grown
- 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
- harvest the GM banana separately from other crops

- clean all equipment used in connection with the GMOs
- monitor the field site for at least 12 months after harvest and destroy any volunteer banana plants that may grow
- destroy all GM plant material, including fruit, not required for further analysis
- transport and store all GMOs in accordance with the Regulator's guidelines
- not permit any GM banana plant material to be used in human food or animal feed.

3.2 Other risk management considerations

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

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

3.2.1 Applicant suitability

232. 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.

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

234. The licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability to hold a licence.

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

3.2.2 Contingency plan

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

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

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

238. 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.

3.2.4 Reporting requirements

239. 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.

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

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

3.2.5 Monitoring for Compliance

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

242. 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.

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

Section 4 Issues to be addressed for future releases

244. Additional information has been identified that may be required to assess an application for a large scale or commercial release of these GM banana lines, or to justify a reduction in containment conditions. This includes:

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

Section 5 Conclusions of the RARMP

245. The risk assessment concluded that this proposed limited and controlled release of up to 151 GM banana lines on a maximum total area of 1.5 ha over four years at one site in the Litchfield Municipality LGA, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

246. The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, location and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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Appendix A Summary of issues raised in submissions received from prescribed experts, agencies and authorities¹² on the consultation RARMP for DIR 107

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

Summary of issues raised	Comments
The trial site is within 50 m of a drain. The drain is not a natural waterway but still could flood the trial site. Does not consider that flooding would spread GM bananas but suggest it is important to clarify the distance between the drain and trial site to remove ambiguity.	<p>The field location is bordered on one side by a drainage channel which is not connected to any waterway.</p> <p>Licence conditions stipulate that the outer edge of the trial site must not be within 50 m of permanent natural waterways or man-made waterways that flow into natural waterways. Irrigation channels that do not flow into natural waterways, such as the drainage channel bordering the field location, are not considered waterways for the purpose of the licence.</p> <p>The potential for dispersal of reproductive plant materials through various means, including flooding, was considered in Risk Scenario 3 and was not assessed as a risk that warranted further assessment.</p>
Does not support the release of anything genetically modified in the area for the proposed release.	Noted. The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology.
<p>Notes that no products from the proposed dealing will be used for human or animal consumption. Concur with the assessment that the dealing constitutes negligible risk to human health and the environment under the proposed licence conditions.</p> <p>Notes that tests for toxicity and allergenicity of the GM banana lines and the purified encoded protein have not been undertaken. Understands that these tests will be required should approval be sought for the GMOs or their products to be used for human consumption.</p>	<p>Noted.</p> <p>The regulation of GM foods is the responsibility of FSANZ. FSANZ approval would need to be obtained before materials from these GM banana lines could be sold as food.</p>
QUT should be required to surround the trial site with a secure fence to clearly separate the GM field trial location from the rest of the commercial farm	<p>The proposed trial site is on the property of the Darwin Banana Farming Company (DBFC). The entire DBFC farm is surrounded by a fence with lockable gates, limiting unauthorised access by people. Only trained and authorised staff will be permitted to access the trial site. The applicant has stated that all staff of the DBFC not involved with the field trial will be instructed not to enter the trial site.</p> <p>The field site is physically bordered on the west by a large shed and permanent dirt road, and on the east by a deep drainage channel. In addition, licence conditions require the field location to be surrounded by a 10 m isolation zone in which no bananas may be grown, providing a clear boundary to the trial site.</p> <p>An additional licence condition has been added requiring that the field location</p>

¹² GTTAC, State and Territory Governments, Australian Government agencies, LGAs and the Minister for the Environment.

