

# **Risk Assessment and Risk Management Plan**

Application for licence for dealings involving an intentional  
release into the environment

**DIR 026/2002**

**Title: Field trial for evaluation of GM papaya to delay fruit  
ripening and test the expression of the introduced genes**

**Applicant: The University of Queensland**

**June 2003**



Office of the  
**Gene Technology Regulator**

## Abbreviations

ACC	1-amino-cyclopropane-1-carboxylic acid
ANZFA	Australia New Zealand Food Authority (now FSANZ)
APHIS	Animal and Plant Health Inspection Service
ATP	adenosine tri-phosphate
<i>bla</i>	beta-lactamase
CaMV	cauliflower mosaic virus
<i>capacs</i>	<i>Carica papaya</i> ACC synthase
DIR	dealing involving intentional release
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
<i>ein</i>	ethylene insensitive
EMBL	European Molecular Biology Laboratory
<i>etr</i>	ethylene receptor
FAO	Food and Agriculture Organisation of the United Nations
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
g	gram
GM	genetically modified
GMAC	Genetic Manipulation Advisory Committee
GMO	genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
GUS	$\beta$ -glucuronidase
ha	hectare
IgE	immunoglobulin E
IgG	immunoglobulin G
Insect-proof enclosure	A self-supporting enclosure comprising transparent nylon netting no greater than 2.0 mm <sup>2</sup> pore size that is sealed to the ground, designed to prevent key pollinating insects from accessing the GM papayas.
IOGTR	Interim Office of the Gene Technology Regulator
<i>Lac Z</i>	$\beta$ -galactosidase
<i>leacs</i>	<i>Lycopersicon esculentum</i> ACC synthase
mg/kg	milligrams per kilogram
MTA	5-methylthioadenosine
mRNA	messenger ribonucleic acid
<i>nos</i>	nopaline synthase
<i>nptII</i>	neomycin phosphotransferase II
NLRD	Notifiable Low Risk Dealing
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase Chain Reaction
<i>pdk</i>	pyruvate orthophosphate dikinase
<i>pga</i>	polygalacturonase
PRSV	Papaya Ring Spot Virus
SAM	S-adenosylmethionine
USDA	United States Department of Agriculture
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
UQ	University of Queensland
WHO	World Health Organisation

*uidA*  
QLD

$\beta$ -glucuronidase gene  
Queensland

# TABLE OF CONTENTS

EXECUTIVE SUMMARY.....	I
CHAPTER 1 BACKGROUND.....	1
SECTION 1 THE APPLICATION.....	1
Section 1.1 The proposed dealings.....	2
Section 1.2 Parent organism.....	2
Section 1.3 Genetic modification and its effect.....	3
Section 1.4 Method of gene transfer.....	4
SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS.....	4
Section 2.1 Previous Australian releases.....	4
Section 2.2 Australian approvals for other GM crops with a delayed fruit ripening trait.....	4
Section 2.3 Approvals by other Australian government agencies.....	4
Section 2.4 International approvals for GM papayas and other GM crops with delayed fruit ripening trait .....	5
CHAPTER 2 SUMMARY OF THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN .....	6
SECTION 1 ISSUES RAISED IN SUBMISSIONS ON THE APPLICATION AND THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN .....	6
SECTION 2 FINALISATION OF THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN.....	7
SECTION 3 DECISION ON THE APPLICATION.....	7
SECTION 3 IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES .....	8
APPENDIX 1 INFORMATION ABOUT THE GMO .....	12
SECTION 1 SUMMARY INFORMATION ABOUT THE GMOs.....	12
Section 1.1 The role of ethylene in plant biology .....	12
Section 1.2 GM papaya with delayed fruit ripening.....	13
1.2.1 Down-regulation of ethylene production.....	13
1.2.2 Disruption of ethylene perception.....	14
Section 1.3 Selectable marker and reporter genes.....	14
SECTION 2 THE PARENT ORGANISM.....	15
SECTION 3 THE INTRODUCED GENES .....	15
Section 3.1 The <i>capacs 1</i> and <i>capacs 2</i> genes.....	15
3.1.1 Mechanisms for altering <i>capacs 1</i> and <i>capacs 2</i> activity.....	16
Section 3.2 The <i>etr1-1</i> gene.....	17
3.2.1 Ethylene perception and plant disease resistance.....	17
Section 3.3 The <i>uidA</i> gene.....	18
Section 3.4 Antibiotic resistance genes .....	19
Section 3.5 Regulatory sequences .....	19
SECTION 4 METHOD OF GENE TRANSFER .....	20
SECTION 5 CHARACTERISATION OF THE INSERTED GENETIC MATERIAL AND STABILITY OF THE GENETIC MODIFICATION .....	20
Section 5.1 Characterisation by Southern blot and PCR analysis.....	20
SECTION 6 EXPRESSION OF THE INTRODUCED PROTEINS.....	21
SECTION 7 EVALUATION OF PLEIOTROPIC EFFECTS OF GENETIC MODIFICATION.....	22

APPENDIX 2 TOXICITY AND ALLERGENICITY TO HUMANS AND OTHER ORGANISMS .....	23
SECTION 1 NATURE OF THE POTENTIAL TOXICITY AND ALLERGENICITY HAZARD .....	23
Section 1.1 Exposure of people to GM papaya .....	23
Section 1.2 Exposure of other organisms to GM papaya .....	24
SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING.....	24
Section 2.1 Toxicity and allergenicity of non-GM papaya .....	24
Section 2.2 Toxicity and allergenicity of the introduced proteins and of GM papaya fruit	25
2.2.1 Acute Toxicity Studies.....	25
2.2.2 Stability of the introduced proteins in the human digestive tract.....	25
2.2.3 Homology with known allergens.....	26
2.2.4 Other sources of the introduced proteins in food .....	27
2.2.5 Assessment by other agencies.....	27
2.2.6 Toxicity to invertebrates and microorganisms .....	28
SECTION 3 CONCLUSIONS REGARDING TOXICITY AND ALLERGENICITY.....	29
APPENDIX 3 ENVIRONMENTAL SAFETY – WEEDINESS .....	30
SECTION 1 NATURE OF THE WEEDINESS HAZARD .....	30
SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING.....	30
Section 2.1 Weediness of non-GM papaya .....	30
Section 2.2 Potential for enhanced weediness of the GM papayas .....	31
2.2.1 Effects of modified ethylene biosynthesis on weediness.....	31
2.2.2 Effects of modified ethylene perception on weediness .....	31
2.2.3 Effect of antibiotic resistance and GUS expression on weediness.....	32
Section 2.3 Spread of GM papayas in the environment .....	33
2.3.1 Spread of pollen from the GM papayas.....	33
2.3.2 Spread of fruit and seeds from the GM papayas .....	33
Section 2.4 Persistence of the GM papayas at the release site .....	34
SECTION 3 CONCLUSIONS REGARDING WEEDINESS .....	34
APPENDIX 4 ENVIRONMENTAL SAFETY - TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS .....	36
SECTION 1 TRANSFER OF INTRODUCED GENES TO OTHER PLANTS .....	37
Section 1.1 Nature of the gene transfer hazard.....	37
1.1.1 Transfer of genes to other papayas.....	37
1.1.2 Transfer of genes to other plant species.....	37
Section 1.2 Likelihood of gene transfer to other papayas, or other plant species .....	38
1.2.1 Proximity to other papayas.....	39
SECTION 2 TRANSFER OF INTRODUCED GENES TO MICROORGANISMS .....	40
Section 2.1 Nature of the gene transfer hazard.....	40
Section 2.2 Likelihood of gene transfer from the GM papayas to microorganisms.....	41
2.2.1 Bacteria.....	41
2.2.2 Viruses.....	43
2.2.3 Fungi.....	43
SECTION 3 TRANSFER OF INTRODUCED GENES TO ANIMALS .....	43
Section 3.1 Nature of the gene transfer hazard.....	43
Section 3.2 Likelihood of gene transfer from the GM papayas to animals.....	44
3.2.1 Humans.....	44
TRANSFER TO HUMANS VIA BACTERIA.....	44
3.2.2 Animals.....	45
SECTION 4 CONCLUSIONS REGARDING GENE TRANSFER TO OTHER ORGANISMS.....	46

