



Australian Government

Department of Health and Ageing

Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 109

**Limited and controlled release of banana
genetically modified for enhanced nutrition**

Applicant: Queensland University of Technology

August 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 109 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 enhanced nutrition. The field trial is authorised to take place at one site in the Shire of Johnstone, Queensland (QLD), on a maximum area of 2.0 ha between August 2011 and August 2013.

The purpose of the trial is to assess pro-vitamin A and/or iron levels in the GM banana fruit and agronomic performance of GM banana plants grown under field conditions. The GM bananas will not be permitted to enter the commercial human food or animal feed supply chains.

A total of up to 1241 lines² of GM banana comprising of 1221 GM Cavendish and 20 GM Lady Finger banana lines are intended for release. Up to 1211 of the GM banana lines contain from one up to three genes that are expected to enhance pro-vitamin A and/or iron content in banana. The genes are derived from a range of organisms including viruses, bacteria, algae and plants.

The remaining 30 GM banana lines contain a gene from a common gut bacterium used to study gene expression patterns in banana fruit.

In addition, all of the GM banana lines contain an antibiotic resistance gene, also derived from a common gut bacterium, which was used to select genetically modified plant cells and 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 took into account information in the application (including proposed containment measures), relevant previous approvals and current 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 those that warrant detailed characterisation are determined. This process is described as risk identification.

Seven 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 seven 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 imposed licence conditions.

As none of the seven risk scenarios characterised in the risk assessment identified any risk that requires 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 2.0 ha at one site between August 2011 and August 2013, inclusive. The **control** measures include containment provisions at the trial site; preventing the use of GM plant materials in commercial 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 or other specific condition; 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 1241 lines of GM banana on a maximum total area of 2.0 ha over two years in the Shire of Johnstone, QLD, 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

ABA	Absciscic acid
Act	The <i>Gene Technology Act 2000</i>
ACO	1-aminocyclopropane-1-carboxylate oxidase
ACS	1-aminocyclopropane-1-carboxylate synthase
<i>APsy2a</i>	Phytoene synthase 2 gene from banana cultivar ‘Asupina’
ATCC	American type culture collection
CaMV	Cauliflower mosaic virus
<i>Cat</i>	Catalase gene
CMSSP	<i>Chrysanthemum morifolium small subunit protein</i>
<i>CrtB</i>	Phytoene synthase
<i>CrtI</i>	Phytoene desaturase
DEEDI	Department of Employment Economic Development and Innovation, QLD
DIR	Dealings involving Intentional Release
DMA	Deoxy-mugineic acid
DMPP	Dimethylallyl di-phosphate
DNA	Deoxyribonucleic Acid
DOXP	1-Deoxy-xylulose-5-phosphate
<i>DXS</i>	1-Deoxy-xylulose-5-phosphate synthase
EFSA	European Food Safety Authority
<i>Exp</i>	Expansin
<i>Ext</i>	Extensin
FAO	Food and Agriculture Organization
Fe	Iron
<i>FEA1</i>	Fe-assimilation 1
FSANZ	Food Standards Australia New Zealand
GGPP	Geranylgeranyl di-phosphate
GA	Gibberellic acid
GM	Genetically Modified
GMO	Genetically Modified Organism
GUS	β -glucuronidase
ha	Hectare
HGT	Horizontal Gene Transfer
IPP	Isopentenyl di-phosphate
JECFA	Joint FAO and WHO Expert Committee on Food Additives
<i>LYCB</i>	Lycopene β -cyclase
MEP	Methyl erythritol phosphate
<i>MT2A</i>	<i>Metallothionein</i>
NA	Nicotianamine
<i>NAS</i>	Nicotianamine synthase
NH&MRC	National Health & Medical Research Council
NLRD	Notifiable Low Risk Dealing
<i>Nos</i>	Nopaline synthase
<i>nptII</i>	Neomycin phosphotransferase type II gene
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment level 2

<i>Psy1Q60</i>	Phytoene synthase 1
PCR	Polymerase Chain Reaction
<i>PsyB73</i>	Phytoene synthase 1
QLD	Queensland
QUT	Queensland University of Technology
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
Rbcs-CTP	Rubisco small subunit – chloroplast targeting peptide
RE	Retinol equivalent
SJRS	South Johnstone Research Station
T-DNA	Transfer DNA
TGA	Therapeutic Goods Administration
the Act	The <i>Gene Technology Act 2000</i>
<i>tra</i>	Transfer gene(s)
<i>Ubi</i>	Polyubiquitin
<i>uidA</i>	β -glucuronidase gene
UL	Upper limit of intake
<i>vir</i>	Virulence gene(s)
WHO	World Health Organization

Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of application DIR 109 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 enhanced nutrition. The field trial is authorised to take place at one site in the Shire of Johnstone, Queensland (QLD), on a maximum area of 2.0 ha between August 2011 and August 2013.

The purpose of the trial is to assess pro-vitamin A and/or iron levels in the GM banana fruit and agronomic performance of GM banana plants grown under field conditions. This would help identifying gene(s) and promoter(s) combinations that will enhance nutritional quality of banana fruits without the associated negative effects on GM plant growth and development observed in a previous trial. The GM bananas will not be permitted to enter the commercial human food or animal feed supply chains.

A total of up to 1241 lines⁴ comprising of 1221 GM Cavendish (cultivars Williams and Dwarf Cavendish) and 20 GM Pome (cultivar Lady Finger) banana lines are intended for release.

Up to 836 of the GM banana lines contain from one up to three of the six genes involved in pro-vitamin A (carotene) biosynthesis that were isolated from range of plants and bacteria.

Up to 215 of the GM banana lines contain either one or two of three genes that are implicated in iron-assimilation in plants and algae.

Additionally, up to 160 lines contain genes for both pro-vitamin A biosynthesis and iron-assimilation. These lines contain at least one gene for each trait with a maximum of three genes in total.

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 30 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 under Exp-1 promoter will be used to study spatial and temporal gene expression pattern under this promoter.

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), *Musa acuminata* (banana) and *Ricinus communis* (castor bean); the soil bacterium *Agrobacterium tumefaciens*; and the plant virus Cauliflower mosaic virus (CaMV). Although *A. tumefaciens* and CaMV are plant pathogens and maize and castor bean both contain known allergens or toxins, the regulatory sequences comprise only a small part of their respective total genomes, and are not in themselves capable of causing disease.

In addition, genetic elements encoding short signal peptides have been introduced to direct one of the introduced proteins to chloroplasts. These elements are derived from *Pisum sativum* (pea) and *Chrysanthemum morifolium* (chrysanthemum). These signal sequences are removed from the pre-protein upon reaching the chloroplast, and hence are not present in the mature functional protein.

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

Risk assessment

The risk assessment took 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 those that warrant detailed characterisation are determined. This process is described as risk identification.

Seven 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 seven 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

- 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 enter commercial human food or animal feed supply chains
- 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 seven risk scenarios characterised in the risk assessment identified any risk that requires 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 2.0 ha per year at one site in the Shire of Johnstone, QLD, between August 2011 and August 2013.
- locate the trial site at least 50 m away from waterways
- 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 until no volunteers are found for at least six months, 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 or other specific conditions
- not permit any GM banana plant material to enter commercial human food or animal feed supply chains.

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, including potential for non-specific uptake of metals other than iron.

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 consultation RARMP

The risk assessment concluded that this limited and controlled release of up to 1241 lines of GM banana on a maximum total area of 2.0 ha over two years in the Shire of Johnstone, QLD, 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

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>>.

by the applicant as these were important considerations in establishing the context for assessing the risks.

Chapter 1 Risk assessment context

Section 1 Background

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

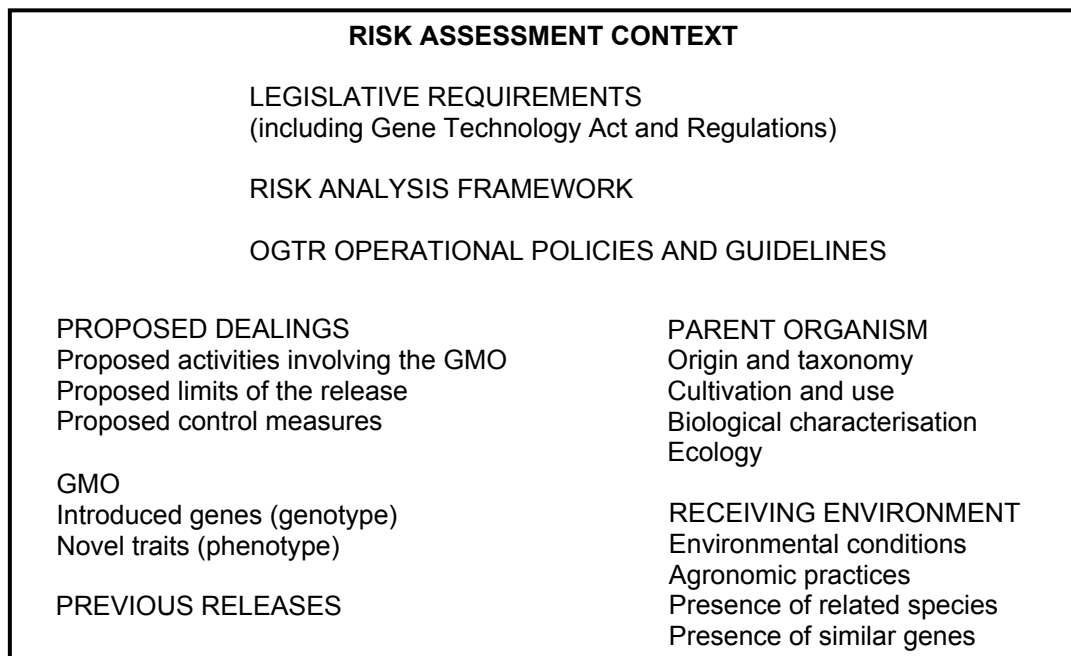


Figure 1. Parameters used to establish the risk assessment context

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 available at the OGTR website <<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 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 on the application and one on consultation RARMP and the issues raised and their considerations are 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 1241 lines⁶ of GM banana, which have been genetically modified (GM) for enhanced nutrition, 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 main purpose of the trial is to conduct experiments to refine strategies for enhancing pro-vitamin A and iron content in banana such that these micro-nutrients can be increased while minimising potential negative effects on growth and development of banana plants. A number of genes will be tested for each of these traits and a number of promoters will also be tested to identify those that achieve best expression of the introduced genes in the fruit. The trial will also address whether both the traits can be combined in the same plant.

12. Up to 1180 newly developed GM banana lines (sub-group Cavendish; cultivar Williams) will be released with one plant per line. The trial will also include 41 lines of GM Dwarf Cavendish banana (sub-group Cavendish) and 20 lines of GM Lady’s Finger banana (sub-group Pome) that were previously released under the licence for DIR 076/2007. For previously released GM banana lines, 10 clones (replicates) per line would be released making the total up to 1790 GM plants (see Table 3). Non-GM bananas would also be planted as controls.

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

13. GM and non-GM bananas would be transported in tissue culture vessels (flasks) from QUT to the proposed release site, South Johnstone Research Centre (SJRS), Department of Employment Economic Development and Innovation (DEEDI), QLD, where they would be de-flasked and acclimatised (hardened off) in a shadehouse for 2–4 months. Acclimatised plants would be moved approximately 500 m to the field location where they would be planted.

14. For lines previously examined under DIR 076/2007 and needing further evaluation, suckers from plants will be harvested from the adjacent field location within SJRS where they were originally released. Harvested suckers would be transported to the shadehouse, cleaned and hardened by air drying. Hardened suckers will be transported back to the new location and planted. In case sufficient suckers (10 per line) could not be obtained, GM plant material which has been maintained *in vitro*, or taken from the field, will be used to regenerate plants in a tissue culture facility in QUT. Such micro-propagated plants would be used for planting after hardening as described above.

15. All the GM banana lines will be maintained using similar cultural practices to those applied to non-GM banana. A description of the relevant agricultural activities proposed for this release can be found in Section 6.3.

16. The GM banana plants would be progressively released. Plants are intended to be grown for at least two cycles (approximately 20–22 months); that is, fruit would be obtained from the plant crop and one ratoon crop (see Section 6.3).

17. GM banana plants will be visually inspected for abnormalities e.g. stunting, chlorosis, necrosis, leaf deformation etc. Some plant material will be harvested for analysis and will be transported to PC2 facilities for experimental purposes. These and any excess harvested material will be destroyed at the end of experiments.

18. Plant material from better performing lines may be harvested to re-initiate and maintain plants in tissue culture. Any such samples taken from the GM banana lines will be transported back to contained facilities at the QUT for tissue culture and any excess material will be destroyed. Plant material not required for further analysis or experiments would be decomposed at the trial site.

19. QUT intends to apply for a separate licence to use pro-vitamin A and/or iron rich GM bananas from this trial in a small scale controlled human nutritional study. Any such application would be assessed separately by the Regulator. However, the GM bananas would not be permitted to enter the commercial human food or animal feed supply chains.

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

20. The release is proposed to take place at one site at SJRS in the Shire of Johnstone, QLD. Within the site there are two field locations, a shadehouse (to be used for hardening off tissue-culture generated plants before they are transferred to the field location) and a packing shed (to be used for packing and temporarily storing plant material harvested for analysis before transport to PC2 facilities at QUT); that together will occupy a maximum total area of 2.0 ha. The release is proposed to occur between August 2011 and August 2013.

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

21. 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 approximately 250 m away from natural waterways and 18 m above sea level
- utilising a parent plant that is essentially female and male sterile
- applying bunch covers to restrict 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 QLD State 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
- transporting GM plant materials to and from the proposed trial site in accordance with Regulator's guidelines or other procedures approved by the Regulator
- 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
- not permitting any GM banana plant material to enter commercial human food or animal feed supply chains.