Summary of issues raised	Comments
	be signed to indicate that the bananas being grown there are for research purposes only, that only authorised persons may access the field location, and that plant material is not to be removed from the site except as authorised by the licence.
Any interstate transport of the GM plant materials must be undertaken in compliance with the quarantine protocols of relevant States	<p>The cultivation and movement of banana planting material is regulated in the NT and Queensland by the NT Department of Resources and the Queensland Department of Primary Industries and Fisheries, respectively. The requirements of State regulators apply whether or not included in the licence issued by the Regulator.</p> <p>The applicant has stated that they will consult with NT and Queensland authorities regarding the movement and planting of GM and non-GM banana material for this trial and adhere to all relevant regulations. In addition, transport of GM plant material off the site must be in accordance with the Regulator's <i>Guidelines for the Transport of GMOs</i>.</p>
Paragraph 12, page 6 refers to the movement of GM and non-GM bananas from QUT to the release site. There is no indication that the virus indexing of all motherstock was completed as per the Queensland Banana Accredited Nursery protocols prior to cells being placed into tissue culture	<p>All of the GM and non-GM bananas being transported to the trial site have been virus indexed, either following the standard Queensland Banana Accredited Nursery (QBAN) protocols or using another protocol that has also been approved by QBAN and Biosecurity Queensland. Ch 1 of the RARMP has been modified to clarify this.</p> <p>The applicant has stated that no virus has ever been detected by them, or the independent testing authority, in any plants before or after moving plants to Northern Queensland. The applicant expects this to also be the case for the plants associated with the proposed release.</p> <p>The applicant will consult with Queensland and NT authorities regarding the movement and planting of GM and non-GM banana material for this trial and adhere to all relevant regulations.</p>
<p>The point of deflasking is not clear. It is assumed from the context that the plantlets will be transported in flask and de-flasked on site (refer also paragraph 97, page 19). This requires clarification.</p> <p>Further detail regarding the security arrangements which will be in place at the shadehouse is required.</p>	<p>Tissue cultured GM and non-GM control bananas will be transported in tissue culture vessels (flasks) from QUT to the proposed trial site, as required by QBAN, where they will be de-flasked and acclimatised for 2-4 months in a secure area within the DBFC shadehouse (see below). Ch 1 of the RARMP has been modified to clarify this.</p> <p>Licence conditions require that the area of the shadehouse used for acclimatising the GMOs must be:</p> <ul style="list-style-type: none"> • separate and lockable; and • signed so as to indicate that GM Plant Material is present; and • closed and locked at all times except when being used for specific purposes; and • cleaned as soon as practicable after use and before it is used for any other purpose.
Paragraph 15, page 7 mentions that only trained and authorised staff will be permitted access to the proposed site. Information about staff training is necessary.	<p>The applicant has stated that training of staff will include standard health and safety induction and also training in practices relevant to DIR licence condition, including practices in handling and disposal of material on site and sample packaging, labelling and transport. Staff will also be instructed that all plants within GM areas (field location or shadehouse) are to be regarded as GM. These details have been added to Ch 1 of the RARMP for clarification.</p> <p>In addition, licence conditions require the licence holder to inform each person covered by the licence of the conditions of the licence, and to obtain a signed statement that the person has been informed of the condition, and has understood and agreed to be bound by it.</p> <p>Licence conditions also require transport of GM plant material off the site to occur in accordance with the Regulator's <i>Guidelines for the Transport of GMOs</i>, which include a requirement that any person undertaking transport of GM plants be trained in the conditions of transport which apply to the GM material being transported.</p>
Some detail about how movements of people and machinery onto the site (which is subject to NT quarantine restriction) is required. For example, is it intended for machinery to be washed and inspected with every movement on	Proposed licence conditions require that any equipment used in connection with the GMOs or plant material must be cleaned as soon as practicable and before it is used for any other purpose. In addition, any area used to clean equipment used in connection with the GMOs or Plant Material must also be cleaned as soon as practicable and before it is used for any other purpose. Cleaning is