Section 4.1	Conclusions regarding gene transfer to other plants .....	46
Section 4.2	Conclusions regarding gene transfer to microorganisms .....	47
Section 4.3	Conclusions regarding gene transfer to animals, including humans .....	47
APPENDIX 5 LICENCE CONDITIONS .....		48
	REASONS FOR LICENCE CONDITIONS .....	57
APPENDIX 6 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS		
INVOLVING INTENTIONAL RELEASES .....		
SECTION 1	THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA .....	60
SECTION 2	THE LICENCE APPLICATION .....	60
SECTION 3	THE INITIAL CONSULTATION PROCESSES .....	61
SECTION 4	THE EVALUATION PROCESSES .....	62
SECTION 5	FURTHER CONSULTATION .....	63
SECTION 6	DECISION ON LICENCE .....	63
APPENDIX 7 SUMMARY OF PUBLIC SUBMISSIONS ON THE RISK ASSESSMENT		
AND RISK MANAGEMENT PLAN .....		65
APPENDIX 8 REFERENCES .....		66

# **RISK ASSESSMENT AND RISK MANAGEMENT PLAN FOR INTENTIONAL RELEASE OF GMOs INTO THE ENVIRONMENT: Application No. DIR 026/2002**

## **EXECUTIVE SUMMARY**

### **INTRODUCTION**

The *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001* (the Regulations) set out requirements which the Gene Technology Regulator (the Regulator) must follow when considering an application for a licence to intentionally release a genetically modified organism (GMO) into the environment.

For a licence to be issued, the Regulator must be satisfied that the release will not pose any risks to human health and safety or the environment that can not be managed. To this end, Section 51 of the Act requires the Regulator to prepare a risk assessment and risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and stakeholders.

### **THE APPLICATION**

The University of Queensland (UQ) applied for a licence (application number DIR 026) for the limited and controlled release of genetically modified (GM) papaya in the Shire of Redlands, Queensland. Seven lines of GM papaya have been modified to delay the process of fruit ripening, while an eighth line contains a 'reporter' gene that will allow evaluation of the operation of the gene regulatory elements used in the other GM papayas. Up to 300 plants of these eight GM papaya lines will be grown in an area of one hectare, from June 2003 to December 2006.

Papaya fruits have poor storage qualities and delayed ripening may prevent spoilage during transportation and storage. Six of these GM papaya lines have modifications which are expected to decrease production of the plant hormone ethylene, which is the 'trigger' for initiation of the ripening process in papaya fruit. The seventh of these GM papayas has a modified ethylene receptor, expected to reduce sensitivity to ethylene. All seven GM papaya lines are expected to exhibit delayed fruit ripening.

All of the GM papayas also contain bacterial antibiotic resistance genes, which were used solely to aid in selection of genetically modified cells in the initial laboratory stages of development of the GM papayas.

The applicant will gather key information from the release about the effect of the genetic modification and the function of the inserted genes. The UQ indicated that these data could not be generated without a field release as fruit production is not possible in glasshouse-grown papaya trees, which can grow to several metres in height before reaching reproductive maturity. However, the release will be strictly limited and controlled. The GM papaya plants in the release will be grown within a self-supporting 'insect-proof' enclosure<sup>†</sup>, which will also prevent access by animals. None of the papaya plants from the release, or their by-products, will be

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<sup>†</sup> The term 'insect-proof enclosure' is used throughout the risk assessment and risk management plan and refers to an enclosure designed to prevent key pollinating insects from accessing the GM papayas and to exclude wildlife, particularly animals that may feed on or disperse papaya fruit and seeds, such as bats and possums.

used for human or animal feed. Fruit and some other plant tissues generated in the release will be analysed in the laboratory for physiological, nutritional and quality attributes and for expression of the inserted genes.

Two limited and controlled releases of GM papaya were approved in Australia under the previous voluntary system that was overseen by the Genetic Manipulation Advisory Committee. These releases received 'deemed' licences under the new regulatory system that are due to expire on 21 June 2003. There have been no reports of adverse effects on human health or the environment resulting from these releases of GM papaya.

Licence application DIR 026/2002 covers three of the lines that were previously authorised for release (PR-128) at this site. The issuing of a licence in respect of this application enables the continued evaluation of 20 plants of these three lines, as well as the release of up to five new lines of GM papaya.

## THE EVALUATION PROCESS

Licence application DIR 026/2002 from the University of Queensland has been evaluated, and a risk assessment and risk management plan (RARMP) prepared, in accordance with the Act and the Regulations, using a Risk Analysis Framework. This framework was developed by the Regulator in consultation with the public and key State, Territory and Commonwealth government stakeholders and the Gene Technology Technical Advisory Committee, and is available at [www.ogtr.gov.au/pdf/public/raffinal.pdf](http://www.ogtr.gov.au/pdf/public/raffinal.pdf).

Details of the process that the Regulator must follow, including the prescribed consultation process on the application, and the matters that must be considered in preparing a RARMP, are set out in Appendix 6 of the RARMP. The complete RARMP can be obtained from the OGTR or from the OGTR's web site at [www.ogtr.gov.au](http://www.ogtr.gov.au).

The risk assessment considered information contained in the application (including information required by Act and the Regulations on the GMO, the parent organism, the proposed dealings and on potential impacts on human health and safety and the environment), submissions received during consultation and current scientific knowledge.

Through this process, potential hazards to human health and safety or the environment that may be posed by release of GM papayas were identified. These have been evaluated on the basis of the likelihood of each hazard occurring and the likely impact of the hazard were it to be realised. The identified potential hazards relate to:

- **toxicity or allergenicity to humans and other organisms:** could the GM papayas with delayed fruit ripening or reporter gene expression be more toxic or allergenic than non-GM papaya, as a result of the novel gene products or because of unforeseen or unintended effects;
- **weediness:** could the GM papayas be harmful to the environment because of inherent weediness or increased potential for weediness; and
- **transfer of introduced genes to other organisms:** could the new genes introduced into the GM papayas transfer to non-GM papaya or to other organisms, with adverse consequences.

## CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator considers that the limited and controlled release of GM papayas with delayed fruit ripening or reporter gene expression will not pose a significant risk to public health and

safety, or to the Australian environment, that cannot be managed. The assessment of each potential hazard identified above is summarised under a separate heading below.

### **Toxicity or allergenicity to humans and other organisms**

The GM papayas are unlikely to prove more toxic or allergenic to humans or other organisms than conventional papaya, because none of the introduced proteins, or the native proteins with modified expression in the GM papaya, have any known intrinsic toxicity or allergenicity. However, detailed toxicity and allergenicity studies have yet to be conducted. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment, and FSANZ approval would need to be obtained before these GM papayas could be used in human food. Currently, the applicant has not applied to FSANZ for evaluation of material from the GM papayas for use in human food.

### **Weediness**

The risk of GM papayas establishing as a weed is low and not likely to be greater than that of conventional papaya. Papaya is not a problematic weed of either agriculture or of natural ecosystems and the genetic modifications are unlikely to alter those aspects of papaya's biology that may potentially affect its weediness. Other than human transportation of papaya fruit, the most likely means of *C. papaya* being dispersed in the environment is by flying foxes (*Poliocephalus* spp.), a known pest of papaya plantations. The use of an insect-proof enclosure, as proposed by the applicant, will prevent flying foxes and other animals from accessing the GM papayas.

### **Transfer of introduced genes to other organisms**

Gene transfer from the GM papayas to non-GM papaya is possible by pollination. The most likely means by which pollen could be transferred to other plants is via hawkmoths (Lepidoptera: Sphingidae), which are the only significant pollinators of papaya in Queensland. The use of an insect-proof enclosure, as proposed by the applicant, will minimise the potential for pollen movement by pollinators. Thus, the risk of gene transfer from GM papayas to cultivated papayas is negligible.

The likelihood of transfer of the introduced genes to other organisms (including microorganisms) is negligible, but even if such transfer occurred, it would be unlikely to pose any hazard to human health and safety or the environment, as the introduced genes are naturally present in microorganisms or in papaya.