22. 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

23. 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 QLD. They are also grown in Western Australia around Carnarvon, Kununurra and Broome and in the NT near Darwin.

24. 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).

25. 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

26. The applicant proposes to release up to 1241 GM banana lines, comprising of 1221 GM Cavendish (cultivar Dwarf Cavendish or Williams) and 20 GM Pome (cultivar Lady Finger) banana lines. The genes introduced into the GM banana lines proposed for release are listed in Table 1 and the promoters and other genetic elements used for expressing these genes are listed in Table 2. Details of the constructs used to generate the GM banana lines are provided in Table 3.

27. Up to 836 of the GM banana lines contain from one up to three genes that are expected to enhance β -carotene content in banana while 215 lines contain one or two genes to increase iron. Up

to 160 lines engineered for enhancing both iron and β -carotene will contain at least one gene for each trait and up to three genes in total.

28. The remaining 30 GM banana lines contain the *uidA* reporter gene under the *Exp1* promoter. These plants will be used to study tissue specificity of the *Exp1* promoter function.

29. 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.

30. Short regulatory sequences would be used to control expression of the introduced genes or to direct proteins to appropriate cellular compartments. These sequences are derived from plants (maize, banana, castor bean, pea and chrysanthemum), a soil bacterium (*Agrobacterium tumefaciens*) and the plant virus cauliflower mosaic virus (CaMV; see Table 2).

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

Gene	Gene – full name	Source organism	Intended function
<i>DXS</i>	Deoxy-xylulose-5-phosphate synthase	Thale cress (<i>Arabidopsis thaliana</i>)	pro-vitamin A biosynthesis
<i>PsyB73</i>	Phytoene synthase 1	Maize (<i>Zea mays</i>) inbred line B73	pro-vitamin A biosynthesis
<i>Psy1Q60</i>	Phytoene synthase 1	Maize (<i>Z. mays</i>) inbred line Q60	pro-vitamin A biosynthesis
<i>APsy2a</i>	Phytoene synthase 2	Banana (<i>Musa spp.</i>) cultivar Asupina	pro-vitamin A biosynthesis
<i>CrtI</i>	Phytoene desaturase	<i>Erwinia uredovora</i>	pro-vitamin A biosynthesis
<i>LYCB</i>	Lycopene β -cyclase	Rice (<i>Oryza sativa</i>)	pro-vitamin A biosynthesis
<i>Ferritin</i>	Ferritin	Wild soybean (<i>Glycine soja</i>)	Fe-assimilation
<i>NAS</i>	Nicotianamine synthase	Rice (<i>O. sativa</i>)	Fe-assimilation
<i>FEA1</i>	Fe-assimilation 1	<i>Chlamydomonas reinhardtii</i>	Fe-assimilation
<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. Genetic elements used in GM banana lines proposed for release.

Element	Source gene	Function	Source organism
<i>Nos</i>	Nopaline synthase	Promoter	<i>Agrobacterium tumefaciens</i>
<i>35S</i>	Cauliflower mosaic virus 35S	Promoter	Cauliflower mosaic virus
<i>Exp1</i>	Expansin 1	Promoter	Banana (<i>Musa acuminata</i>) cultivar Williams
<i>Exp4</i>	Expansin 4	Promoter	Banana (<i>Musa acuminata</i>) cultivar Cavendish
<i>Ext</i>	Extensin	Promoter	Banana (<i>Musa acuminata</i>) cultivar Cavendish
<i>MT2A</i>	Metallothionein	Promoter	Banana (<i>Musa acuminata</i>) cultivar Cavendish
<i>ACS</i>	1-aminocyclopropane-1-carboxylate synthase	Promoter	Banana (<i>Musa acuminata</i>) cultivar Williams
<i>ACO</i>	1-aminocyclopropane-1-carboxylate oxidase	Promoter	Banana (<i>Musa acuminata</i>) cultivar Williams
<i>Ubi</i>	Polyubiquitin	Promoter	Maize (<i>Zea mays</i>)
<i>Nos</i>	Nopaline synthase	Terminator	<i>Agrobacterium tumefaciens</i>
<i>35S</i>	Cauliflower mosaic virus 35S	Terminator	Cauliflower mosaic virus
<i>Cat</i>	Catalase	Intron	Castor bean (<i>Ricinus communis</i>)
CMSSP	<i>Chrysanthemum morifolium</i> small subunit protein	Signal peptide	Chrysanthemum (<i>Chrysanthemum morifolium</i>)
Rbcs-CTP	Rubisco small subunit – chloroplast targeting peptide	Signal peptide	Pea (<i>Pisum sativum</i>)

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

Lines previously released under DIR 076/2007							
Cultivar	Construct(s)	Gene(s)	Promoter(s)	Additional genetic elements	Max. lines	Replicates per line	Total plants
Dwarf Cavendish	pCAM-Exp1-APsy2a	<i>APsy2A</i>	<i>Exp1</i>	<i>Nos</i>	5	10	50
	pCAM-ACO-APSy2a	<i>APsy2A</i>	<i>ACO</i>	<i>Nos</i>	5	10	50
	pCAM-Ubi-APSy2a	<i>APsy2A</i>	<i>Ubi</i>	<i>Nos</i>	2	10	20
	pCAM-Exp1-PsyB73	<i>PsyB73</i>	<i>Exp1</i>	<i>Nos</i>	5	10	50
	pCAM-ACO- PsyB73	<i>PsyB73</i>	<i>ACO</i>	<i>Nos</i>	5	10	50
	pCAM-Ubi-PsyB73	<i>PsyB73</i>	<i>Ubi</i>	<i>Nos</i>	4	10	40
	pCAM-ACO-APSy2a pBin-Exp1-Crt1	<i>APsy2A</i> <i>Crt1</i>	<i>ACO</i> <i>Exp1</i>	<i>Nos</i> CMSSP, <i>Nos</i>	5	10	50
	pCAM-ACO-PsyB73 pBin-Exp1-Crt1	<i>PsyB73</i> <i>Crt1</i>	<i>ACO</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	5	10	50
	pCAM-Exp1-Ferritin	<i>Ferritin</i>	<i>Exp1</i>	<i>Nos</i>	5	10	50
	pCAM-Exp1-APsy2a	<i>APsy2A</i>	<i>Exp1</i>	<i>Nos</i>	5	10	50
Lady Finger	pCAM-ACO-APSy2a	<i>APsy2A</i>	<i>ACO</i>	<i>Nos</i>	5	10	50
	pCAM-Exp1-PsyB73	<i>PsyB73</i>	<i>Exp1</i>	<i>Nos</i>	5	10	50
	pCAM-ACO- PsyB73	<i>PsyB73</i>	<i>ACO</i>	<i>Nos</i>	5	10	50
	pCAM-Exp1-APsy2a	<i>APsy2A</i>	<i>Exp1</i>	<i>Nos</i>	5	10	50
Lines newly developed for release							
Williams	pOPT-A	<i>Psy1Q60</i> <i>Crt1</i>	<i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Rbcs-CTP, Nos</i>	10	1	10
	pOPT-B	<i>Psy1Q60</i> <i>Crt1</i>	<i>Exp1</i> <i>Exp4</i>	<i>Nos</i> <i>Nos</i>	10	1	10
	pOPT-C	<i>Psy1Q60</i> <i>Crt1</i> <i>Ferritin</i>	<i>Exp1</i> <i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i> <i>Nos</i>	10	1	10
	pOPT-D	<i>APsy2A</i> <i>Crt1</i>	<i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	10	1	10
	pOPT-F	<i>APsy2A</i>	<i>Exp4</i>	<i>Nos</i>	30	1	30
	pOPT-G	<i>uidA-Cat</i>	<i>Exp1</i>	<i>Cat, Nos</i>	30	1	30
	pOPT-H	<i>Psy1 Q60</i> <i>Ferritin</i>	<i>ACS</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	30	1	30
	pOPT-I	<i>Psy1 Q60</i>	<i>ACS</i>	<i>Nos</i>	30	1	30
	pOPT-J	<i>Psy1 Q60</i>	<i>Exp1</i>	<i>Nos</i>	30	1	30
	pOPT-K	<i>APsy2A</i>	<i>Exp1</i>	<i>Nos</i>	30	1	30
	pOPT-L	<i>Psy1 Q60</i> <i>Ferritin</i>	<i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	30	1	30
	pGen2-A	<i>Apsy2a</i>	<i>Exp1</i>	<i>Nos</i>	30	1	30
	pGen2-B	<i>Psy1 Q60</i>	<i>Exp1</i>	<i>Nos</i>	30	1	30
	pGen2-C	<i>Ferritin</i>	<i>Exp1</i>	<i>Nos</i>	30	1	30
	pGen2-D	<i>Ferritin</i>	<i>MT2a</i>	<i>Nos</i>	30	1	30
	pGen2-E	<i>Ferritin</i>	<i>ACO</i>	<i>Nos</i>	30	1	30
	pGen2-F	<i>Apsy2a</i>	<i>MT2a</i>	<i>Nos</i>	30	1	30
	pGen2-G	<i>Apsy2a</i>	<i>Ext</i>	<i>Nos</i>	30	1	30
	pGen2-CA	<i>Ferritin</i> <i>Apsy2a</i>	<i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	30	1	30
	pGen2-DA	<i>Ferritin</i> <i>Apsy2a</i>	<i>MT2a</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	30	1	30
	pGen2-AF	<i>Apsy2a</i> <i>Apsy2a</i>	<i>Exp1</i> <i>MT2a</i>	<i>Nos</i> <i>Nos</i>	30	1	30
	pGen2-CD	<i>Ferritin</i> <i>Ferritin</i>	<i>Exp1</i> <i>MT2a</i>	<i>Nos</i> <i>Nos</i>	30	1	30
	pGen3-EA	<i>Ferritin</i> <i>Apsy2a</i>	<i>ACO</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	30	1	30
	pGen3-H	<i>DXS</i>	<i>Exp1</i>	<i>Nos</i>	30	1	30
	pGen2-I	<i>LYCB</i>	<i>Exp1</i>	<i>Nos</i>	30	1	30
	PGen3-HI	<i>DXS</i> <i>LYCB</i>	<i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	30	1	30

pGen3-HA	<i>DXS</i> <i>Apsy2a</i>	<i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	30	1	30
pGen3-AI	<i>Apsy2a</i> <i>LYCB</i>	<i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	30	1	30
pGen3-HAI	<i>DXS</i> <i>Apsy2a</i> <i>LYCB</i>	<i>Exp1</i> <i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i> <i>Nos</i>	30	1	30
pGen3-J	<i>DXS</i>	<i>MT2a</i>	<i>Nos</i>	30	1	30
pGen3-K	<i>LYCB</i>	<i>MT2a</i>	<i>Nos</i>	30	1	30
pGen-JK	<i>DXS</i> <i>LYCB</i>	<i>MT2a</i> <i>MT2a</i>	<i>Nos</i> <i>Nos</i>	30	1	30
pGen3-JF	<i>DXS</i> <i>Apsy2a</i>	<i>MT2a</i> <i>MT2a</i>	<i>Nos</i> <i>Nos</i>	30	1	30
pGen3-FK	<i>Apsy2a</i> <i>LYCB</i>	<i>MT2a</i> <i>MT2a</i>	<i>Nos</i> <i>Nos</i>	30	1	30
pGen3-JFK	<i>DXS</i> <i>APsy2A</i> <i>LYCB</i>	<i>MT2a</i> <i>MT2a</i> <i>MT2a</i>	<i>Nos</i> <i>Nos</i> <i>Nos</i>	30	1	30
pGen2-L	<i>APsy2A</i>	<i>ACO</i>	<i>Nos</i>	30	1	30
pGen2-M	<i>Psy1Q60</i>	<i>ACO</i>	<i>Nos</i>	30	1	30
pGen2-OL	<i>APsy2a</i> <i>Crtl</i>	<i>ACO</i> <i>Exp1</i>	<i>Nos</i> <i>Rbcs-CTP, Nos</i>	30	1	30
pGen2-OM	<i>Psy1Q60</i> <i>Crtl</i>	<i>ACO</i> <i>Exp1</i>	<i>Nos</i> <i>Rbcs-CTP, Nos</i>	30	1	30
pGen4-PC	<i>Ferritin</i> <i>NAS</i>	<i>Exp1</i> <i>Ubi</i>	<i>Nos</i> <i>Nos</i>	30	1	30
pGen4-QC	<i>Ferritin</i> <i>NAS</i>	<i>Exp1</i> <i>Exp1</i>	<i>Nos</i> <i>Nos</i>	30	1	30
pGen4-RC	<i>Ferritin</i> <i>FEA1</i>	<i>Exp1</i> <i>Ubi</i>	<i>Nos</i> <i>Nos</i>	30	1	30

* All lines contain the *nptII* gene with promoter and terminator from either the CaMV 35S or the *A. tumefaciens nos* gene

5.2 Introduction to pro-vitamin A and its biosynthesis

31. Vitamin A is an essential nutrient needed in small amounts for normal functioning of the visual system, growth, differentiation, maturation, reproduction and immunity among others (FAO/WHO 2001). Vitamin A is derived from pro-vitamin A carotenoids which are synthesized by plants and many species of bacteria, fungi and archaea. Human and most animals cannot produce these carotenoids and their need for vitamin A must be met through dietary intake. Carotenoid biosynthesis genes have recently been discovered in aphids (Moran & Jarvik 2010).