Summary of issues raised	Comments
and off the site?	<p>defined in the licence as the removal and/or destruction of the GMOs and plant material. These licence conditions have been imposed to minimise dispersal of GM plant material.</p> <p>The applicant is also required to adhere to all relevant State and Territory legislation regarding the cultivation and movement of bananas, including quarantine measures imposed under the NT <i>Plant Diseases Control Act</i>.</p>
<p>Paragraph 103 refers to the use of 2,4-D and glyphosate to kill banana plants and any volunteers. Both of these chemicals are registered for use on banana in the NT. Glyphosate is known to be more effective in controlling the spread of banana in the NT as less regrowth is likely.</p>	<p>At the end of the trial, GM banana plants containing meristematic tissue must be cut off the main plant at ground level and destroyed with kerosene, distillate or herbicide. The field location must then be monitored, and any volunteers destroyed, for a minimum of 12 months and until the site is clear of volunteers for at least six months. These measures will prevent persistence of the GMOs in the environment.</p>
<p>The RARMP does not address the risks associated with the movement of infected material back to Queensland.</p> <p>The arrangements for the diagnostics to determine which tissues are infected by TR4 and information surrounding the regulatory requirements for the movement of samples back to Queensland should also be considered due to the potential for impacts on the environment in that location.</p> <p>If TR4 resistant lines are identified, then the arrangements for material to be returned to Queensland to allow multiplication of the selected lines need to be clarified (the NT's regulatory requirements in relation to interstate movement of material will also need to be met).</p>	<p>The applicant has stated that they do not intend to remove any infected plant material from the trial site. A small number of samples may be taken from the upper part of non-symptomatic GM banana plants containing the GUS reporter gene for the purpose of GUS assays or nucleic acid extraction. Ch 1 and Ch 2 of the RARMP have been modified to clarify this.</p> <p>The disease status of the GM banana plants would be determined visually at the field location. Ratings are given to external symptoms (e.g. number of yellow leaves, degree of stem splitting), and to internal symptoms by cutting across the stem and assessing the percentage of discolouration. These details have been added to Ch 1 of the RARMP.</p> <p>Licence conditions require transport of any GM plant material off the site to occur accordance with the Regulator's <i>Guidelines for the Transport of GMOs</i>.</p> <p>The applicant is also required to adhere to all relevant State and Territory regulatory requirements regarding the movement of bananas and banana plant material.</p>
<p>No new relevant data has been provided since the previous trial under DIR 079/2007. Has concerns regarding:</p> <ul style="list-style-type: none"> • the potential for an increase in sensitivity to pathogens by altering how bananas respond to infection • the potential for selection of a more severe pathogen • the potential for the introduced proteins to have a negative effect on organisms that come into contact with them. <p>Considers the likelihood of these events being realised remains and is greater in the current trial than that assessed in the RARMP. This is due to the increased number of introduced genes and GM banana lines and the longer duration of the trial.</p>	<p>Each application for a DIR licence is assessed on a case by case basis. DIR 107 involves an experimental release of small size and limited duration and with a number of proposed controls, and the Regulator assessed it within this context.</p> <p>The potential for expression of the introduced anti-apoptotic genes to result in an increase in sensitivity to pathogens or to allow the selection of a more virulent pathogen was assessed in Risk Scenario 6 in the context of all relevant information, including observations from the DIR 079/2007 field trial and recent scientific publications, and was not identified as a risk that warranted further detailed assessment.</p> <p>The potential for toxicity and/or allergenicity in people and other organisms as a result of exposure to GM plant materials was assessed in Risk Scenario 1 in the context of the limits and control measures proposed by the applicant and was not identified as a risk that warranted further assessment (see below).</p>
<p>The introduced anti-apoptotic genes may result in the GM banana lines being both disease resistant and abiotic stress tolerant, leading to unintended effects on the biology of banana plants and wider changes in ecosystem dynamics.</p>	<p>Ch 2, Section 2.5 refers to the potential for unintended effects in the GM plants. Risk Scenario 6 discusses the possibility that the GM banana plants expressing the anti-apoptotic genes will show enhanced tolerance to biotic and abiotic stresses. The potential for harm as a result of unintended effects was assessed in this scenario and was not identified as a risk that warranted further detailed assessment.</p> <p>The RARMP notes that uncertainty exists in relation to unintended effects as a result of changes in biochemistry, physiology or ecology of the GM plants. Additional data to address these uncertainties may be required to assess possible future applications for a larger scale or commercial release (Chapter 3, Section 5).</p> <p>The likelihood of any unintended 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. It is a condition of all DIR licences that any unexpected or unintended</p>