## **THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)**

As part of the evaluation process for this licence application, a risk management plan has been developed to address the risks identified (refer to Conclusion of the risk assessment, above). This plan is given effect by the licence conditions imposed. The key licence conditions are outlined below.

### **Toxicity or allergenicity to humans and other organisms**

Licence conditions have been imposed to:

- restrict access to the site to authorised personnel;

- fully enclose the GM papaya plants in a self-supporting insect-proof enclosure that is secured at ground level (this condition has been imposed largely to manage the risks of weediness and gene flow (see below) but also limits the potential for realisation of any risk of toxicity or allergenicity);
- provide appropriate signage at the release site to indicate that GM papayas are being grown within the enclosure and that plants or other material (eg. fruit) must not be removed, except for laboratory analysis as expressly authorised by the licence;
- prohibit the use of the GM papayas and any of their by-products for human or animal food; and
- establish a system for accounting for all fruit produced by the GM papayas and record instances of damage or removal of such fruit.

### **Weediness**

Licence conditions have been imposed to:

- enclose the release site within a self-supporting insect-proof enclosure (which also excludes flying foxes or other animals which may otherwise disperse seed); and
- monitor the release site for 12 months after removal of the GM papaya, and remove any papaya plants that regrow on the site.

### **Transfer of introduced genes to other organisms**

Licence conditions have been imposed which require the applicant to:

- enclose the release site within a self-supporting insect-proof enclosure (which excludes key pollinators);
- remove all male flowers before they open, to prevent dispersal of pollen;
- immediately remove and destroy all flowers and fruits to prevent dispersal of pollen (or seeds), in the event that the insect-proof enclosure is damaged and cannot be repaired immediately; and
- install insect-light traps capable of attracting and trapping key potential pollinators within the enclosure, to monitor the efficacy with which the insect-proof netting excludes such insects.

### **General licence conditions**

Any licence issued by the Regulator also contains a number of general conditions, which are also relevant to risk management. These include, for example:

- identification of the persons or classes of person covered by the licence;
- a requirement that the applicant allow access to the release sites by the Regulator, or persons authorised by the Regulator, for the purposes of monitoring or auditing; and
- a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to human health or safety or to the environment.

## **Monitoring and enforcement of compliance by the OGTR**

As well as the legislative capacity to enforce compliance with licence conditions, the Regulator has additional options for risk management. The Regulator can direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment. The OGTR also independently monitors releases that it has authorised. At least 20% of all release sites will be inspected each year, in accordance with a monitoring and compliance strategy based on risk profiling, to determine whether licence holders are complying with the licence conditions, or whether there are any unforeseen problems.

## **FURTHER INFORMATION**

Detailed information on the evaluation of the application, including the licence conditions, is available in the risk assessment and risk management plan document for this application, which can be obtained from the web site of the Office of the Gene Technology Regulator ([www.ogtr.gov.au](http://www.ogtr.gov.au)), or by calling 1800 181 030 (please quote application number DIR 026/2002).

## CHAPTER 1 BACKGROUND

1. This chapter provides information about the background to the application and previous releases of relevant GMOs into the environment.

### SECTION 1 THE APPLICATION

<b>Project Title:</b>	<b>Field trial for evaluation of GM papaya to delay fruit ripening and test the expression of the introduced genes</b>
<b>Applicant:</b>	University of Queensland St. Lucia Brisbane QLD 4072
<b>Common name of the parent organism:</b>	Papaya or pawpaw
<b>Scientific name of the parent organism:</b>	<i>Carica papaya</i> L.
<b>Modified trait(s):</b>	Delayed fruit ripening, reporter gene expression and antibiotic resistance
<b>Identity of the gene(s) responsible for the modified trait(s):</b>	<ul style="list-style-type: none"><li>• <i>capacs 1</i> and <i>capacs 2</i> genes from papaya (genes associated with ethylene production and fruit ripening)</li><li>• <i>etr1-1</i> gene from <i>Arabidopsis thaliana</i> (ethylene perception and fruit ripening)</li><li>• <math>\beta</math>-glucuronidase gene (<i>uidA</i>) from <i>Escherichia coli</i> (reporter gene)</li><li>• <i>npIII</i> gene from bacterial Tn5 transposon (antibiotic resistance gene)</li></ul>
<b>Proposed Release Location:</b>	Shire of Redlands (QLD).
<b>Proposed Release Size:</b>	1 hectare
<b>Proposed Release Date:</b>	June 2003 – December 2006. Continued evaluation of 20 GM papayas planted since 2002 and release of up to 300 new papaya plants in August 2003.

2. The OGTR has received an application (licence application number DIR 026) from the University of Queensland (UQ) for the intentional release of genetically modified (GM) papayas into the environment in the shire of Redlands Queensland (Qld). Approval would enable the continued evaluation of three types of GM papayas planted since 2002. The release was authorised by the previous voluntary system and under ‘deemed’ licence PR-128, which expires in June 2003 under the transitional arrangements of Section 190 of the Gene Technology Act 2000 (the Act). The applicant also proposes the release of five new types of GM papaya plants, in August 2003. Up to 300 GM papaya plants will be grown on a single site covering one hectare for a three year period.

3. The proposed release aims to evaluate modified fruit ripening characteristics of eight different GM papayas. However, fruit production is not possible in glasshouse-grown papaya trees, which can grow to several metres in height before reproductive maturity. Accordingly, the applicant seeks a licence to release the GM papaya plants into the field under limited and controlled conditions to evaluate fruit ripening characteristics.

## Section 1.1 The proposed dealings

4. The UQ seeks approval to grow six different types of GM papayas that have been genetically modified to delay fruit ripening by decreasing the natural production ('down-regulating') ACC (1-amino-cyclopropane-1-carboxylic acid) synthase, an intermediate enzyme in the biosynthesis of the plant hormone, ethylene (see Table 1, Appendix 1). In addition, UQ proposes to grow one type of GM papaya that has been modified to delay fruit ripening by a change in the perception of ethylene. Another type of GM papaya has also been modified to express a reporter gene that will allow evaluation of the effectiveness of the promoter controlling expression of the introduced genes.
5. The proposed limited and controlled trial involves planting up to 300 glasshouse-grown GM papaya plants into the field at one site in the Shire of Redlands, Qld. The GM papaya plants will be planted inside an insect-proof netting enclosure\*.
6. Papaya fruit has poor storage qualities. If fruit ripening is delayed over several days to weeks it may be possible to decrease spoilage due to over-ripening during transportation and storage. The applicant proposes to grow the GM papayas under insect-proof netting in the field for fruit production, to monitor the rate of fruit ripening. In this regard, a majority of fruit will be harvested before they are fully ripe, but for a limited number of fruit, the rate of ripening will be assessed on the tree. It is anticipated that key information regarding the genetic modifications and physiological, nutritional and quality attributes of the fruit will be obtained. The expression levels of the naturally occurring and introduced ACC synthase genes and the introduced ethylene perception gene will also be determined. Reporter gene expression will also be evaluated to assess the effectiveness of the regulatory sequence (promoter) that controls the expression of the introduced ACC synthase genes.
7. None of the fruit produced from these trials is intended for human or animal consumption.

## Section 1.2 Parent organism

8. The parent organism is *Carica papaya* L. (papaya, or paw paw), which is exotic to Australia, but is grown both commercially and in domestic gardens in tropical and sub-tropical parts of Australia from Western Australia to New South Wales.
9. *C. papaya* is not a pathogenic organism and the genetic modifications of the papaya plants proposed for release will not alter this. More detailed information on papaya can be found in a review document 'The Biology and Ecology of Papaya (paw paw), *Carica papaya* L., in Australia' that was produced in order to inform this risk assessment process. This document is available at the OGTR website (<http://www.ogtr.gov.au>).

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\* The term 'insect-proof enclosure' is used throughout the risk assessment and risk management plan and refers to an enclosure designed to prevent key pollinating insects from accessing the GM papayas and to exclude wildlife, particularly animals that may feed on or disperse papaya fruit and seeds, such as bats and possums.