32. Dietary needs of vitamin A are normally supplied by the consumption of pre-formed vitamin A (from foods derived from animals e.g. milk, meats, liver and egg yolks) and pro-vitamin A carotenoids (from foods derived from fruits and vegetables e.g. green leafy vegetables and yellow/orange fruits such as pumpkin, carrot, mango and papaya). The recommended daily intake (RDI) of vitamin A as Retinol Equivalents (RE) for adult (19+ years) men is 900 µg/day and for adult women is 700 µg/day (NH&MRC 2006).

33. Of the major pro-vitamin A carotenoids (α -carotene, β -carotene and β -cryptoxanthene), β -carotene is most efficiently converted to vitamin A (Hess et al. 2005). Some examples of the levels of β -carotene contained in commonly consumed plant (or plant-derived) foods are given in Table 4. The Fe'i banana (*Musa troglodytorum* L.) cultivar 'Asupina' that is consumed in Micronesia, and from which the *APsy2a* gene has been sourced, contains 1,412 µg β -carotene/100 g (259 RE) and meets the estimated vitamin A requirements for an adult woman within normal consumption patterns (Englberger et al. 2006a). Several other Fe'i cultivars also contain very high levels of β -carotene with 'Utin Iap' and 'Utimwas' containing approximately 8,000 µg β -carotene/100 g and 'Karat' containing approximately 2,000 µg β -carotene/100 g (Englberger et al. 2006b).

Table 4. Levels of β -carotene, retinol equivalents (RE) and iron in commonly consumed plant-based foods (value per 100g of fresh weight)*.

Food Source	β -carotene (μ g)	RE (μ g) ¹	Iron (mg)
Banana - Cavendish (peeled fruit)	64	11	0.5
Blackberry - fresh	150	53	0.4
Bok Choy (stir-fried)	692	116	2.1
Breakfast cereal (mixed grain flakes), iron-fortified	3	0	8.1
Broccoli (boiled)	273	46	0.8
Canola Oil	0	0	0
Capsicum (red, fresh)	282	215	0.3
Carrot (fresh, mature)	5,996	1,316	0.3
Cashew	6	1	5.0
Kidney bean (canned)	0	0	2.1
Lettuce - Iceberg	120	23	0.6
Mango (fresh)	2,195	366	0.3
Olive Oil	13	2	0
Pasta (fresh, white flour)	0	0	0.7
Soybean (canned)	0	0	1.8
Spinach – English (boiled)	2,201	376	3.5
Walnut	21	4	2.5

* data taken from NUTTAB 2006 (FSANZ 2006)

¹ Vitamin A intakes or requirements are expressed as Retinol Equivalents (RE) (NH&MRC 2006)

34. In higher plants carotenoids are synthesised in the plastids from five carbon isopentyl di-phosphate (IPP) units (Giuliano et al. 2008; Figure 2). In plastids IPP is generated via the methyl erythritol phosphate (MEP) pathway (Rodriguez-Concepcion & Boronat 2002; Cunnigham 2002). The first step of this pathway is condensation of pyruvic acid and glyceraldehyde-3-phosphate to form 1-deoxy-xylulose-5-phosphate (DOXP). This is the rate limiting step of this pathway and is catalysed by 1-deoxy-xylulose-5-phosphate synthase (Estevez et al. 2001; Cazzonelli & Pogson 2010). DOXP is then converted to MEP, the first pathway specific compound, through function of DOXP reductoisomerase. MEP is finally converted to IPP and its isomer dimethylallyl di-phosphate (DMPP), through several pathway intermediates.

35. IPP is the building block for terpenoids, quinones, chlorophyll, plant hormones like gibberellins, cytokinins and various pigments including carotenoids (Roberts 2007; Tanaka et al. 2008; Giuliano et al. 2008). In the carotenoid biosynthesis pathway (Cunnigham 2002; Giuliano et al. 2008) four molecules of IPP are condensed to the 20 carbon molecule geranylgeranyl di-phosphate (GGPP) in a reaction catalysed by GGPP synthase. Two GGPP molecules are further coupled by the enzyme phytoene synthase to form phytoene, the first dedicated compound of the carotenoid pathway. Phytoene is then converted into lycopene (tetra-*cis* isoform) through sequential action of phytoene desaturase and ζ -carotene desaturase. Carotenoid *cis-trans* isomerase enzymes then convert the tetra-*cis*-lycopene to all-*trans*-lycopene. Bacterial phytoene desaturase can perform all these desaturase and isomerase functions and hence alone can convert phytoene to all-*trans*-lycopene (Ye et al. 2000). All-*trans* lycopene is the substrate for two competing enzymes, lycopene ϵ -cyclase and β -cyclase. These two enzymes can act in concert to convert all-*trans* lycopene to α -carotene by addition of one ϵ -ring at one end and one β -ring at the other. Alternatively lycopene β -cyclase alone can add two β -rings, one at each end of all-*trans* lycopene, to form β -carotene (DellaPenna & Pogson 2006). The relative abundance of α - or β -carotene thus depends on the relative activities of these two enzymes.

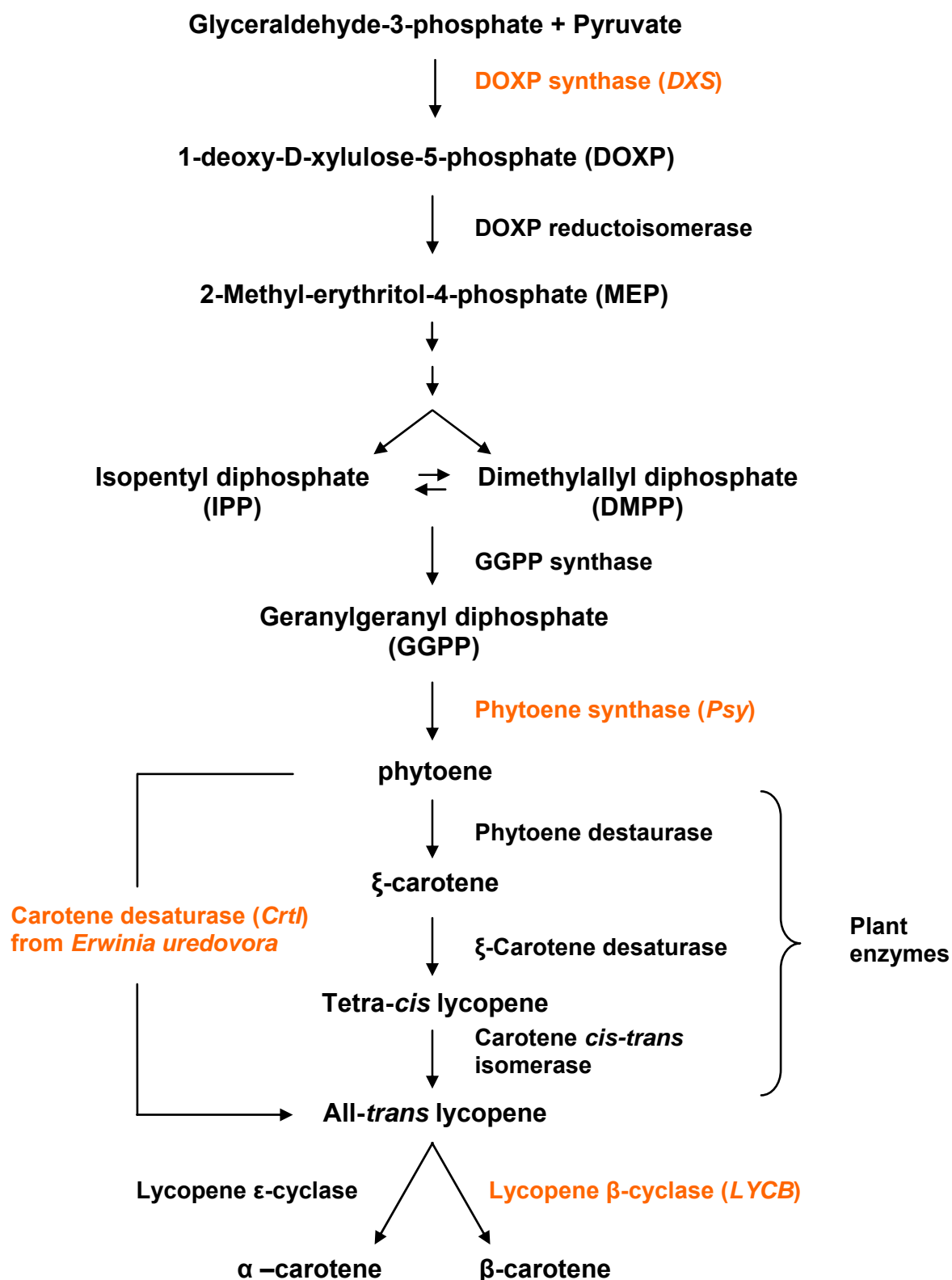


Figure 2. Biosynthesis of β -carotene.

Enzymes and encoding genes used in GM banana lines proposed for release are in orange. Refer to text (Section 5.2) for details.

5.3 Introduction to iron and its assimilation in plants

36. Iron (Fe) is an important micronutrient in all living organisms for its role in numerous cellular functions. In animals it serves as a carrier of oxygen from the lungs to the tissues by red blood cell haemoglobin; as a transport medium for electrons within cells; and as an integrated part of important enzyme systems in various tissues (FAO/WHO, 2001). At the same time excess iron has toxic effects in cells through generation of free radicals via the Fenton reaction (Hell & Stephan 2003; Palmer & Guerinot 2009).

37. Iron deficiency is a major concern to human nutrition all over the world. The RDI of iron is 18 mg/day in women aged 19 – 50 years and is 8 mg/day in adult men (19+ years) and women over 50. The upper level of intake (UL) for adult men and women is set at 45 mg/day (NH&MRC 2006).

38. Haem iron is found only in meat, fish and poultry and is absorbed much more easily than non-haem iron, which is found primarily in fruits, vegetables, dried beans, nuts and grain products (NH&MRC 2006). Some plant dietary sources of iron are given in Table 4. By comparison with these plant sources, the level of iron in lean red meat such as lamb or beef, which is regarded as the best dietary source of iron, varies from 2.2 – 3.3 mg/100 g (FSANZ 2006).

39. Due to high toxicity associated with excess iron, its uptake, transport and storage are highly regulated. One of the major ways of dealing with excess iron in cells is sequestration. Ferritin is the principal iron storage protein in all organisms which forms a nanocage capable of binding 4500 atoms of iron in its interior. When orally administered, ferritin can provide a source for iron. Over-expressing ferritin has been attempted in a bid to increase iron content in rice with modest success (Goto et al. 1999; Vasconcelos et al. 2003; Drakakaki et al. 2005). However, these studies also suggest that enhancing iron uptake as well as translocation is needed for further bio-fortification of rice with this essential mineral.

40. The main difficulty associated with iron uptake in plants is low solubility of the Fe^{3+} form prevalent in soil (Hell & Stephan 2003; Kim & Guerinot 2007). To circumvent this, non-graminaceous plants employ a reduction based strategy (Hell & Stephan 2003; Kim & Guerinot 2007). These plants release H^+ ions into surrounding soils which reduce soil pH and increase solubility of Fe^{3+} ions. Soluble Fe^{3+} is then reduced to Fe^{2+} by Fe^{3+} -chelate reductase and taken up into the cells by iron transporters. Graminaceous plants e.g. rice, wheat etc. employ an alternate chelation-based strategy (Hell & Stephan 2003; Kim & Guerinot 2007) where they secrete small molecular weight compounds known as mugineic acid (MA) to chelate Fe^{3+} in the rhizosphere. These Fe^{3+} -MA complexes are then transported by specific transporter proteins.

41. In graminaceous plants mugineic acids e.g. deoxy-mugineic acid (DMA) are derived from nicotianamine (Hell & Stephan 2003; Kim & Guerinot 2007). Nicotianamine is synthesised from S-adenosyl methionine by the enzyme nicotianamine synthase. Apart from that, nicotianamine is also a universal chelator of metal ions including iron, zinc etc. and hence plays an important role in metal translocation and homeostasis. Thus, an increase in nicotianamine through genetic modification is envisaged as another strategy to increase iron in rice (Bashir et al. 2010).

5.4 The introduced genes and their encoded proteins

42. 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.

5.4.1 The introduced genes for pro-vitamin A biosynthesis and their encoded proteins

43. These genes encode various enzymes that are involved in biosynthesis of pro-vitamin A and are expected to increase pro-vitamin A content in GM banana. Some of these genes are currently being trialled under DIR 076/2007 as indicated below.

The *DXS* gene

44. The *DXS* gene encodes the deoxy-D-xylulose 5-phosphate synthase enzyme which catalyses the first step of the MEP pathway of IPP biosynthesis and is derived from *A. thaliana*. Constitutive over-expression of the endogenous gene in *Arabidopsis* enhanced levels of many plastidic isoprenoids including carotenoids (Estevez et al. 2001). Fruit-specific expression of *E. coli DXS* gene in tomato also resulted in up to 1.6 fold increase in phytoene and β -carotene (Enfissi et al. 2005). Over-expression of *E. coli DXS* gene also enhanced carotenoid levels in potato tubers (Morris et al. 2006). In contrast, expression of the daffodil *DXS* gene in rice did not achieve the expected increase in β -carotene level (Al-Babili & Beyer 2005).

The *PsyB73*, *Psy1Q60* and *APsy2a* genes

45. Synthesis of phytoene by phytoene synthase is the first committed step of carotenoid biosynthesis and is generally accepted as the most important regulatory enzyme in the pathway (Cazzonelli & Pogson 2010). This enzyme is encoded by the phytoene synthase gene (*Psy*) and over-expression of this gene has been a successful strategy for increasing carotenoid levels in a number of crop plants.