Summary of issues raised	Comments
<p>The risks of exposure to frugivores and flower feeding animals have been discussed in the RARMP. However, most of the invertebrate pests of banana are neither frugivores nor flower-feeders. Native invertebrate species could be exposed to other parts of the GM bananas (root, stem, foliage). Suggests that the species composition and abundance of invertebrates found on banana plants in the trial be monitored and compared with an appropriate control plot. A significant difference could indicate a possible toxic, allergenic or other effect.</p>	<p>effects of the GMO be reported to the Regulator.</p> <p>Ch 2 of the RARMP notes that organisms including vertebrates, invertebrates, symbiotic microorganisms and/or pathogenic fungi may be exposed directly or indirectly to the proteins encoded by the introduced genes. However, the limited scale, short duration and other proposed limits and controls will minimise exposure of vertebrate and invertebrate species to the GM plant material.</p> <p>The potential for toxicity and/or allergenicity in people and other organisms as a result of exposure to GM plant materials was assessed in the context of the limits and control measures proposed by the applicant and was not identified as a risk that warranted further assessment.</p> <p>The proposed release involves early stage research, and the RARMP notes that uncertainty exists, including in relation to potential increases in the toxicity or allergenicity of the GM banana plants as a result of the introduced genes. Additional data to address these uncertainties would be required to assess future applications for a larger scale trial, reduced containment conditions, or the commercial release of these GM banana lines if they are selected for further development.</p>
<p>The Regulator should consider means of clearly separating GM and non-GM bananas.</p>	<p>Licence conditions require that the area of the shadehouse used for acclimatising the GMOs must be:</p> <ul style="list-style-type: none"> • separate and lockable; and • signed so as to indicate that GM Plant Material is present; and • closed and locked at all times except when being used for specific purposes; and • cleaned as soon as practicable after use and before it is used for any other purpose. <p>The field location is physically bordered on the west by a large shed and permanent dirt road, and on the east by a deep drainage channel. In addition, licence conditions require the field location to be surrounded by a 10 m isolation zone in which no bananas may be grown, providing a clear boundary to the field site.</p> <p>An additional licence condition has been added requiring that the field location be signed to indicate that the bananas being grown there are for research purposes only, that only authorised persons may access the field location, and that plant material is not to be removed from the site except as authorised by the licence</p>

Appendix B Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 107

The Regulator received one submission from the public on the consultation RARMP. This submission, 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: H: Human health; C: Consultation

Other abbreviations: FSANZ: Food Standards Australia New Zealand; GM: Genetically Modified; GMO: Genetically Modified Organism; RARMP: Risk Assessment and Risk Management Plan.

Type: I: individual.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	x	H	Proposed release threatens human health and safety by being part of a process designed to impose artificial food on human populations. Believes it is irresponsible to have trials for agronomic performance when long term health effects are not known.	DIR 107 is a limited and controlled release for the purpose of conducting experiments, including assessing the disease response of the GM bananas. The RARMP concluded that this limited and controlled release poses negligible risks to people or the environment. The Regulator has imposed a range of measures to minimise exposure to the GMOs and their genetic material, including preventing use in human food and animal feed. FSANZ approval would be required before products from the GM banana lines could be sold as food.
			C	Other government bodies should not support this trial.	The <i>Gene Technology Act 2000</i> requires extensive consultation on the RARMP with the public as well as a wide range of experts, agencies and authorities. These comprise all State and Territory Governments, the Gene Technology Technical Advisory Committee, prescribed Australian Government agencies, the Environment Minister and relevant local councils. All issues relating to the health and safety of people and the protection of the environment raised during the consultation process were thoroughly considered in the context of current scientific knowledge before finalising the RARMP and making the decision to issue the licence.