### Section 1.3 Genetic modification and its effect

10. Six types of GM papayas that have been genetically modified to delay fruit ripening which contain additional copies of genes encoding the enzyme ACC synthase (both sense and antisense versions of the *capacs 1* or *capacs 2* genes from *Carica papaya*). These additional copies initiate down-regulation of naturally occurring ACC synthase gene activity through two different methods. Together, these methods are known as ‘gene silencing’, as they prevent a target plant gene producing the protein that it normally makes, thus ‘silencing’ the activity of the gene.

11. The ACC synthase genes are associated with biosynthesis of ethylene. It is expected that production of ethylene will be decreased due to inhibition of production of ACC, a metabolic intermediate required for the natural synthesis of ethylene in plants. One response to decreased ethylene production is to reduce the rate at which fruit ripen.

12. One type of GM papaya will test a different approach for delaying fruit ripening. It has been genetically modified to contain the *etr1-1* gene, encoding a modified ethylene receptor protein from *Arabidopsis thaliana*. The *etr1-1* gene encodes a non-functional version of the ethylene receptor gene *etr1*. In GM papaya plants containing the *etr1-1* gene, the fruits are expected to be partially insensitive to ethylene, and thereby, delay ripening.

13. Some plants proposed for release express the *uidA* gene from the bacterium, *Escherichia coli*. This gene codes for the enzyme  $\beta$ -glucuronidase (GUS). The GUS enzyme converts a colourless substrate into a blue colour in a simple laboratory assay and is used as a reporter or ‘marker’ to detect tissues that have been successfully genetically modified. Exposure of plant tissues containing the GUS gene to this substrate facilitates measurement of the expression of the *uidA* gene. The applicant proposes to release some papaya plants that have been modified by inserting the *uidA* gene, instead of the fruit ripening genes, as a means of confirming the effectiveness of the promoter that is used to control the expression of the ACC synthase genes. GUS activity will also be used to indicate the plant tissue(s) in which the genes are expressed.

14. The plants also contain bacterial genes conferring resistance to the antibiotics kanamycin and neomycin (*nptII* gene) and ampicillin (*bla* gene). These genes were used to select bacteria and plants containing the desired fruit ripening and GUS genes, in the laboratory. The *bla* gene is under the control of a bacterial promoter and therefore it will not be expressed in the GM papayas.

15. Short regulatory sequences that are required to control the functioning of the genes are also present in the GM papayas. These are derived from the cauliflower mosaic virus, *Agrobacterium tumefaciens* (a common soil bacterium) and apple (*Malus domestica*). Although the first two of these organisms are plant pathogens, the regulatory sequences comprise only a small part of their total genome and are not, in themselves, capable of causing disease.

16. Further details about the genetic modification process and the introduced genes are provided in Appendix 1, Sections 3 and 4.

## **Section 1.4 Method of gene transfer**

17. Each of the genes and their associated regulatory sequences were introduced into papaya by microprojectile bombardment. This technique involves coating the DNA containing the genes onto very small tungsten or gold particles which are ‘shot’ into the papaya tissue. Particle bombardment has been widely used in Australia and overseas for introducing new genes into plants.

## **SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS**

### **Section 2.1 Previous Australian releases**

18. Under the former voluntary system overseen by the GMAC, there were two releases of GM papayas:

- PR-108 (Queensland Department of Primary Industries) comprised GM papaya plants resistant to Papaya Ring Spot Virus (PRSV) and involved up to 100 plants released in an area up to 0.15 hectares; and
- PR-128 (University of Queensland) comprised 20 GM papaya plants modified for delayed fruit ripening released in an area up to 1 hectare (originally, approval was given to grow 1000 GM papaya plants).

19. As noted previously, the current application involves the continued evaluation of three types of GM papayas approved for release under PR-128, and the release of five additional types of GM papayas.

20. There have been no applications for general (commercial) release of GM papayas in Australia to date.

### **Section 2.2 Australian approvals for other GM crops with a delayed fruit ripening trait**

21. As noted above, there have been no applications for general (commercial) release of other GM fruit crops with delayed ripening in Australia to date. However, a GM carnation with down-regulation of ethylene production via insertion of a truncated ACC synthase gene, which has enhanced vase life, was approved for commercial release in Australia under the former voluntary system (GR-1). The limited and controlled release of pineapples with decreased ethylene production was authorised by the previous voluntary system under deemed licence PR-95. Applications to continue both these releases are currently under consideration by the Regulator.

### **Section 2.3 Approvals by other Australian government agencies**

22. The OGTR is responsible for assessing the biosafety risks to human health and the environment associated with development and use of GMOs. Other government regulatory requirements must also be met in respect of the release of the GMOs, and the use of products of the GMO, including the requirements of Food Standards Australia New Zealand (FSANZ).

23. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment. As the trials proposed in this application represent an early 'proof of concept' stage of development, the applicant has not as yet applied to FSANZ for evaluation of material from the GM papayas for use in human food. FSANZ approval would need to be obtained before it could be used in human food.

24. Further information about food safety and food labelling are available from FSANZ:

Food Standard Australia New Zealand  
PO Box 7186  
Canberra Mail Centre ACT 2610  
Phone: (02) 6271 2222  
Fax: (02) 6271 2278  
E-mail: [info@foodstandards.gov.au](mailto:info@foodstandards.gov.au)  
<http://www.foodstandards.gov.au>

#### **Section 2.4 International approvals for GM papayas and other GM crops with delayed fruit ripening trait**

25. GM papaya lines resistant to papaya ring spot virus (PRSV) are grown in Hawaii and were approved for commercial use in the USA in 1996.

26. Other fruit species genetically modified for delayed fruit ripening have been approved for commercial release in the USA and Canada:

- Tomato (*Lycopersicon esculentum*) modified for delayed fruit ripening via insertion of a truncated ACC synthase gene was approved for commercial release in the USA and Canada in 1995;
- Tomato modified for delayed fruit ripening via degradation of essential components of ethylene biosynthesis by introduced enzymes (ACC deaminase or S-adenosylmethionine (SAM) hydrolase) were approved for commercial release in the USA in 1995 and 1996, respectively;
- Two types of tomato modified for delayed fruit ripening via suppression of the activity of fruit softening (polygalacturonase genes) were approved for commercial release in the USA (1994) and Canada (1995 and 1996);
- Approval for melon (*Cucumis melo*) modified for delayed fruit ripening via expression of the SAM hydrolase gene, is currently pending in the USA.

## **CHAPTER 2 SUMMARY OF THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN**

27. The Act and the Regulations require that risks associated with dealings with GMOs are identified and assessed as to whether they can be managed to protect human health and safety and the environment (see Appendix 6).

### **SECTION 1 ISSUES RAISED IN SUBMISSIONS ON THE APPLICATION AND THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN**

28. Comments received in response to the consultation on the risk assessment and risk management plan undertaken with expert groups and key stakeholders, as required by Section 50, of the Act and with the public, as required by Section 52 of the Act (see Appendix 6), were very important in shaping this risk assessment and risk management plan, which formed the basis of the final decision on the application.

29. Written submissions received from the agencies and authorities prescribed by Section 50 of the Act in relation to DIR application number 026/2002 suggested that the following issues should be addressed in the risk assessment and the risk management plan:

- toxicity of the introduced proteins to soil microorganisms, invertebrates, birds and mammals including humans (Appendix 2 refers);
- persistence or accumulation of the introduced proteins in the environment and potential for adverse impacts resulting from the presence of the introduced proteins in the environment (Appendices 1 and 2 refer);
- potential for transfer of the introduced genes from the GM papaya to other papaya plants, related plants and microorganisms and potential for subsequent ecological impacts (Appendix 4 refers);
- potential for the genetic modifications to alter the level, formation or breakdown of potentially toxic plant metabolites, for example components of the ethylene biosynthesis pathway, or to have other unintended impacts on the plant phenotype (Appendices 1 and 2 refer);
- measures to prevent the unintended exposure of humans or other organisms to the GMOs and the unintended spread of the GMOs, pollen, seed or vegetative propagules beyond the release site (Appendix 5 refers);
- measures to prevent the occurrence of GM papaya volunteers at the release site after completion of the release (Appendix 5 refers); and
- data requirements for the future development of the GMOs (Chapter 2, Section 3 refers).