46. The *PsyB73* gene derived from maize inbred line B73 and the *Psy1Q60* gene from maize cultivar Q60 both encode phytoene synthase enzymes and have identical amino acid sequences (Buckner et al. 1996). The *PsyB73* gene was used in development of the high β -carotene Golden Rice II (Buckner et al. 1996; Paine et al. 2005). The *APsy2a* is the phytoene synthase II gene from banana cultivar Asupina and has not been reported in the literature. *PsyB73* and *APsy2a* genes are currently being evaluated under DIR 076/2007 and preliminary data suggests that GM banana expressing either of these two genes have higher pro-vitamin A content (information supplied by the applicant). This also indicates that proteins encoded by *PsyB73* and *APsy2a* genes perform the same function.

47. Over-expression of phytoene desaturase from a number of sources, e.g. daffodil (*Narcissus pseudonarcissus*), maize, tomato, rice, has been studied for modifying β -carotene levels in rice (Ye et al. 2000; Paine et al. 2005). The maize *Psy1B73* gene was also used for increased carotenoid expression in GM wheat (Cong et al. 2009). The bacterial homolog of *Psy* gene, *crtB* from *E. uredoovora* has been used for increasing total carotenoid as well as β -carotene content in tomato (Fray et al. 1995; Fraser et al. 2002), canola (Shewmaker et al. 1999), potato (Ducreux et al. 2005) and maize (Aluru et al. 2008).

The *CrtI* gene

48. *CrtI* gene from *E. uredoovora* codes for phytoene desaturase and generates all-*trans*-lycopene directly from phytoene by combining the functions of phytoene desaturase, ζ -carotene desaturase and lycopene-*cis-trans*-isomerase (Giuliano et al. 2008). This gene will be expressed in GM banana as a fusion protein with the chloroplast targeting signal peptide of ribulose-1,5-bisphosphate carboxylase small subunit from chrysanthemum (CMSSP; Outchkourov et al. 2003) or pea (Rbcs-CTP; Coruzzi et al. 1984; Paine et al. 2005). The *CrtI* gene fused to Rbcs-CTP was employed along with *Psy* genes to enhance pro-vitamin A level in Golden Rice and Golden Rice II (Ye et al. 2000; Paine et al. 2005). A similar strategy has been successfully employed to increase total carotenoids and β -carotene levels in potato and maize and total carotenoids in wheat (Diretto et al. 2007; Aluru et al. 2008; Cong et al. 2009). This gene fused with either of the chloroplast targeting signals is currently being assessed under DIR 076/2007.

The *LYBC* gene

49. The *LYBC* (lycopene β cyclase) gene is obtained from rice and the encoded enzyme catalyses preferential conversion of lycopene to β -carotene over α -carotene (DellaPenna & Pogson 2006). However, over-expression of *LYBC* gene from daffodil was found not to be essential in increasing pro-vitamin A levels in Golden Rice as endogenous expression of this gene was

sufficient for the conversion (Ye et al. 2000; Al-Babili & Beyer 2005). In contrast, plastidic expression of this daffodil gene in GM tomato resulted in higher conversion of lycopene to β -carotene (Apel & Bock 2009). The homologous *CrtY* gene from *E. uredovora* was also used in conjunction with *CrtB* and *CrtI* to enhance β -carotene levels in GM potato, although individual contributions from these genes were not studied in detail (Diretto et al. 2007).

5.4.2 The introduced genes for increasing iron content and their encoded proteins

The Ferritin gene

50. The *Ferritin* gene from wild soybean codes for a ferritin protein identical to the ferritin from cultivated soybean (*Glycine max*). Ferritin is a major iron-storage protein in both plants and animals. In plants, the level of ferritin is regulated at the transcriptional level and hence over-expression of this gene is a straightforward strategy to increase ferritin content. The *Ferritin* gene from cultivated soybean has been used for endosperm specific over-expression in rice, resulting in up to 3-fold increase in iron content in rice seeds (Goto et al. 1999; Vasconcelos et al. 2003). There was also an accompanying increase in zinc concentration (Vasconcelos et al. 2003). However, when constitutively over-expressed in rice and wheat, iron levels were increased in leaves but not grains (Drakakaki et al. 2000).

The NAS gene

51. Some of the GM bananas in the proposed release will contain a *NAS* gene from rice. The *NAS* gene encodes the enzyme nicotianamine synthase, which catalyses formation of nicotianamine (NA), a chelator of metal ions including iron and a precursor of plant deoxy-mugineic acid (DMA), another metal ion-chelator. Over-expression of the *NAS* gene as a strategy to increase NA levels, and hence iron uptake, has been attempted in several cases. Ectopic over-expression of the *Arabidopsis NAS1* gene in tobacco resulted in higher NA concentration, higher iron, zinc and manganese concentration in leaves and better tolerance to iron deficiency (Douchkov et al. 2005). Similarly, rice plants over-expressing a barley *NAS1* gene demonstrated higher levels of endogenous NA and DMA and a 2–3 fold increase in iron and zinc levels in seeds (Masuda et al. 2009). Wirth and colleagues (2009) reported up to 6-fold increase in seed iron content in GM rice co-expressing *Arabidopsis NAS1* and bean (*Phaseolus vulgaris*) *Ferritin* genes. Endosperm specific over-expression of the endogenous *NAS1* gene in GM rice not only lead to increased iron and zinc levels in unpolished grains, but also leads to increased bioavailability of iron in *in-vitro* studies (Zheng et al. 2010).

The FEA1 gene

52. The *FEA1* gene is cloned from *C. reinhardtii*, a photosynthetic alga in which it is highly expressed under iron deficient conditions (Rubinelli et al. 2002). The encoded protein FEA (Fe assimilation) is secreted into the medium and is hypothesised to bind iron to facilitate its uptake by iron transporters (Allen et al. 2007). Over-expression of *FEA1* in *Arabidopsis* rescued lethal iron transporter mutations and also GM plants demonstrated better growth than wild type under iron deficient conditions (Chiu 2007). Similar better performance under iron-limiting growth conditions suggesting better iron uptake was observed in GM cassava expressing *FEA1* (information supplied by the applicant). GM banana plants proposed for release will contain a *FEA1* gene that is codon optimized for expression in plants. However, the amino acid sequence of the encoded protein is identical to the *C. reinhardtii* FEA1 protein.

5.4.3 The reporter gene (*uidA*) and its encoded protein (*GUS*)

53. The *uidA* gene encodes the enzyme β -glucuronidase (GUS), which is derived from the common gut bacterium *E. coli*. The active GUS enzyme is a homo-tetramer of 68 kDa protein sub-units and catalyses the hydrolysis of β -glucuronides and, less efficiently, some β -galacturonides. Hence, *E. coli* can use these compounds as its main carbon and energy source. The *uidA* gene is a widely used reporter gene in GM plants (Miki & McHugh 2004) as it allows GM tissues to be identified using a simple visual assay.

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

54. The *nptII* gene was initially isolated from the transposon Tn5 that was present in the bacterium *E. coli* strain K12 and codes neomycin phosphotransferase type II. This is an aminoglycoside phosphotransferase enzyme which catalyses transfer of the γ -phosphate group of ATP to specific hydroxyl group of aminoglycoside antibiotics e.g. kanamycin, neomycin etc. and hence detoxifies them. Plants expressing the enzyme can tolerate certain concentrations of these antibiotics which lead to bleaching and growth inhibition in non-transformed plants. The *nptII* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004).

5.5 Toxicity/allergenicity of the proteins associated with the introduced genes

55. Homologues of the introduced proteins, and proteins with similar sequences and 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.

56. The GUS enzyme is from *E. coli* which lives in the digestive tract of vertebrates, including humans (Jefferson et al. 1986). GUS activity has been described in all vertebrates, numerous bacteria and many invertebrate species (Gilissen et al 1998). A number of GM crops containing the *uidA* gene have been approved for limited and controlled release in Australia, including GM bananas (DIR 076/2007, DIR 107), GM maize (DIR 086/2008) and GM sugarcane (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 2003; FSANZ 2002).

57. All of the GM banana lines contain the *nptII* gene. 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/> 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).

58. No toxicity/allergenicity tests have been performed on the other introduced proteins or on the GM banana lines as the proposed trial is still at proof of concept stage. Such tests may need 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). Recently a small scale human nutritional trial was conducted in which five adult human subjects consumed Golden Rice-II, a GM rice engineered for increased β -carotene (Paine et al. 2005; Tang et al. 2009). Golden Rice-II expresses *PsyB73* gene from maize and *CrtI* gene from *E. uredovora* (Paine et al. 2005). No adverse effects, including allergic reactions and gastrointestinal disturbances, were noted in this trial (Tang et al. 2009).

59. 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 are used to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The applicant compared the predicted amino acid sequences of the proteins encoded by each of the genes introduced for enhanced nutrition to a database of known allergens, the Food Allergy Research and Resource Program allergen protein database AllergenOnline (<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 uses a sliding window of 80

amino acids, looking for identities greater than 35%, which is a threshold often used to highlight an allergenicity concern (Fiers et al. 2004). None of the proteins to be expressed in GM banana has any sequence/structure homology to any allergen catalogued at the AllergenOnline database.

60. 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.

5.6 Toxicity of end products associated with the introduced genes for nutrient enhancement

61. Pro-vitamin A carotenoids (such as β -carotene) and iron occur naturally in the environment by virtue of their widespread presence in plant and/or animal material. In addition, carotene forms of vitamin A and iron are permitted food additives in Australia (FSANZ 2007). On this basis, people and other organisms have a long history of exposure to these end products. While soluble iron salts present in soils can be toxic to plants, there is no indication that the forms and levels of iron found naturally in plant or animal material are toxic (Table 4).

62. The mammalian toxicity of β -carotene and iron has been independently assessed by regulatory agencies or health advisory bodies in Australia and overseas for the purposes of determining safe levels of intake via food or supplements. Assessments of these compounds has been conducted by the National Health & Medical Research Council (NH&MRC 2006), the UK's Expert Group on Vitamins and Minerals (Expert Group on Vitamins and Minerals 2003), the European Commission (EC 2000) and the Joint Food and Agricultural Organisation of the United Nations/World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA 1974; JECFA 1975; JECFA 1983; JECFA 1993). These assessments have examined both animal and human data.

63. Excess intake of preformed vitamin A (retinol) can cause hypervitaminosis A leading to birth defects, liver abnormalities, reduced mineral density in bones and central nervous system disorders. The upper intake levels (UL) for retinol is 600 $\mu\text{g/day}$ for infants up to 3000 $\mu\text{g/day}$ for lactating adults (NH&MRC 2006). However, the conversion of β -carotene to retinol in the body is tightly regulated by vitamin A status and there is not enough data to establish an UL for β -carotene (NH&MRC 2006).

64. Although β -carotenes can function as anti-oxidants, and epidemiological studies associated high β -carotene uptake with reduced risk of many chronic diseases, excess intake of β -carotene (20 mg/day) can cause some harm to smokers or people exposed to asbestos (Rao & Rao 2007; Gallicchio et al. 2008; Goralczyk 2009; Druesne-Pecollo et al. 2010).

65. A comprehensive search of the scientific literature found no information or data to suggest that pro-vitamin A carotenoids (such as β -carotene) have any toxicity potential in other organisms. However, as carotenoids can be converted to toxic cleavage products, high level of carotenoids in diets may have other negative effects particularly under oxidative stress conditions. American gold finches supplemented with high amounts of carotenoid (3000 $\mu\text{g/day}$) were more colourful, but showed more skeletal muscle deterioration and reduced flight performance during the post-supplementation period than birds receiving low doses of carotenoid supplements (30 $\mu\text{g/day}$) (Huggins et al. 2010). Thus, in the long term, maintaining high level of carotenoids for bright colouration may pose a challenge to these birds (Vinkler & Albrecht 2010).

66. There is considerable scope of iron toxicity as it can accumulate in the body and its effect can range from gastrointestinal irritation to systemic toxicity (NH&MRC 2006). This is more pronounced for people with haemochromatosis. The prescribed UL for iron is 20 mg/day for healthy infants and children (up to 3 yr), 40 mg/day for children up to 13 yr and 45 mg/day for people up to 50 years of age (NH&MRC 2006).

67. The levels of these compounds generated in the GM banana plants are not expected to exceed the levels found naturally in edible plants (Table 4). An indication of likely increases may be gained

from similar studies with other GM plants. For example, Golden Rice II which expresses *PsyB73* and *CrtI* genes in rice endosperm increased the total carotenoid content to a maximum of 37 µg/g (23-fold) of dry seed weight (Paine et al. 2005). Similar attempts in maize resulted in up to 34-fold increase in total carotenoids content (46.5 µg/g seed dry weight) as compared to parental lines and a preferential accumulation of β-carotene (13.8 µg/g seed dry weight maximum; Aluru et al. 2008). Expressing bacterial *CrtB* (phytoene synthase), *CrtI* and *CrtY* (lycopene β-cyclase) in GM potato lead to a 20-fold increase in total carotenoids (Diretto et al. 2007) and the level of β-carotene was significantly higher than parental lines (47 µg/g dry weight, 3600 fold increase).

68. Expression of soybean *Ferritin* gene in rice lead to a 2–3 fold increase in iron content in unpolished GM rice seeds (Goto et al. 1999; Vasconcelos et al. 2003). Constitutive expression of the barley *NAS* gene in rice resulted in a maximum of 4.5-fold increase in iron content in seeds (Masuda et al. 2009). When the *Ferritin* gene was expressed in conjunction with *NAS* and *Phytase* genes, it led to more than six-fold increase in iron concentration in rice seeds (approximately 7 µg/g dry weight; Wirth et al. 2009); but is still over 10-fold less than iron-fortified breakfast cereals (81 µg/g fresh weight; Table 4). Over-expression of these genes also lead to increased accumulation of zinc, manganese and nickel (Vasconcelos et al. 2003; Douchkov et al. 2005; Pianelli et al. 2005; Masuda et al. 2009).