30. In total the Regulator received three submissions from the public on this application and the associated risk assessment and risk management plan. None of the submissions specifically addressed matters relating to the protection of human health and safety or the environment. A summary of these written submissions is provided in Appendix 7.

## **SECTION 2 FINALISATION OF THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN**

31. In accordance with Section 51 of the Act, the Regulator has taken into account all written submissions that related to human health and safety and the environment in finalising the risk assessment and risk management plan. The issues raised were considered carefully and weighed against the body of current scientific information in reaching the conclusions set out in this document.

32. The risk assessment process, detailed in Appendix 8, identified a number of hazards that may be posed by the proposed dealings. The risks posed by these hazards were assessed by considering:

- the likelihood of the hazard occurring;
- the likely consequences (impact) of the hazard, were it to be realised; and
- risk management options to mitigate any identified risks.

33. The categories used, according to the level of risk are ‘negligible’, ‘very low’, ‘low’, ‘moderate’, ‘high’ or ‘very high’.

34. The following table, Table 1, lists each of the potential hazards that were considered during the risk assessment process in the *Hazard Identification* column and summarises the assessment of each hazard under the column headed *Risk Level*. A comprehensive assessment of each identified hazard is provided in Appendices 2 - 6, as cross-referenced in the column headed *Conclusions of the Risk Assessment*.

35. Where it is considered that risk management is necessary to protect the health and safety of humans and/or the environment, the table also summarises risk management options for each hazard (*Risk Management (RM) Options*), identifies the method that has been selected (*Preferred RM Method*) and summarises the reason for selecting a particular method (*Reason for selecting RM Method*). The risk management plan for the proposed dealing will be given effect by specific conditions within the licence. These conditions are summarised in the final column, headed Licence Conditions, and detailed in Appendix 5.

## **SECTION 3 DECISION ON THE APPLICATION**

36. The matters that the Regulator must consider in making a decision are detailed in Appendix 6. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings are able to be managed so as to protect human health and safety and the environment.

37. It is concluded that there are no significant risks to human health and safety or to the Australian environment arising from the proposed release of GM papayas that cannot be managed. Detailed risk analyses based on the available scientific information are provided in Appendices 2 - 4 in support of this conclusion.

38. In accordance with the matters required to be considered under section 58 of the Act, the Regulator has determined that the University of Queensland is suitable to hold a licence for a dealing involving intentional release of a GMO into the environment. Further information on the process of assessing the suitability of the applicant is contained in Appendix 6.

39. Therefore, the Regulator has issued licence number DIR 026/2002.

### SECTION 3 IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES

40. Before any application for a larger scale release and/or reduced containment measures involving these GM papayas could be considered, more detailed information would be required on:

- the levels of expression in both fruit and non-fruit tissues of the introduced *etr1-1*, *uidA*, *nptII* genes, and the introduced and endogenous *capacs 1* and *capacs 2* genes;
- genetic segregation and molecular characterisation of the inserted genetic material, including determination of the non-coding vector sequence, if present in the GM papayas;
- the potential toxicity and allergenicity of the GM papaya for humans, including more information on the toxicity and allergenicity of the introduced ETR1-1 protein and any alterations in the toxicity and allergenicity of the papaya fruit as a result of the modified ripening characteristics;
- potential toxicity of the GM papaya for non-target organisms including mammals, pests, beneficial organisms and communities of soil microorganisms;
- the potential for the novel proteins to persist in the environment;
- the foraging range of known pollinators, particularly hawkmoths;
- the distances that frugivores disperse papaya fruit and the viability of papaya seeds that may be ingested by these animals;
- the extent and significance of *C. papaya* soil seed banks for the population dynamics of naturalised *C. papaya* populations; and
- impacts of the genetic modifications on attributes of *C. papaya* that may affect its weediness including impacts on seed germinability, fruit and seed productivity, susceptibility to pathogens and responses to herbivory.

41. It should be noted that, as a condition of licence, the applicant must develop a research program, in consultation with the OGTR, to produce much of this data. As a minimum, this program must include research to confirm the genes that have been introduced into the GM papayas and the research results must be reported to the OGTR annually. These data could not be collected prior to the proposed limited and controlled release because, as indicated in Chapter 1 and Appendix 1, papaya fruit cannot be produced easily under glasshouse conditions and much of the critical information regarding the GM papayas proposed for release must be derived from the analysis of fruit tissues.

42. Provision of the above data during the proposed release is not required to ensure the management of risks to human health and safety and the environment. The risk management measures summarised in Table 1 and given effect by the proposed specific licence conditions effectively manage these risks.

**Table 1 Summary of the risk assessment and the risk management plan (including summary of proposed licence conditions).**

GM papaya: the genetically modified papaya proposed for release

Hazard Identification	Risk Level (combines 'likelihood' and 'impact')	Conclusions of the Risk Assessment (refer to appendices for details)	Does risk require management?	Risk Management (RM) Options [* Preferred RM Method(s)]	Reason(s) for selecting RM method	Is risk managed?	Licence conditions (see Appendix 5 for detailed licence conditions)
<b>TOXICITY AND ALLERGENICITY FOR HUMANS: Food</b>	Low	See Appendix 2 <ul style="list-style-type: none"> <li>the GM papayas are not likely to become more toxic or allergenic than non-GM papayas as a result of the introduced proteins or the down-regulation of ethylene biosynthesis or altered ethylene perception;</li> <li>available data to date suggest that the risk of toxicity and allergenicity is low but detailed toxicity and allergenicity studies have not yet been conducted;</li> <li>exposure of humans to the GM papayas will be minimal due to the limited scale and enclosure of the proposed trial within insect-proof netting; and</li> <li>none of the GM material from the release will be used for human consumption.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>* Limit scale of release; and</li> <li>* Prohibit use of GM papaya or by-products for human food; and</li> <li>* Impose containment, transport and storage conditions on release.</li> <li>Disallow release.</li> </ul>	<ol style="list-style-type: none"> <li>Limit scale: decreases likelihood of exposure;</li> <li>Prohibit use as human food: prevents human exposure through food;</li> <li>Impose containment, transport and storage conditions: decreases likelihood of exposure.</li> <li>Require research: will enable OGTR to evaluate risks to human health and safety.</li> </ol>	Yes	<ol style="list-style-type: none"> <li>Limit scale: restrict area to one hectare at one site in the Shire of Redlands;</li> <li>Prohibit use as human food: no fruit from the release to be consumed by humans and all fruit and other GM materials not required for analysis to be destroyed;</li> <li>Impose containment, transport and storage conditions: GM papaya to be grown under insect-proof netting enclosure that is secured at ground level and to be transported and stored according to OGTR requirements; GM products to be prevented from entering waterways;</li> <li>Research program developed in consultation with OGTR.</li> </ol>
<b>TOXICITY AND ALLERGENICITY FOR HUMANS: Occupational exposure</b>	Low	See Appendix 2 <ul style="list-style-type: none"> <li>the GM papayas are not likely to become more toxic or allergenic than non-GM papayas as a result of the introduced proteins or the down-regulation of ethylene biosynthesis or altered ethylene perception;</li> <li>available data to date suggest that the risk of toxicity and allergenicity is low but detailed toxicity and allergenicity studies have not yet been conducted;</li> <li>exposure of humans to the GM papayas will be minimal due to the limited scale and enclosure of the proposed trial within insect-proof netting; and</li> <li>none of the GM material from the release will be used for human consumption.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>* Limit scale of release; and</li> <li>* Require applicant to notify people associated with the release of possible allergenicity/toxicity of the GMOs with appropriate signage and to report allergic/toxic responses to the OGTR; and</li> <li>* Require research to be conducted into toxicity and allergenicity.</li> <li>Disallow release.</li> </ul>	<ol style="list-style-type: none"> <li>Limit scale: decreases likelihood of exposure;</li> <li>Notification/signage: ensures that people working with the GMOs are aware of possible allergenicity/toxicity;</li> <li>Reporting allergic/toxic responses: indicates nature and frequency of any allergic/toxic responses and allows Regulator to adapt risk management measures, if necessary.</li> </ol>	Yes	<ol style="list-style-type: none"> <li>Limit scale: as above;</li> <li>Require applicant to ensure that people working with the GMOs are aware of possible allergenicity/toxicity, with a sign on the door of the enclosure and laboratory;</li> <li>Require immediate reporting of unusual allergic/toxic responses in workers associated with the release.</li> </ol>
<b>TOXICITY FOR OTHER ORGANISMS: Mammals, birds and invertebrates</b>	Low	See Appendix 2 <ul style="list-style-type: none"> <li>the GM papayas are not likely to become more toxic or allergenic than non-GM papayas as a result of the introduced proteins or the down-regulation of ethylene biosynthesis or altered ethylene perception;</li> <li>available data to date suggest that the risk of toxicity and allergenicity is low but detailed toxicity and allergenicity studies have not yet been conducted;</li> <li>exposure of other organisms to the GM papayas will be minimal due to the limited scale and enclosure of the trial within an insect-proof enclosure that also prevents animals that may feed on the fruit from entering the release site; and</li> <li>none of the GM material from the release will be used for animal consumption;</li> </ul>	Yes	<ul style="list-style-type: none"> <li>* Limit scale of release; and</li> <li>* Prohibit use of GM papaya or by-products as animal food;</li> <li>* Require release to be enclosed in insect-proof netting; and</li> <li>* Research to be conducted into non-target impacts and persistence in the environment.</li> <li>Disallow release.</li> </ul>	<ol style="list-style-type: none"> <li>Limit scale: decreases likelihood of exposure;</li> <li>Prohibit use as animal food: prevents animal exposure through feed</li> <li>Enclose release: decreases likelihood of exposure.</li> <li>Require research: enables OGTR to evaluate risks to other organisms.</li> </ol>	Yes	<ol style="list-style-type: none"> <li>Limit scale: as above;</li> <li>Prohibit use as animal food: no fruit or by-products from the GMOs to be used as animal feed and all fruit GM materials not required for analysis to be destroyed;</li> <li>Enclose release: GM papaya to be grown within insect-proof netting enclosure that is secured at ground level. Integrity of enclosure to be monitored every day and repaired immediately, if necessary, throughout release; and</li> <li>Research program developed in consultation with OGTR</li> </ol>