5.7 Other effects associated with genetic modifications for enhanced nutrition

69. Many of these genes involved in β-carotene biosynthesis and described under Section 5.4.1 have been expressed in various GM plants either singly or in combination, and effects on endogenous genes and metabolic pathways have been investigated. Constitutive expression of an additional *Psy* gene in GM tomato resulted in changed expression of other genes involved in the pathway (Fraser et al. 2007). Over-expression of a *DXS* gene in potato tubers also resulted in over-expression of the endogenous *PsyI* gene (Morris et al. 2006). Re-creation of the β-carotene biosynthetic pathway in potato using *E. caratovora* genes generally resulted in down-regulation of the endogenous *PsyI* gene but induction of some other genes e.g. phytoene desaturase in carotenoid pathway (Diretto et al. 2010). In contrast, no significant changes in expression of endogenous genes involved in carotenoid biosynthesis were observed in GM wheat expressing maize *PsyI* and *E. caratovora CrtI* genes.

70. These changes in gene expression were also reflected in changes in metabolites including composition of total carotenoids (Lindgren et al. 2003; Ducreux et al. 2005). For example, in tomato, constitutive over-expression of *PsyI* gene resulted in changes in total carotenoid compositions and other metabolites generally associated with ripening processes (Fraser et al. 2007). In contrast expression of the *PsyI* gene from daffodil in rice led to an increase in phytoene but not the downstream carotenoids (Burkhardt et al. 1997). In the case of potato (Diretto et al. 2007; Diretto et al. 2010) expression of bacterial β-carotene biosynthesis genes also resulted in perturbation of leaf carotenoid metabolites. There were changes in expression levels of related pathway genes and high transcript-metabolite correlations reflecting a co-ordinated control of carotenoid biosynthesis pathways involving both positive and negative feedback mechanisms.

71. However, no novel metabolite has been reported in any of these studies and a comprehensive search of scientific literature also did not reveal any such cases.

72. Since isoprenes are also building blocks for a number of plant hormones including abscisic acid (ABA), gibberelic acids (GA) and some cytokinins as well as pigments like chlorophyll, manipulation of the carotenoid biosynthetic pathway has also resulted in changes in hormone balances and phenotypes. Constitutive over-expression of the *PsyI* gene in tomato resulted in dwarfism, loss of chlorophyll and up to 30-fold reduction in GA1 (Fray et al. 1995). Similar phenotypes were also observed in GM tobacco over-expressing endogenous *PsyI* and *Psy2* genes (Busch et al. 2002). Over-expression of bacterial *DXS* genes in potato tubers also led to significantly elongated tubers and a reduction in tuber dormancy (Morris et al. 2006). A significant

increase in *trans*-zeatin riboside, a cytokinin, was observed in these lines while GA1 and ABA levels were not significantly affected. In contrast, seed specific over-expression of *Psy1* and *Psy2* in *Arabidopsis* resulted in delayed germination and increase in total carotenoids, chlorophyll and ABA (Lindgren et al. 2003).

73. The applicant has stated that in the case of GM banana lines released under DIR 076/2007 some changes in plant phenotype e.g. leaf yellowing, appearance of necrotic spots and stunting were observed in some of the lines. Also, some GM banana somatic embryos expressing *Psy1* and/or *CrtI* genes developed orange colourations, presumably due to accumulation of high β -carotene, and failed to regenerate (information supplied by applicant).

74. Endosperm specific expression of ferritin in rice also resulted in concomitant increase in zinc (Vasconcelos et al. 2003). Similar increases in metal ions like zinc and manganese were also observed in tobacco expressing a soybean *Ferritin* gene (Yoshihara et al. 2003).

75. The mugineic acid family of metal chelators including NA can also bind to other metal ions and hence can contribute to their uptake in plants (Palmer & Guerinot 2009). Such increase in zinc content, although less pronounced than iron, was observed when *Arabidopsis NAS* and soybean *Ferritin* genes were expressed in rice (Wirth et al. 2009). Similarly, both iron and zinc levels were increased in rice seeds expressing the *NAS* gene from barley (Masuda et al. 2009). Over-expression of *NAS* also led to increased heavy metal tolerance (Douchkov et al. 2005; Pianelli et al. 2005) and paradoxically, in one case, increased sensitivity to iron deficiency which was due to decreased bioavailability of iron in NA over-accumulating plants (Cassin et al. 2009).

76. Additionally iron status of plants can influence the soil microbial community (Jin et al. 2010). Growth of tobacco plants over-expressing ferritin in plastids can lead to reduced iron availability to soil microflora, particularly in iron deficient soils, and can significantly alter the microbial community structure under such conditions (Robin et al. 2006a; Robin et al. 2006b). This led to selection of fluorescent pseudomonads that can synthesize siderophores with high affinity to iron and hence are less susceptible to iron stress (Robin et al. 2007).

5.8 The regulatory sequences

77. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct gene transcription. Also required for gene expression in plants is a transcription termination region, including a polyadenylation signal. Other sequences, such as introns and protein targeting sequences, may contribute to the expression pattern of a given gene. The regulatory sequences used in the GM banana lines are listed in Table 2 and are detailed below.

78. Although some of regulatory sequences are derived from plant pathogens (CaMV 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 (maize and castor bean) are not in themselves toxic or allergenic.

5.8.1 Regulatory sequences for expression of the introduced genes for enhanced nutrition

79. Expression of all the introduced genes are either driven by a constitutive promoter (*Ubi*) or promoters expected to provide high expression specifically in banana fruit tissues (*Exp1*, *Exp4*, *Ext*, *MT2a*, *ACO* and *ACS*).

80. The *Ubi* promoter is from the maize poly-ubiquitin gene. This is a constitutive promoter and is expected to direct the genes to be expressed in most plant tissues and throughout the plant life cycle (Christensen et al. 1992). This promoter has been extensively used to drive gene expression in a large number of GM monocotyledonous plants (Christensen & Quail 1996).

81. *Exp1* and *Exp4* promoters are derived from banana expansin genes (*Exp1* and *Exp4*; Trivedi & Nath 2004; Asha et al. 2007). Expansins are proteins that play a role in cell wall loosening and extension and their expression is differentially regulated during stages of plant growth and

development including fruit development and ripening (Choi et al. 2006). The *Exp1* gene from banana is expressed only in fruit at later stages of ripening and the transcript accumulates steadily over the climacteric ripening phase (Trivedi & Nath 2004). In contrast *Exp4* is expressed steadily in fruit throughout development and ripening (Asha et al. 2007). The promoter of the banana *Exp1* gene was used in GM banana plants release under DIR 076/2007 but no data on expression pattern or level is yet available.

82. The *Ext* promoter is derived from a banana extensin gene. The *Ext* genes code for extensin proteins which are hydroxyproline-rich glycoproteins thought to be involved in cell wall extension (Wilson & Fry 1986). In banana, one *Ext* gene was observed to be down-regulated in pulp of ripening fruit (Medina-Suarez et al. 1997), while another study reported a peel specific up-regulation of another *Ext* gene during ripening (Drury et al. 1999). The *Ext* promoter element is from the *Ext* gene from banana which is up-regulated during ripening (Drury et al. 1999).

83. The *MT2a* promoter is derived from the corresponding metallothionein (*MT*) gene in banana fruit. Metallothioneins are ubiquitous, cysteine-rich heavy metal binding proteins that play an important role in heavy metal detoxification and homeostasis. They are grouped into different classes and types based on number and arrangement of cysteine containing motifs and are encoded by a family of genes with different temporal as well as spatial expression patterns and which also respond to different environmental cues (Cobbett & Goldsbrough 2002; Hassinen et al. 2011). For instance, in banana, *MT2a* gene expression is higher in green fruits and then declines as the fruit ripens whereas *MT2b* and *MT3* genes are expressed later in ripening (Liu et al. 2002). Ethylene has an inhibitory effect on *MT2a*, but not on *MT2b* or *MT3* and only *MT3* expression is enhanced in response to heavy metals like copper, zinc or cadmium (Liu et al. 2002). Unpublished data from transient expression experiments suggest that the *MT2a* promoter can drive transgene expression in fruit (information supplied by applicant).

84. *ACO* and *ACS* promoters are derived from the banana *ACC oxidase (ACO)* and *ACC synthase (ACS)* genes. The enzymes encoded by these genes are involved in biosynthesis of the fruit ripening hormone ethylene (Do et al. 2005; Huang et al. 2006). There are at least nine members of the *ACS* gene family in banana of which *MaACS1* is strongly over-expressed in ripening banana fruits (Liu et al. 1999; Huang et al. 2006). Two members of the *ACO* gene family have been described so far and both show high expression in banana fruits during ripening (Liu et al. 1999; Do et al. 2005). Only fruit specific expression was observed when promoter regions of *ACS1* and *ACO1* genes were used to drive transient GUS expression in various tissues of banana (Wang & Peng 2001a; Wang & Peng 2001b). These promoters have been used to regulate gene expression in GM banana lines released under DIR 076/2007 but data on their expression pattern is not yet available.

85. All the introduced genes have the terminator region from *A. tumefaciens nos* gene (Depicker et al. 1982). The *nos* terminator has been used in a wide variety of constructs used for plant genetic modifications (Reiting et al. 2007).

5.8.2 Other genetic elements for expression of the introduced genes for enhanced nutrition

86. Two different plastid targeting signal sequences will be used to direct the phytoene desaturase enzyme from *E. uredovora* to chloroplasts where it functions. These signal sequences are obtained from Rubisco small subunit gene (*RBCS1*) either from chrysanthemum or pea (Coruzzi et al. 1984; Wong et al. 1992). These gene sequences will be translated but removed during post-translational modification, and hence will be absent from the mature functional protein.

5.8.3 Regulatory sequences for expression of the uidA reporter gene

87. The *uidA* reporter gene in GM bananas is driven by the *Exp1* promoter and has a downstream terminator from the *A. tumefaciens nos* gene. The *uidA* gene also contains an intron from the castor bean *catalase* gene (*Cat*; Ohta et al. 1990). 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 *Agrobacterium*. The *Cat* intron can also enhance expression of introduced genes in plants (Tanaka et al. 1990).

5.8.4 Regulatory sequences for expression of the *nptII* marker genes

88. All of the GM bananas contain the *nptII* gene under the control of the promoter and mRNA termination region of the CaMV 35S gene (Odell et al. 1985) or *A. tumefaciens nos* gene (Depicker et al. 1982). These promoters are widely used to drive constitutive expression of genes in GMOs. The 35S and *nos* terminators have also been used widely in GM plants (Mitsuhara et al. 1996; Reiting et al. 2007).

5.9 Method of genetic modification

89. *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 used 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).

90. 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.

91. 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).

92. For each transformation, embryonic cell suspensions of banana are co-cultivated with *A. tumefaciens* AGL1 carrying one of the gene constructs listed in Table 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 shade house before being transferred to soil.

5.10 Characterisation of the GMOs

5.10.1 Stability and molecular characterisation

93. Not all of the GM banana lines proposed for release have been generated prior to submission of this application. All gene constructs used for transformation have been fully sequenced (information supplied by applicant).

94. As the project is at an early stage, full molecular characterisation of the GM banana lines has not been carried out. Of the lines proposed for release, lines previously released under DIR 076/2007 were screened for the presence of the introduced genes using PCR. These plants were also tested for presence of *Agrobacterium* by PCR using primers specific to non-T-DNA

regions. As the remaining GM banana lines are being generated, they will also be screened for the introduced genes as well as *Agrobacterium* by PCR and no plant testing positive for *Agrobacterium* will be released.

95. Southern hybridisation for further confirmation and copy number determination have been performed with only some of the lines previously released under DIR 076/2007 and intended for further evaluation under the proposed release. From one up to 11 copies of the introduced genes have been detected in the lines tested.

96. The exact location of the inserted genes within the banana genome is not known. *A. tumefaciens* inserts genetic material into plant genomic DNA via illegitimate recombination, which can potentially result in insertion of the introduced genes 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.

5.10.2 Characterisation of the phenotype of the GM banana lines

97. The purpose of the trial is to assess pro-vitamin A and/or iron levels in the GM banana fruit and agronomic performance of GM banana plants grown under field conditions. This would help identifying gene(s) and promoter(s) combinations that will enhance nutritional quality of banana fruits without the associated negative effects on GM plant growth and development observed in a previous trial. The applicant states that it is not possible to grow numerous large banana plants until fruiting in a glasshouse to assess pro-vitamin A and iron contents in fruit or to study the phenotypes of such large plants. Such nutrient content and phenotypic data will be collected during the proposed trial.

98. Most of the GM banana lines proposed for release are being developed and have not been phenotypically characterised. Of the GM banana plants released under DIR 076/2007, some over-expressing *APsy2a* or *PsyB73* genes showed higher pro-vitamin A levels. However, some of these lines also showed leaf yellowing, stunting and development of necrotic spots. The lines selected for further evaluation under the proposed release have higher pro-vitamin A content but no adverse effect on growth or development. Nutrient content and phenotypic data for GM banana lines containing the *CrtI* and *Ferritin* genes are not yet available. However, almost all of the GM banana lines proposed to be released are expected to over-express the genes for pro-vitamin A biosynthesis and/or iron homeostasis in a fruit specific manner. This is intended to mitigate some of the negative effects e.g. chlorosis, stunting observed in GM banana plants trialled under DIR 076/2007. The applicant states that the GM banana plants will be monitored for aberrant phenotypes during the proposed trial.