Hazard Identification	Risk Level (combines 'likelihood' and 'impact')	Conclusions of the Risk Assessment (refer to appendices for details)	Does risk require management?	Risk Management (RM) Options [* Preferred RM Method(s)]	Reason(s) for selecting RM method	Is risk managed?	Licence conditions (see Appendix 5 for detailed licence conditions)
<b>TOXICITY FOR OTHER ORGANISMS: Micro-organisms</b>	Low	<p>See Appendix 2</p> <ul style="list-style-type: none"> <li>▪ the GM papayas are not likely to become more toxic or allergenic than non-GM papayas as a result of the introduced proteins or the down-regulation of ethylene biosynthesis or altered ethylene perception;</li> <li>▪ available data to date suggest that the risk of toxicity and allergenicity is low but detailed toxicity and allergenicity studies have not yet been conducted;</li> <li>▪ exposure of microorganisms to the GM papayas will be minimal due to the limited scale of the proposed trial;</li> <li>▪ fruit produced during the release will be removed from the site to prevent fruit proteins from entering the soil.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>▪ *Prevent fruit from falling to ground; and</li> <li>▪ * Limit scale of release; and</li> <li>▪ * Research to be conducted into non-target impacts and persistence in the environment. Disallow release.</li> </ul>	<ol style="list-style-type: none"> <li>1) Limit scale: decreases likelihood of exposure and potential impact.</li> <li>2) Prevent fruit falling to ground: decreases likelihood of proteins entering soil and affecting non-target species;</li> <li>3) Require research: enables OGTR to evaluate risks to other organisms.</li> </ol>	Yes	<ol style="list-style-type: none"> <li>1) Limit scale: as above;</li> <li>2) Ensure that fruits formed during the trial are harvested before they fall to the ground; and</li> <li>3) Research program developed in consultation with OGTR</li> </ol>
<b>WEEDINESS: Spread in the environment</b>	Low	<p>See Appendix 3</p> <ul style="list-style-type: none"> <li>▪ the down-regulation of ethylene biosynthesis, the modification to ethylene perception and the other introduced genes are unlikely to affect attributes of the GM papayas proposed for release that may alter potential weediness;</li> <li>▪ papaya is not a problematic weed in Australia;</li> <li>▪ no closely related species are problematic weeds in Australia;</li> <li>▪ the proposed release is restricted to one hectare; and</li> <li>▪ dispersal of the GM papaya seeds will be limited by containing the release in an insect-proof enclosure that also prevents larger animals accessing the GM plants and their fruit.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>▪ * Prevent movement of vegetative propagules or seeds beyond release site; and</li> <li>▪ * Account for all fruit produced; and</li> <li>▪ * Destroy viable material not required for analysis or subsequent release and monitor site to enable removal of volunteers or regrowth. Disallow release.</li> </ul>	<ol style="list-style-type: none"> <li>1) Prevent movement: limits spread of the GM papaya beyond the release site.</li> <li>2) Account for fruit: decreases likelihood of spread beyond the release site.</li> <li>3) Destroy and monitor: limits spread beyond the release site.</li> </ol>	Yes	<ol style="list-style-type: none"> <li>1) Prevent movement: impose containment, transport and storage conditions, enclose release (as above).</li> <li>2) Account for fruit: all fruit set to be numbered, recorded and accounted for and instances of damage or removal of fruit to be recorded.</li> <li>3) Destroy and monitor: destroy all viable material not required for analysis or subsequent release and monitor the release site after the release and remove any papaya plants that regrow.</li> </ol>
<b>WEEDINESS: Persistence in the environment</b>	Low	<p>See Appendix 3</p> <ul style="list-style-type: none"> <li>▪ the down-regulation of ethylene biosynthesis, the modification to ethylene perception and the other introduced genes are unlikely to affect attributes of the GM papayas proposed for release that may alter potential weediness;</li> <li>▪ papaya is not a problematic weed in Australia;</li> <li>▪ no closely related species are problematic weeds in Australia;</li> <li>▪ the proposed release is restricted to one hectare; and</li> <li>▪ dispersal of the GM papaya seeds will be limited by containing the release in an insect-proof enclosure that also prevents larger animals accessing the GM plants and their fruit.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>▪ * Prevent movement of vegetative propagules or seeds beyond release site; and</li> <li>▪ * Account for all fruit produced; and</li> <li>▪ * Destroy viable material not required for analysis or subsequent release and monitor site to enable removal of volunteers or regrowth. Disallow release.</li> </ul>	<ol style="list-style-type: none"> <li>1) Prevent movement: prevents spread of the GM papaya beyond the release site.</li> <li>2) Account for fruit: decreases likelihood of spread beyond the release site.</li> </ol>	Yes	<ol style="list-style-type: none"> <li>1) Prevent movement: impose containment, transport and storage conditions, enclose release (as above).</li> <li>2) Account for fruit: (as above)</li> <li>3) Destroy and monitor: destroy all viable material not required for analysis or subsequent release and monitor the release site after the release and remove any papaya plants that regrow.</li> </ol>