Section 6 The receiving environment

99. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs 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

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

101. The release is proposed to take place at one site in the SJRS, a property of DEEDI, in the Shire of Johnstone, QLD.

102. The applicant proposes to locate the GM banana trial site 250 m from the South Johnstone river, the nearest natural waterway. The proposed release site is 18 m above sea level and the

highest flood level recorded at the closed flood gauge is 11 m in 1967 (10.5 m in 2011; <http://www.bom.gov.au/hydro/flood/qld/brochures/johnstone/johnstone.shtml#PreviousFlooding>). 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 and does not abut any public road.

103. The town of Innisfail and surrounding area including SJRS were affected by Cyclone Yasi in January 2011. GM banana plants released under DIR 076/2007 growing on a site adjacent to the proposed release site were severely damaged. However, none of the plants or suckers was uprooted and carried away by the cyclonic wind (information supplied by applicant). This was confirmed during an OGTR monitoring visit to the trial site in February 2011.

6.2 Relevant biotic factors

104. 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 within a major banana growing region. The closest population centre to the proposed release site is South Johnstone (1 km), which has a population of approximately 200–300 people. The town of Innisfail is approximately 12 km from the release site and has an estimated population of 15,000 people.
- ♦ This region is in the tropical rainforest climatic type (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology) in which 70% of Australia's banana production occurs (Biosecurity Australia 2007). Other major agricultural activities around the site are growing sugarcane and cattle.
- ♦ The proposed release site is within a research station in which other GM bananas released under DIR 076/2007 are grown as well as non-GM and native bananas; but none for commercial purposes. There are some 200 *Musa* spp. accessions growing as part of a germplasm collection at the Research Station in which the proposed site is located. Under QLD State legislation these are not permitted to fruit or produce male flowers. The GM banana lines released under DIR 076/2007 are either not allowed to flower or the flowers are bagged to prevent any pollination.
- ♦ Native *Musa* species are present in QLD and the nearest observed population of native bananas is approximately 10 km from the site (information supplied by the applicant).
- ♦ 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
 - ♦ feral pigs have access to the site but, while they have been observed knocking down and eating small plants, there is no evidence that they uproot plants, detach suckers or eat fruit that is still on the plant. The applicant states that trapping to control the pigs commenced at the Research Station in January 2008. There are no domestic livestock at the Research Station on which the proposed site is located.

6.3 Relevant agricultural practices

105. The cultivation and movement of banana planting material is regulated in QLD by Biosecurity Queensland within DEEDI (<http://www2.dpi.qld.gov.au/health/14775.html>).

106. 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).

107. 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 a field location. This is a separate, lockable building located within the research station and approximately 500 m from the proposed field location.

108. The applicant also proposes to use suckers from some of the GM banana lines released under DIR 076/2007. These suckers will be transported from the current field location to the shadehouse mentioned above, air-dried for 2–3 days before transferring back to a new field location.

109. Any excess whole plant or sucker from the shadehouse will be destroyed either by herbicide treatment or shredding and decomposition in a closed container. After destruction, such material would be placed on the ground at the locations.

110. 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 are non-propagative and would be left on the ground at the field location to decompose. Waste non-propagative plant material from the shade house would also be placed on the ground at the field location for decomposition.

111. 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.

112. It is intended that the GM bananas be grown through to fruiting to allow assessment of pro-vitamin A and iron content in fruit. 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).

113. GM banana fruit harvested for further analysis will be packaged for transport to PC2 facilities in QUT. The fruit may also be stored temporarily prior to transport. Packaging and temporary storage of GM banana material will take place in a lockable packaging shed and cold room approximately 300 m from the proposed release site.

114. Harvested fruit will be transported to PC2 facilities and destroyed after assessment. Any excess fruit at the locations or the packing shed would be shredded and placed on the ground at a field location.

115. 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 the site monitored for volunteer banana plants for a period of at least 12 months.

6.4 Presence of related plants in the receiving environment

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

⁷The 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’.

117. 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 QLD. The applicant states that the nearest observed naturally occurring population of this species is approximately 10 km from the proposed site at South Johnstone. Also native vegetation consistent with the type of community in which *M. acuminata* subsp. *banksii* is found occurs much closer to the site. Plantings of *M. acuminata* subsp. *banksii* are also present in a field collection at SJRS.

118. *M. acuminata* subsp. *banksii* has the potential to cross with cultivated triploid and tetraploid cultivars with a *M. acuminata* background (such as the cultivar ‘Williams’). However, the applicant has stated that there are no reports of seed formation in commercial banana plantations growing in northern QLD where the native species are in close proximity. Further, under compliance with the State legislation for banana disease control (Queensland Government 1999), *M. acuminata* subsp. *banksii* grown at SJRS are not allowed to flower.

119. *M. jackeyi* has been found in Bellenden Ker (approximately 50 km north of South Johnstone) and Cooktown (approximately 350 km north of South Johnstone; information supplied by the applicant).

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

120. The genes and regulatory sequences introduced in the GM banana lines for nutrient enhancement were originally isolated from naturally occurring organisms that are already widespread and prevalent in the environment.

121. All of the genes introduced in GM banana are derived from plant or bacterial species widely occurring in the environment. Three of these species are the common crop or fruit plants rice, maize and banana; wild soybean (*G. soja*) is closely related to cultivated soybean (*G. max*) and *Arabidopsis thaliana* is a well characterised common plant used in experimental studies.

122. Humans, herbivores and microorganisms would encounter the *CrtI* gene from *Erwinia uredovora* by consumption of fruits and a vegetable infected with the bacterium and also, as the bacterium exists in soil, from exposure to soil/soil water splashes. As the gene codes for an enzyme that is involved in pro-vitamin A carotenoid synthesis, the biosynthetic products of the action of the enzyme would be essentially similar to those produced in plant pro-vitamin A carotenoid synthesis and hence would be routinely encountered by humans, herbivores and microorganisms.

123. The *FEA1* gene is obtained from *C. reinhardtii*, a unicellular green algae widely distributed in soil and fresh water and a well characterized organism extensively used in laboratories. A database search identified *FEA1* homologues in *Volvox carteri* f. *nargiensis* (multicellular green fresh water algae) and *Chlorococcum littorale* (unicellular marine algae). Hence, humans, herbivores and microorganisms would also encounter the FEA1 protein from consumption of fruits and vegetables contaminated with such algae and/or from exposure to soil/soil water splashes.

124. The biosynthetic pathway for pro-vitamin A carotenoids is ubiquitous in photosynthetic organisms and most plants contain *NAS* and *Ferritin* genes involved in iron uptake and transport. Thus the introduced genes for enhanced nutrition (except *CrtI* and *FEA1* genes) are most likely orthologous to genes in all other plants. Therefore, it is expected that humans, herbivores/omnivores and microorganisms routinely encounter the introduced genes and their gene products, or their homologues, through contact with plants and food derived from plants. As shown in Table 4, the levels of pro-vitamin A and iron that occur in a range of plant parts and plant-derived products can be high; these plant parts are consumed by humans and animals without harm.

125. 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.

126. The *uidA* reporter gene is also derived from *E. coli*. GUS enzyme activity has been detected in numerous microbial, plant and animal species including humans (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.

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

127. Previously QUT received approval for limited and controlled release of GM banana lines modified for enhanced pro-vitamin A, vitamin E and iron content under the licence DIR 076/2007. These lines were derived from Cavendish and Lady Finger cultivars and some of them express *APsy2a*, *PsyB73*, *CrtI* and *Ferritin* genes, singly or in combination under control of *Ubi*, *ACO*, *ACS* or *Exp* promoters. The approved GM banana lines were released at one site at the same research station, SJRS in QLD on up to 1.4 ha in May 2008. Sixty one of these previously released lines are intended for another release under the current proposal. There has been no release of the remaining GM banana lines in Australia.

128. The Regulator also issued two licences DIR 079/2007 and DIR 107 to QUT for limited and controlled release of banana genetically modified for disease resistance. Plants under DIR 079/2007 were trialled at one site of up to 1.4 ha in SJRS, QLD between July 2008 and April 2010. Under DIR 107 GM banana lines were approved for release at one site of up to 1.5 ha in the Northern Territory between January 2011 and November 2014.

7.1.2 Approvals by other government agencies

129. 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.

130. 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.

131. 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.

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>>.

7.2 International approvals of GM banana

132. There has been no release of these GM banana lines or other banana lines modified for nutrient content internationally. However, there have been some international field trials of GM banana lines modified for traits including disease resistance⁹ including one study in the USA (Vishnevetsky et al. 2011).

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

Chapter 2 Risk assessment

Section 1 Introduction

133. 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 3). 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.

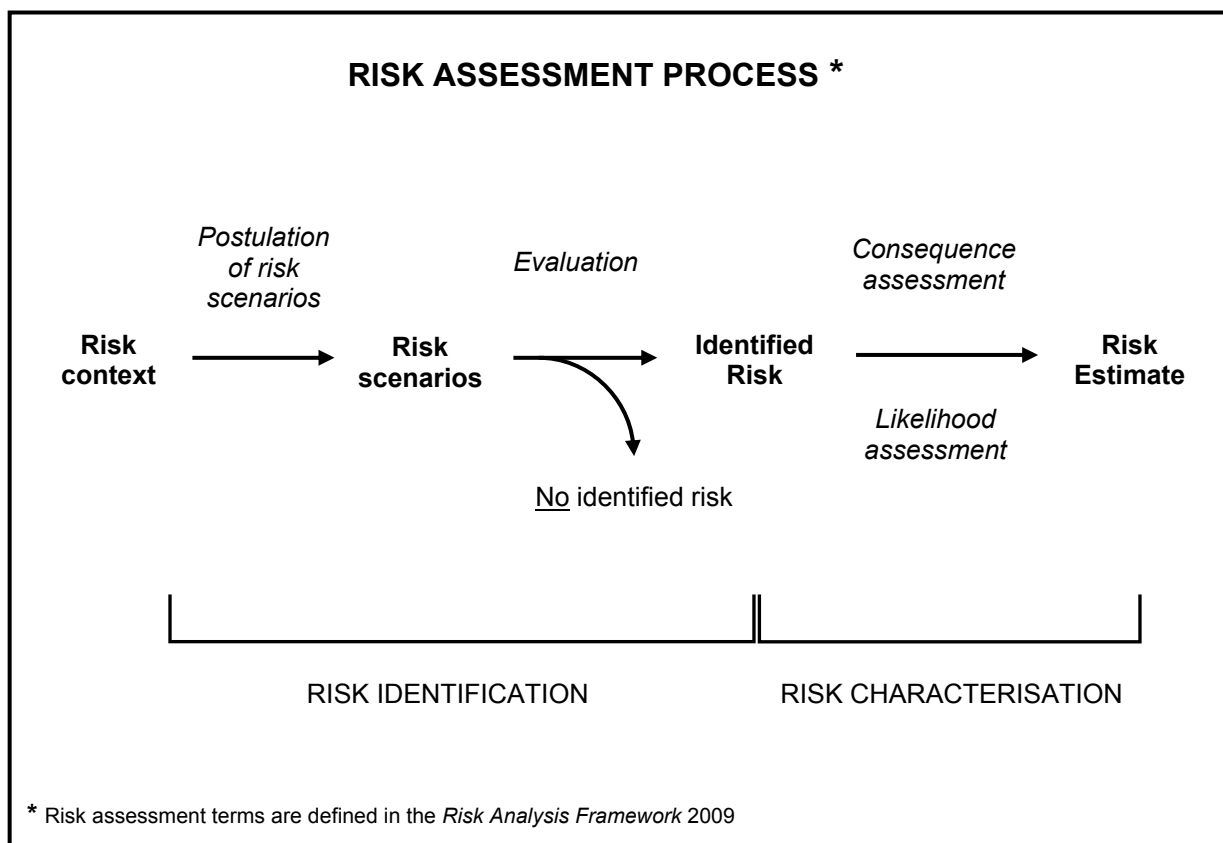


Figure 3. The risk assessment process.

134. 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).

135. 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.

136. 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.

137. 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

138. 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.

139. Seven risk scenarios were identified and evaluated. These are summarised in Table 5, 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.

140. As discussed in Chapter 1, Sections 5.4.3 and 5.4.4, 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.

141. All of the introduced regulatory sequences are derived from common plants, bacteria and a virus. Similar regulatory elements are naturally present in banana and the introduced elements are expected to operate in similar ways as endogenous ones. Therefore, although the transfer of introduced regulatory sequences to other sexually compatible plants could result in unpredictable effects, the impact is not likely to be greater than that arising from transfer of endogenous regulatory elements. Hence, these potential effects will not be further assessed for this application.

142. The potential for horizontal gene transfer and any possible adverse outcomes has been reviewed in literature (Keese 2008) as well as assessed in many previous RARMPs (most recently for GM banana in DIR 107; available at <http://www.ogtr.gov.au> or by contacting the OGTR). No risk was identified as the genes are already present in the environment and available for transfer via demonstrated natural mechanisms. This is also the case for the genes to be introduced in the GM banana for this application so this risk category will not be assessed further.

Table 5. Summary of risk scenarios from dealings with GM banana genetically modified for enhanced nutrition.

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 and the metabolites produced by them, where the introduced gene codes for an enzyme, 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 commercial 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. The genetic modifications increasing the ability of the GMOs to persist at the proposed trial site/s beyond the proposed DIR.	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.
	3. The genetic modifications increasing the ability of reproductive GM plant material to spread and/or persist outside the proposed release site.	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 or other specified conditions.
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 Unintended changes in biochemistry, physiology or ecology	5. 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 be detected and eliminated during the trial. Unintended adverse effects, if any, would be minimised by the proposed limits and controls.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.5 Unintended presence in the environment of <i>Agrobacterium tumefaciens</i> containing the introduced genes	6. 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 plant found to contain <i>Agrobacterium</i> will not be released. The GM <i>Agrobacterium</i> used is disarmed and can no longer lead to the formation of crown galls on plants, therefore any infection would be limited to individual cells and there would be no production/regeneration of whole GM plants. The introduced genes or similar genes and regulatory sequences are already present in the environment. Risk Scenarios 1–3 were not considered to give rise to identified risks.
Section 2.6 Unauthorised activities	7. 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

143. 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).

144. 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).

145. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced genes for enhanced nutrition. 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) or indirectly through the food chain.

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

146. Expression of the introduced genes for enhanced nutrition 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.

147. In the context of the proposed dealings, both of the following requirements would have to be met for GM banana to have any toxic or allergenic effect:

- the genetic modification would have to result in production of toxic or allergenic proteins or compounds not present in commercially grown banana varieties, and

- humans or other organisms would have to be exposed to the GM banana plants through contact, ingestion or inhalation.

148. 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.

149. The introduced genes for enhanced nutrition were isolated from naturally occurring organisms that are already widespread and prevalent in the environment e.g. common food plants including banana and microbial organisms from soil and water (Chapter 1, Section 5.4). Therefore, people and animals are exposed to the same or similar proteins through their diet and the environment.

150. Although no toxicity or allergenicity studies have been performed on the GM banana plant material, no information was found to suggest that the proteins encoded by the introduced genes are toxic or allergenic to people or to other organisms (Chapter 1, Section 5.5).

151. Based on the information available on occurrences and functions of the introduced proteins (Chapter 1, Section 5.4), it is not expected that any novel products would be produced as a result of expression of the introduced genes in GM banana.

152. The proposed limits and controls of the trial (Chapter 1, Section 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 applicant's proposal to bag fruits and to either bag or remove male inflorescences.

153. The proposed trial site is within a property that is surrounded by a fence and sign posted for authorised entry only. 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 banana plant material will be permitted to enter commercial human food or animal feed supply chains. In the future, some GM banana fruits from this trial may be used for small scale, controlled human nutritional studies. However, any such experiment will be conducted, subject to approval by OGTR, under a separate licence and hence is not considered further in this assessment.

154. 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 (2011–2013) and small size (up to 2.0 ha) of the proposed trial would also limit the potential for exposure to the GM plant material.

155. **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 Potential for spread and persistence of the GM banana plants in the environment

156. This section addresses the question of whether or not the proposed dealings with the GMOs may lead to harm to human health and safety or the environment as a result of an increased potential for spread and/or persistence due to the genetic modification.

157. All plants have the potential to lead to harm in certain environments. Harms that may arise from a certain plant species in a particular environment include:

- adverse effects on the health of people and/or animals
- reduction in the establishment, yield and/or quality of desired plants
- restriction in the physical movement of people, animals, vehicles, machinery and/or water

- adverse effects on environmental health, such as adverse changes to strata levels, nutrient levels, fire regime, soil salinity, soil stability, or by providing food and/or shelter to pests, pathogens and/or diseases.

158. For the purpose of this document, plant species causing significant levels of one or more of these harms are called ‘weeds’. A plant species may be weedy in one or more land uses, such as cropping or nature conservation.

159. Characteristics that influence the spread (dispersal of the plant or its genetic material) and persistence (establishment, survival and reproduction) of a plant species impact on the degree of its invasiveness. These characteristics include the ability to establish in competition with other plants, to tolerate standard weed management practices, to reproduce quickly, prolifically and asexually as well as sexually, and to be dispersed over long distances by natural and/or human means. The degree of invasiveness of a plant species in a particular environment gives an indication of the likelihood of its weediness in that environment. In addition to local experience, a history of weediness overseas can be used as an indicator for weediness in Australia.

160. Baseline information on the weediness of banana, including factors limiting the spread and persistence of non-GM banana plants, 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.

161. Scenarios relating to altered spread and persistence of the GM bananas, compared to non-GM bananas, include:

- the genetic modification enabling the GM bananas to persist at the release site beyond the proposed dealings, leading to an increased level of harm relative to non-GM banana varieties
- the genetic modification enabling reproductive GM plant material to spread outside the proposed release site, and to persist in the environment leading to an increased level of harm relative to non-GM banana varieties.

Risk Scenario 2. The genetic modification increasing the ability of the GMOs to persist at the proposed trial site beyond the proposed dealings

162. If the genetic modifications were to provide the GM banana plants with a selective advantage, relative to non-GM banana varieties, and if they were to persist at the proposed trial site, this would increase exposure of the environment, including people and other organisms, to the GMOs. This may give rise to an increase in the level of one or more of the potential harms associated with weeds, relative to non-GM banana varieties. Persistence may also provide increased opportunity for the GMOs to be dispersed beyond the release site.

163. An increase in the level of harm relative to commercially grown banana varieties could only occur where a plausible pathway to harm exists. For this to occur in the context of the proposed dealings, both of the following requirements would have to be met:

- the genetic modification would have to provide the GMOs with a selective advantage, relative to commercially grown banana varieties
- the GM plants would have to be able to persist at the proposed trial site leading to some harm.

164. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of the proposed dealings has been considered in risk scenario 1 and was not identified as a risk that warrants further assessment.

165. As the proposed release is for early stage research, the potential of most of the GMOs to have a selective advantage (e.g. increased rates of establishment, survival and reproduction) relative to non-GM bananas has not been investigated and is an area of uncertainty. In a previous trial under DIR 076/2007 some GM banana lines engineered for increase levels of pro-vitamin A displayed some stunting, leaf yellowing and necrosis, characteristics likely to reduce their potential for persistence.

166. As discussed above, commercial banana cultivars do not possess characteristics that are usually associated with weediness nor are they considered as weeds. Based on information on functions of introduced proteins (Chapter 1, Section 5.4), those involved in increasing the content of pro-vitamin A are not expected to provide any selective advantage to the GM banana plants. GM banana plants with increased level of NAS, ferritin or those expressing the FEA1 protein may have some advantage under iron limiting conditions due to their enhanced ability to take up and store iron. There is no information available on the soil iron content at the field locations at the release site.

167. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the persistence of the GMOs regardless of any selective advantage that they might have under iron limiting conditions. The applicant proposes a number of control measures, including: destruction of all plant materials not required for further analysis; post harvest monitoring of the trial sites for at least 12 months and destruction of all volunteer banana plants. Suitability of the proposed limits and controls are assessed in Chapter 3.

168. **Conclusion:** The potential for an increase in the level of harm as a result of the genetic modification for enhanced nutrition enabling the GM banana plants to persist at the trial sites is not identified as a risk that warrants further assessment.

Risk Scenario 3. The genetic modification increasing the ability of reproductive GM plant material to spread and/or persist outside the proposed release site

169. If the GM banana lines were to be dispersed from the release site, and persist in the wider environment, this could increase exposure of the environment, including people and other organisms. Such exposures could lead to an increase in the level of one or more of the potential harms associated with weeds, relative to non-GM banana varieties.

170. To realise any increase in the level of harm relative to non-GM bananas as a result of spread and persistence of the GMOs outside the trial site in the course of the proposed dealings, either of the following requirements would have to be met:

- the GMOs would have to be able to spread from the trial site, with or without persistence in the wider environment
- the presence of the GMOs in the wider environment would have to lead to some harm.

171. Note that the potential for increased allergenicity in people or toxicity in people and other organisms as a result of the proposed dealings has been considered in risk scenario 1 and was not identified as a risk that warrants further assessment. Additionally, risks that may arise through gene flow via pollen are not considered in this risk scenario as they are addressed in risk scenario 4.

172. As the proposed release is for early stage research and this is the first field trial with most of the GMOs, the potential of the GMOs to spread and persist outside the trial site has not been investigated and is an area of uncertainty.

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

174. Dispersal of other plant material could occur via the activity of frugivores or other animals, or through extremes of weather such as flooding or cyclones. 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.

175. Control measures proposed by the applicant as well as the proposed limits of the release will minimise dispersal outside the trial site (Chapter 1, Section 3.3). GM plant materials will be transported in accordance with the Regulator's transportation guidelines or other specific conditions. Only plant materials needed for experimentation will be transported outside the site and will be contained to prevent any loss of material.

176. 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.

177. 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.

178. Feral pigs and water buffalo can damage plants at ground level. But the applicant states that they have not been recorded uprooting plants or detaching suckers on the land at the site. 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.

179. 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 proposed release site is not prone to heavy run-off or landslips. Dispersal of vegetative propagules has not been observed for other trials of GM banana due to extreme weather conditions (eg. DIR076/2007 and cyclone Yasi - information supplied by the applicant and further verified by OGTR monitoring activities).

180. Based on information on functions of the introduced proteins (Chapter 1, Section 5.4), the genetic modifications are unlikely to impart any selective advantage or characters associated with weediness to the GM banana lines that would improve their persistence outside the proposed trial site over non-GM banana. GM banana plants with increased capacity to take up and store iron may have some advantage over commercial banana under iron stress conditions. The cultivation of banana in QLD is strictly regulated under the state government legislation, which would impose measures to manage any persistence of the GM banana outside the release site.

181. **Conclusion:** The potential for an increased level of harm due to the spread of reproductive GM plant material and persistence of the GMOs outside the trial site is not identified as a risk that warrants further assessment.

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

182. 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 neighbouring crops, plants, related weeds or native plants (Glover 2002).

183. 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.

184. 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

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

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

187. 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.

188. Other *Musa* species growing as part of a germplasm collection at the site are not permitted to flower restricting any possibility of cross-fertilization with GM banana.

189. The nearest sexually compatible native *Musa* species is 10 km from the proposed release site. It should be noted that blossom foraging bats of *Pteropus* spp., which could feed on the flowers of *Musa* spp., could carry pollen that becomes trapped in their head fur as far as 50 km (Eby 1995).

190. However, the likelihood of this occurrence posing a risk with regard to gene flow involving pollen from GM banana flowers is very low because of the low viability of the pollen produced by Cavendish flowers (Fortescue & Turner 2004) and the proposed control measures to remove or bag any male flowers (Chapter 1; Section 3.3).

191. The likelihood of vertical gene transfer occurring will be further reduced by the close monitoring of the GM banana plants during the proposed trial. In addition, limits on the size, location and duration of the proposed trial would minimise the likelihood of vertical gene transfer occurring. 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.

192. Even in the extremely unlikely event of vertical transfer of the genes from GM to non-GM banana or any sexually compatible species, these genes are expected to behave in similar ways as in the GM banana. As discussed under risk scenarios 1 and 2, these genes are unlikely to produce any toxic and/or allergenic substance or any increase in weediness in recipient plants.

193. **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 Unintended changes in biochemistry, physiology or ecology

194. 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

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

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.

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

196. 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.

197. As discussed under Chapter 1, Section 5.7 some physiological and physical abnormalities may arise due to genetic modification of banana plants for nutrient enhancement. Limited data is available on the phenotype of the GM banana plants as the project is in early stages. Phenotypic data is available for only a limited number of GM banana lines modified for enhanced nutrition. Some of these lines showed stunting, yellowing and development of necrotic spots while the rest were normal. The purpose of the proposed trial is to gather additional phenotypic data from GM banana lines modified for enhanced nutrition and to select plants which have higher pro-vitamin A and/or iron content but which are otherwise normal.

198. These genetic modifications can also lead to changes in composition of total carotenoids, and may alter expressions of genes and/or alter levels of metabolites from interrelated pathways (Chapter 2; Section 5.7). However, all these pathway intermediates and metabolites are already present in nature and animals and humans are already exposed to them. Since the introduced genes and their functions are well characterised, it is highly unlikely that any novel compound will be produced as result of these modifications.

199. If metals such as manganese, zinc and cadmium are already present in the soil at the field trial location, the expression of the introduced genes for enhancement of iron may result in elevated levels of these metals in banana tissues if the iron transport proteins are not specific (Chapter 1, Section 5.7). Currently there is no data available for effects of expressions of these genes in banana. Levels of copper, zinc, cobalt, magnesium, manganese, nickel, lead, mercury, cadmium, chromium and aluminium in banana tissues will be measured as part of the data collected from the trial.

200. Iron over-accumulating plants can, under iron limiting conditions, lead to further depletion of soil iron, and thus modify the soil microbial population (Chapter 1, Section 5.7). There is no data available for such a scenario under field condition with GM plants. However, under the proposed release, iron accumulating GM plant materials will be returned to the soil and thus can replenish the iron.

201. *A. tumefaciens* inserts the introduced genes randomly in the plants genome and the outcome of such random insertion event 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, significant unexpected alterations that occur as a result of the genetic modification process are likely to be detected, and other breeding techniques (such as intervarietal hybrids) also induce abundant unexpected changes (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).

202. Adverse effects, if any, arising due to inadvertent changes in biochemistry, physiology or ecology will be minimised by the proposed limits on trial size and duration, and by proposed control measures.

203. **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.

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

204. *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. 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).

205. *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).

206. 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.

207. 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.

208. 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 6. Transfer of the introduced genes from *A. tumefaciens* to other organisms

209. Release of GM banana plants carrying GM *A. tumefaciens* into the environment could result in the transfer of genes to non-target plants or other microorganisms, particularly bacteria and yeast naturally present in the soil at the release site (Leifert 2000; Hammerschlag et al. 2000). For GM banana plants to cause any harm to the receiving environment via *A. tumefaciens*, the GM banana plants released into the environment must carry viable *A. tumefaciens* cells containing the binary vector (plasmid), and the *A. tumefaciens* must then cause harm to the environment, eg through transfer of the introduced genes to non-target organisms.

210. The GM banana lines proposed for release were generated by *Agrobacterium*-mediated transformation following the method of Khanna et al. (2004) (see Chapter 1, Section 5.9). 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.

211. 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 was found during this screening. The applicant states that any plants found to contain *Agrobacterium* will not be released.

212. Even if viable 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 is disarmed and 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 whole GM plants.