Hazard Identification	Risk Level (combines 'likelihood' and 'impact')	Conclusions of the Risk Assessment (refer to appendices for details)	Does risk require management?	Risk Management (RM) Options [* Preferred RM Method(s)]	Reason(s) for selecting RM method	Is risk managed?	Licence conditions (see Appendix 5 for detailed licence conditions)
<b>GENE TRANSFER: Plants</b> <ul style="list-style-type: none"> <li>Other papaya plants, including plantations and naturalised papaya populations</li> </ul>	Negligible	<b>See Appendix 4</b> <ul style="list-style-type: none"> <li>pollen movement will be very limited because the release will be enclosed within an insect-proof enclosure; potential male flowers will be removed, thereby reducing the availability of pollen; and</li> <li>potential gene flow to commercial plantations and known naturalised papaya populations will be limited by geographic isolation.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>* <b>Require release to be enclosed in insect-proof netting;</b></li> <li>* <b>Immediately repair breaches of enclosure;</b></li> <li>* <b>Destroy flowers and fruit if enclosure cannot be repaired immediately;</b></li> <li>* <b>Remove male flowers.</b></li> <li>Disallow release.</li> </ul>	1) Enclose release: limits access for pollinators and movement of pollen beyond release site. 2) Repair breaches: ensures enclosure remains functional 3) Destroy flowers and fruit: prevents gene transfer in event of significant damage to enclosure; 4) Remove male flowers: further limits likelihood of pollen movement beyond release site.	Yes	1) Enclose release: (as above) 2) Immediately repair damage to enclosure. 3) Destroy flowers and fruit if damage to enclosure cannot be repaired immediately. 4) Remove male flowers: all male flowers to be removed prior to anthesis.
<b>GENE TRANSFER: Plants</b> <ul style="list-style-type: none"> <li>Other plant genera</li> </ul>	Negligible	<b>See Appendix 4</b> <ul style="list-style-type: none"> <li>In addition to containing the release in an insect-proof enclosure:</li> <li>genetic incompatibility with papaya's closest relatives (<i>Vasconcella</i> spp.) effectively prevents the formation of hybrids and limits potential for back-crossing to the parental species; and</li> <li>strong and well-demonstrated genetic differences significantly limit gene transfer to more distantly related plant genera.</li> </ul>	No	N/A	N/A	N/A	None required
<b>GENE TRANSFER: Microorganisms</b>	Negligible	<b>See Appendix 4</b> <ul style="list-style-type: none"> <li>horizontal gene transfer is the only possible mechanism for such transfer, yet this has not been demonstrated from plants to microorganisms under natural conditions.</li> </ul> <p>It should be noted that in the extremely unlikely event of such a transfer occurring, human health and safety and the environment are unlikely to be adversely effected.</p>	No	N/A	N/A	N/A	None required
<b>GENE TRANSFER: Humans &amp; other animals</b>	Negligible	<b>See Appendix 4</b> <ul style="list-style-type: none"> <li>simulated ruminant digestion studies with model experimental systems indicate that introduced genes and endogenous plant genes are rapidly degraded, representing a considerable barrier to gene transfer; and</li> <li>vertebrate animals will not be exposed to the GM papaya fruits; and</li> <li>FSANZ approval would need to be obtained before tissues from the GM papayas, including fruits, could be used in human food. As yet the applicant has not applied to FSANZ for evaluation of the GM material due to the early stage of the work.</li> </ul> <p>It should be noted that in the extremely unlikely event of such a transfer occurring, human health and safety and the environment are unlikely to be adversely effected.</p>	No	N/A	N/A	N/A	None required

## **APPENDIX 1 INFORMATION ABOUT THE GMO**

43. In preparing the risk assessment and risk management plan, the Regulator is required under Section 49 (2) of the Act to consider the properties of the parent organism and the effects of genetic modification.

44. This part of the document addresses these matters and provides detailed information about the GMOs for release, the parent organism, the genetic modification process, the genes that have been introduced, the information on genetic constructs, the new proteins that are expressed and altered phenotype of the papayas as a result of the genetic modification.

### **SECTION 1 SUMMARY INFORMATION ABOUT THE GMOS**

45. The University of Queensland (UQ) proposes a continued limited and controlled release of GM papaya (*Carica papaya*) plants with altered fruit ripening characteristics. UQ aims to evaluate the effect on fruit ripening of two genes involved in ethylene production and one gene involved in ethylene perception. Ethylene is a gaseous plant hormone that regulates many aspects of plant growth and development and is responsible for the timing of ripening in fruit (Alexander & Grierson 2002) (see Section 1.1).

46. In total, eight types of GM papayas are proposed for release (see Table 1). Seven of these have been modified to delay the process of fruit ripening and of these, six incorporate either sense, antisense or 'hairpin' (both sense and antisense genes linked in one construct) versions of two genes involved with ethylene biosynthesis. The seventh of these GM papayas with delayed fruit ripening contains a sense version of a gene involved with ethylene perception (see Section 1.2). The eighth type of GM papaya contains a reporter gene that helps to identify plant tissues in which the modified fruit-ripening traits are likely to be expressed.

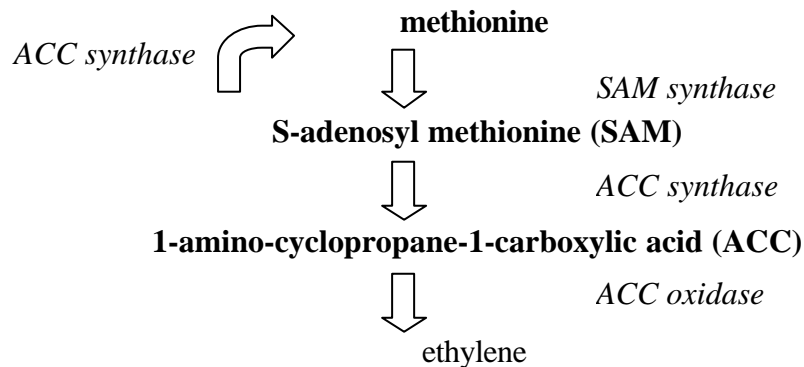
47. Three types of GM papayas, with sense or antisense versions of two genes involved in ethylene biosynthesis, were released into the environment under limited and controlled conditions under licence number PR-128 in 2002, based on approval issued under the previous voluntary system administered by the Genetic Manipulation Advisory Committee (GMAC). Approval of licence application DIR 026/2002 would enable the continued limited and controlled release of the GM papayas licensed under PR-128, as well as the release of the five new types of GM papayas (with 'hairpin' versions of ACC synthase genes, a sense version of an ethylene perception gene or a reporter gene).

#### **Section 1.1 The role of ethylene in plant biology**

48. Ethylene is a plant hormone that plays a role in many different aspects of plant growth and development, including cell elongation, formation of root hairs, induction of seed germination and leaf and flower abscission (1999). Likewise, plant responses to stresses, such as wounding or pathogen attack, involve ethylene (Stepanova & Ecker 2000; Thomma et al. 2001). The process of ripening in many fruit, such as bananas, melons, avocados and tomatoes, is also controlled by ethylene, which is particularly important in the transition from nature green fruit to ripe fruit (Alexander & Grierson 2002).

49. Ethylene is a gas, which is produced in all plant tissues and is able to rapidly diffuse out of the tissue in which it is produced. ACC (1-amino-cyclopropane-1-carboxylic acid) is the immediate precursor of ethylene. The enzyme ACC synthase produces ACC from SAM (S-adenosyl methionine), which in turn is derived from the amino acid methionine via the action of the enzyme SAM synthase (see Figure 1). ACC synthase also converts SAM to 5-methylthioadenosine (MTA), which is used for the re-synthesis of methionine, thus maintaining a constant pool of methionine in the cell.

**Figure 1** The biosynthesis of ethylene in plants. Intermediates in the pathway are in **bold**; enzymes responsible for each step in the pathway are in *italics*.



## Section 1.2 GM papaya with delayed fruit ripening

50. Papayas were genetically modified to delay fruit ripening by either down-regulating ethylene production or disrupting ethylene perception.

### 1.2.1 Down-regulation of ethylene production

51. The GM papaya plants will contain either sense or antisense copies (copies in the correct or inverse orientation) of the native papaya genes, *capacs 1* and *capacs 2*, encoding the enzyme ACC synthase, or 'hairpin' constructs (sense and antisense copies linked in one construct) (Smith et al. 2002) of these genes. Introduction of an additional copy or copies of the native *capacs 1* and *capacs 2* genes in sense and/or antisense orientation is expected to silence the corresponding native genes, resulting in decreased production of ethylene by the plant due to inhibition of ACC production. The resulting reduction in ethylene is expected to prevent or delay ripening of mature papaya fruit.