213. 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 1–5). Homologues of the introduced genes, or proteins with similar function, are already widespread in plants and other organisms and are thus already available for transfer from these sources.

214. **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.

2.6 Unauthorised activities

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

215. 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.

216. **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

217. 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.

218. Seven 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.

219. 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.

220. The characterisation of the seven 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 permitted to enter commercial human food or animal feed supply chains
- 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.

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

222. 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.

223. 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

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.

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.

224. For DIR 109, 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 5, associated with the potential for any unintended effects as a result of changes to biochemistry, physiology or ecology of the GM banana plants.

225. 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.

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

Chapter 3 Risk management plan

Section 1 Background

227. 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.

228. 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.

229. 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.

230. 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

231. 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. These risk scenarios were considered in the context of the scale of the proposed release (a maximum area of 2.0 ha on one site between August 2011 and August 2013), the proposed containment measures (Chapter 1, Section 3), and the receiving environment (Section 6). 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. Therefore, no conditions are imposed to treat these negligible risks.

Section 3 General risk management

232. 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 of this Chapter.

3.1 Licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by QUT

233. 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 seven risk scenarios characterised for the proposed release in Chapter 2. The appropriateness of these controls is considered further below.

234. The proposed release will be limited to a maximum of 2.0 ha on one site in the Johnstone Shire (QLD) and the duration of the proposed release will be limited to two years. Only staff with appropriate training will be allowed to deal with the GMOs.

235. In the future, the applicant intends to conduct a small scale controlled human nutritional study with pro-vitamin A and/or iron rich GM bananas from this trial. This will require a separate application and assessment and be subject to approval by the Regulator. The GM bananas will not be permitted to enter the commercial human food or animal feed supply chains. 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).

236. The proposed trial site is located within a QLD government owned research station. The proposed field locations are at least 250 m from the nearest waterways, level and not prone to flooding, heavy runoff or landslips, which reduce the likelihood of plant material being washed away from the site. 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. These measures will limit scope for the GM bananas to disperse and establish outside the proposed release site (Risk Scenario 3).

237. 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>) or any transport plan approved by the Regulator. The guidelines are standard protocols for the handling of GMOs to minimise exposure of human and other organisms to the GMOs (Risk Scenario 1) and dispersal into the environment (Risk Scenario 3). Other proposed transport plans are discussed below.

238. Most of the GM banana plants will be generated in certified PC2 facilities. These GM plants will be transported to the site according to the Regulator's guidelines. These plants will be hardened at the shadehouse before planting at the field locations. Hardened banana plants growing in pots will be transported from the shadehouse to the field locations in open trailers. This is a distance of approximately 500 m. All the plants will also be accounted for at the field locations. The whole process will be carried out by trained staff and is appropriate to prevent any dispersal of whole banana plants during transport (Risk Scenario 3). This method of transport is currently in place for other banana trials at SJRS, QLD (DIR 076/2007) and in Northern Territory (DIR 107).

239. Some of the GM lines currently being trialled under DIR 076/2007 will be re-assessed under the DIR109 licence. These lines will be propagated from suckers and/or plants generated from parental lines through tissue culture. The micropropagated plants will be treated like newly developed GM lines for transport, hardening and planting (as described above).

240. For lines propagated through suckers, suckers will be removed from GM banana plants growing in the adjacent site and transported in closed, labelled containers to a wash area dedicated for washing of machinery used in the trial. The suckers will then be transported to the shadehouse used for hardening of tissue cultured plants. The suckers will be air-dried for approximately 24–48 hr and then transported back to the release site. The suckers will be planted deep into soil with no part visible and the position of each sucker marked at the location as well as on a site map. At all stages of the operation all the suckers will be accounted for by appropriate labelling and numbering. The whole process will be carried out by trained staff at all stages. This will limit any potential for dispersal of the GM banana lines outside the trial area and potential exposure to animals or humans (Risk Scenario 3).

241. 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 research station. This will also limit the potential for

exposure of human or other organisms to GM banana (Risk Scenario 1) as well as that of spread and dispersal of GM banana beyond the limits of the release site (Risk Scenario 3).

242. 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.

243. The covering of bunches is a standard practice used in commercial 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.

244. 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 at the field locations that is not required for experimental analysis would be destroyed by shredding (or another approved method) 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 QLD (DIR 076/2007) and Northern Territory (DIR 107) 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).

245. 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) and suckers should additionally be sprayed with herbicide, so as to destroy the corm, before being transferred to the field location. Alternatively, they may be destroyed by shredding and decomposition in closed decomposition bins. 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).

246. Fruits required for analysis will be transported to the packing shed in closed containers and packed into sturdy plastic garbage bags and sealed. These bags will further be placed in plain cardboard cartons to avoid any mis-identification with commercially grown bananas which are transported in readily identifiable cartons. The cartons will be sealed and labelled to indicate that they contain GM plant material for research purpose only and are not suitable for human or animal consumption. Packaged fruit may be stored temporarily within the packing shed. The packing shed will not be used for any other purpose while the GM banana fruit is being packed. Once packed, the packing benches will be cleaned of all plant material which, along with any excess fruit, will be destroyed and left to decompose on the ground at the field locations. The whole operation will be carried out by trained staff.

247. Packed fruit may be transported via courier services. The applicant proposes to inform the intended recipient that transport has commenced once the packages are picked up by the courier

service. All the packages will be accounted for at the destination. Additionally, the package labels will contain name and contact number of the licence holder and intended recipient with instructions to contact them as soon as possible in case any package is misplaced or damaged during transport. Banana fruit is not a propagative plant material. Further, transport in secure packages, label information and tracking mechanisms will limit potential exposure of human and other organisms to GM banana fruit or their dispersal during transport (Risk Scenarios 1 and 3).

248. 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 wet season 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 minimise the persistence of the GMOs in the environment (Risk Scenario 2). In addition, a licence condition has been imposed which requires a six-month volunteer free period before monitoring of the field locations can cease to ensure no GM bananas remain at the trial site. This is a condition applied in previous licences for release of GM bananas (DIR 076/2007 and DIR 107).

249. In addition to the above points, QLD 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

250. 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 2.0 ha at one site in the Shire of Johnstone, QLD between August 2011 and August 2014
- locate the trial site at least 50 m away from waterways
- 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 until no volunteers are found for at least six months, 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 or other specific conditions
- not permit any GM banana plant material to enter commercial human food or animal feed supply chains.

3.2 Other risk management considerations

251. 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 and other places for the purpose of monitoring or auditing.

3.2.1 Applicant suitability

252. 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.

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

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

255. 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

256. 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.

257. 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

258. 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. Prior to growing the GMOs, QUT would also be required to provide a list of people and organizations who will be covered, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

259. 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.

260. 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 would include:

- location of trial site

- expected dates of transport of GMOs to the shadehouse
- expected dates of planting at the field locations
- expected and actual dates of cleaning of field locations.

3.2.5 Monitoring for Compliance

261. 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.

262. 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.

263. 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

264. 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 including potential for non-specific uptake of metals other than iron.

Section 5 Conclusions of the RARMP

265. The risk assessment concluded that this proposed limited and controlled release of up to 1241 GM banana lines on a maximum total area of 2.0 ha over two years in the Shire of Johnstone, Qld poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

266. 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 109

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
Suggested that the Regulator should consider alternative containment measures for transport by courier.	For transport by courier, use of plain cartons to package GM bananas rather than ones used for commercially grown bananas will minimise mis-identification. RARMP and licence conditions have been modified accordingly.
Suggested coordinated data collection and risk assessment considerations with the Ugandan competent authority for the release of GM bananas to ensure both the technological transfer and the risk assessment capacity are transferred efficiently.	Applicant has been informed. The OGTR is already actively engaged at an international level in transfer of knowledge on risk assessment of GMOs. The OGTR's risk assessment framework (RAF) is highly regarded internationally and both the RAF and RARMPs are publicly available from the department's website or by contacting the office. Should further communication regarding risk assessment of GM banana lines be needed, the Regulator will be happy to engage with the Ugandan authorities, if requested.
There is some uncertainty regarding Risk Scenario 1 which needs further consideration in the RARMP.	None of the introduced proteins are likely to be toxic or allergenic or produce any metabolite that is toxic or allergenic. Data from other plants modified for nutrient enhancement suggests that GM banana plants are unlikely to accumulate β -carotene and/or iron to levels unsafe for humans or animals. This has been further clarified in the RARMP. Thus production of toxic or allergenic substance was not identified as a risk that requires further assessment.
There is some uncertainty regarding Risk Scenario 5 which needs further consideration in the RARMP.	Likelihood of any unintended changes in the biochemistry, physiology or ecology in GM banana lines and their plausible effects is explained in the RARMP. The proposed limits and controls on the trial will minimise exposure of the environment to the GM banana plants. Thus any unintended change in the biochemistry, physiology or ecology in GM banana lines was not identified as a risk that requires further assessment.
Genetic modifications to enhance uptake of iron may lead to increase in uptake of other metals. At this moment there is no data on level of iron or other metals in GM banana lines. In the case of the release site having elevated levels of toxic metals, such non-specific uptake	The proposed release is limited and controlled for experimental purposes. As part of the trial GM banana plant material, including but not limited to fruit, will be assayed for accumulation of iron and other metal ions. The applicant also stated that the release site does not have heavy metal contamination. Further, any GM banana plant material from the

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

Summary of issues raised	Comments
may lead to increase in dietary uptake of toxic metals.	trial is not permitted to enter commercial human food or animal feed supply chains.
Changes in nutrient status may have subtle effects on herbivores, pathogens, commensals and symbionts associated with plants. Altering nitrogen status of plants was found to change the abundance of aphids or sensitivity to range of pathogens. Effects of banana plants modified for enhanced nutrition remains to be determined. Suggest monitoring of insect population at the trial site to be included as licence condition.	<p>The GM banana plants are expected to have a range of pro-vitamin A and/or iron contents. The majority of these lines are represented by a single plant making it a highly heterogeneous population which would confound any interpretation of data on insect population.</p> <p>The proposed release site is in an area of altering patterns of land usage. The land usage in the research station itself is constantly varying which itself will lead to variations in insect population.</p> <p>Insect control procedures are in place and are similar to those employed in commercial banana cultivations. These control procedures will also affect insect population.</p> <p>Given the limited scope of the trial (both time and size), dynamic land use pattern, pest control processes and heterogeneity of plants, collection of data on insect population at the trial site is unlikely to provide any meaningful information.</p>
It is not correct to say that humans and animals cannot synthesize carotenoids. Genes for carotene biosynthesis are present in aphids. Provided references to these reports.	Corrections made in the RARMP to incorporate this information.
RARMP considered only toxic effects of high levels of carotenoid intake in animals while it can have negative effects other than toxicity. Cited literature reporting high levels of carotenoids in birds exerted detrimental effects roughly equivalent to those observed in the case of carotenoid removal from diet. Increased carotenoid levels may affect insect population and have a flow on effect on environment.	<p>Supporting literature on potential negative effects of β-carotene or vitamin A was reviewed and information incorporated in the RARMP. However, the study demonstrating such negative effects relied on experimental data from birds fed on high levels of carotenoids (3000 $\mu\text{g/day}$ for 60 days); a scenario unlikely to be reflected in natural feeding habits even with banana fruits with enhanced β-carotene levels.</p> <p>Licence conditions require that the banana fruits be covered to protect from frugivores and insect pests limiting exposure of birds and insects to fruits. Also, insect control practices similar to commercial banana crops are in place for GM banana lines.</p>
There is higher level of uncertainty regarding production of novel metabolites in GM banana than suggested in the RARMP as GM banana has not been extensively analysed.	The GM banana lines have not been extensively analysed as this is an early stage trial. This is explained in the RARMP. There is no report of production of novel metabolite production associated with genetic engineering of β -carotene biosynthesis in plants. Thus it is unlikely that GM banana will produce any novel metabolite.
<p>The trial aims to provide information about GM bananas with enhanced nutrients which are to be released in Uganda. Raises concern about safety of this technology to human health and the environment and recommends the following specific changes to future data requirements;</p> <ul style="list-style-type: none"> • Measurement of metal ions (zinc, copper, cobalt, magnesium, nickel, cadmium, lead, mercury, chromium, aluminium) in GM and non-GM banana plants under conditions which simulate contaminated and non-contaminated soil; • Determination of relative ability of the transporter to differentiate and concentrate heavy metals; • Determination of amounts of metal ions required to produce visual growth symptoms for both GM and non-GM bananas • Collection of data on diversity and abundance of invertebrate species on GM and non-GM bananas. 	<p>The GM banana lines under trial are not intended to be released in Uganda. The trial will generate useful information e.g. ideal gene and gene-promoter combinations to enhance nutrient content in banana, for development of independent GM banana lines in Uganda.</p> <p>Additional data requirements have been added for any future releases regarding the specificity of the iron transporter and the possibility that the GM banana lines may accumulate other metal ions.</p> <p>Information on invertebrates on GM banana lines developed from Australian cultivars and released in Australian ecosystem will be of little relevance in the Ugandan context.</p>

Appendix B Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 109

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: E: Environment; H: Human health; P: Public acceptance

Other abbreviations: 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, E	Proposed release threatens environmental stability and it is irresponsible to have trials for agronomic performance when long term effects on the health and environment are unknown.	DIR 109 is a limited and controlled release for the purpose of conducting experiments, including assessing nutrient enhancement in 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 commercial human food and animal feed. FSANZ approval would be required before products from the GM banana lines could be sold as food.
			P	Trials of GM bananas will involve investment of public resources. It is inappropriate to conduct this trial as the public has not approved of the idea of consuming unnatural foods.	The consideration of social and economic issues and the appropriateness of using gene technology is outside the scope of issues to which the Regulator may have regard when deciding whether or not to issue a licence