**Table 1** Summary of the introduced genes present in the eight types of GM papayas proposed for release and the expected phenotypes. **Entries in bold refer to GM papaya types that were released under PR-128.**

Gene	Promoter <sup>1</sup>	Terminator	Selectable marker <sup>2</sup>	Expected phenotype
<i>capacs 1</i> (sense)	35S	<b>nos</b>	<i>nptII, bla</i>	<b>gene silencing; reduction in ethylene production</b>
<i>capacs 1</i> (antisense)	35S	<b>nos</b>	<i>nptII, bla</i>	<b>gene silencing; reduction in ethylene production</b>
<i>capacs 2</i> (sense)	35S	nos	<i>nptII, bla</i>	gene silencing; reduction in ethylene production
<i>capacs 2</i> (antisense)	35S	<b>nos</b>	<i>nptII, bla</i>	<b>gene silencing; reduction in ethylene production</b>
<i>capacs 1</i> (sense and antisense)	35S	nos	<i>nptII, bla</i>	gene silencing; reduction in ethylene production
<i>capacs 2</i> (sense and antisense)	35S	nos	<i>nptII, bla</i>	gene silencing; reduction in ethylene production
<i>etr1-1</i> (sense)	pga	nos	<i>nptII, bla</i>	loss of ability to perceive ethylene
<i>uidA</i> (sense)	35S	nos	<i>nptII, bla</i>	reporter gene expression

<sup>1</sup> see section 3.5 for description of promoters

<sup>2</sup> two different transformation vectors were used for generating the GM papaya plants. Consequently, the GM papaya plants will contain either two versions of the *nptII* gene, one of which is controlled by a bacterial promoter and will not be expressed in the GM papaya plants, or the *nptII* gene and the *bla* gene, which is also under the control of a bacterial promoter and not expressed in the GM papaya plants (see Section 3.4).

### 1.2.2 Disruption of ethylene perception

52. The proposed release also aims to evaluate the fruit ripening characteristics of GM papaya plants carrying a gene required for ethylene perception from *Arabidopsis thaliana* (mustard cress). *Arabidopsis* plants have been identified that carry a non-functional copy of the *etr1* gene (*etr1-1*) (Chang et al. 1993; Bleecker et al. 1988). Plants carrying this non-functional gene are insensitive to ethylene and exhibit delayed fruit ripening and floral senescence.

53. The *capacs 1*, *capacs 2* and *etr1-1* genes are discussed in more detail in Section 3 of this Appendix. Potential hazards relating to transfer of these genes to other papaya plants are discussed in Appendix 4.

### Section 1.3 Selectable marker and reporter genes

54. Some GM papaya plants proposed for release express the *uidA* gene from the bacterium, *Escherichia coli*, instead of the ethylene-related genes. This gene codes for the enzyme  $\beta$ -glucuronidase (GUS). Its expression enables visual identification of plant tissues in which this enzyme is produced and will provide a means of confirming the effectiveness of the regulatory sequence (promoter) that is used to drive expression of the fruit ripening genes. GUS activity will also be used to indicate the tissues in which the genes are expressed.

55. The GM papaya plants also contain antibiotic resistance genes. These genes were used as selectable marker genes in the early laboratory stages of development of the plants, to enable selection of bacteria or plant cells containing the desired genetic modification. The antibiotic resistance genes are the bacterial neomycin phosphotransferase type II (nptII) gene, conferring resistance to the antibiotics kanamycin and neomycin, and the beta-lactamase (bla) gene. The bla gene confers resistance to the antibiotic ampicillin and is linked to a bacterial promoter that does not function in plants, so the protein is not expressed in the GM papayas. The antibiotic resistance genes are discussed in more detail in Section 3 of this Appendix. Potential hazards relating to transfer of these genes to other organisms are discussed in Appendix 4.

## **SECTION 2 THE PARENT ORGANISM**

56. The parent organism is *Carica papaya* L. (papaya, or paw paw), which is exotic to Australia, but is grown both commercially and in domestic gardens in tropical and subtropical parts of Australia from Western Australia to New South Wales. More detailed information on papaya can be found in a review document 'The Biology and Ecology of Papaya (paw paw), *Carica papaya* L., in Australia' that was produced in order to inform this risk assessment process. This document is available at the OGTR website (<http://www.ogtr.gov.au>).

## **SECTION 3 THE INTRODUCED GENES**

### **Section 3.1 The *capacs 1* and *capacs 2* genes**

57. The *capacs 1* and *capacs 2* genes occur naturally in non-GM papayas (Mason & Botella 1997). They encode the enzyme ACC synthase, a component of the ethylene biosynthesis pathway (see Section 1.1). Ethylene is a plant hormone that regulates many aspects of plant growth and development, including fruit ripening (Alexander & Grierson 2002). ACC synthase (1-amino-cyclopropane-1-carboxylic acid synthase) catalyses the synthesis of ACC (1-amino-cyclopropane-1-carboxylic acid), a metabolic intermediate required for the production of ethylene. The *capacs 1* and *capacs 2* genes introduced into the GM papayas proposed for release have been isolated from the *Carica papaya* variety 'Solo' and are re-introduced into the same variety in different forms (see Table 1) to down-regulate papaya's production of ethylene during fruit development.

58. Down-regulating ACC synthase using the technique employed by the applicant has previously been shown to prevent fruit ripening in tomato (Oeller et al. 1991). The inhibition of ripening is reversed if external ethylene is applied to the tomato fruit. Similarly, the down-regulation of ACC oxidase, another enzyme necessary for the production of ethylene, has been shown to prevent ripening in rock melon (cantaloupe) (Ayub et al. 1996). The aim of the proposed release is to examine the effect of down-regulating *capacs 1* and *capacs 2* on the ripening of papaya fruit under normal field conditions.

59. The *capacs 1* gene is active primarily at the initial stages of fruit ripening, while the *capacs 2* gene is most highly active later in ripening (Mason & Botella 1997). The expression pattern of *capacs 2* in ripening papaya fruit is similar to that of a tomato ACC synthase gene known to be crucial to ripening of tomato fruit (Oeller et al. 1991; Mason & Botella 1997). However, the expression pattern of *capacs 1* is unusual for a ACC synthase gene. This is because, unlike *capacs 2* and other common ACC synthase genes such as *leacs 2* from tomato fruit (Lincoln et al. 1993), *capacs 1* is expressed at high levels in mature green papaya fruit, but its expression decreases steadily during ripening (Mason & Botella 1997).

60. The ACC synthase genes targeted in the GM papaya plants are only expressed in fruit, during the ripening process (information provided by the applicant). Thus, the applicant has indicated that down-regulating *capacs 1* and *capacs 2* is not expected to affect any other ethylene-related processes in the plant. However, as the introduced ACC synthase genes are under the control of the 35S promoter (see Section 3.5), it is possible that ethylene production may also be down-regulated in plant tissues other than fruit, such as leaves or seed. For example, in melons genetically modified to down-regulate ethylene biosynthesis via modification of the activity of another enzyme involved in ethylene biosynthesis, ACC oxidase, ethylene production is also down-regulated in leaves (Ayub et al. 1996).

61. Future applications to release these GM papayas (which would be subject to separate applications and assessments) would require information regarding the impact of down-regulating ethylene biosynthesis on key ethylene-related processes in papaya other than fruit ripening, such as plant growth and development or disease susceptibility, before the application could be considered.

### **3.1.1 Mechanisms for altering *capacs 1* and *capacs 2* activity**

62. Two different methods of initiating down regulation of gene activity are used to generate GM papaya plants with altered ACC synthase activity. Together, these methods are known as ‘gene silencing’, as they result in ‘silencing’ of the normal activity of a targeted gene present in the plant.

63. With the first method, silencing of the papaya’s *capacs 1* and *capacs 2* genes is achieved by inserting the genes into the papaya in either the sense or antisense orientation. This means that the inserted genes will be either in the correct (sense) orientation for translation of the gene to produce the protein, or in the opposite (antisense; incorrect) orientation. The presence of genes inserted in the sense or antisense orientation can silence both the inserted gene and the copy of the gene already present in the plant. This phenomenon was first identified in plants in 1990 (Napoli et al. 1990; Van der Krol et al. 1990) and has since been extensively used in the analysis of plant genes (Wang & Waterhouse 2002).

64. Gene silencing via the introduction of sense or antisense copies of several different genes involved in ethylene synthesis and other aspects of fruit ripening has been demonstrated in other fruit such as tomato and melon (for example (Oeller et al. 1991; Smith et al. 1990; Ayub et al. 1996). In Australia, the limited and controlled release of pineapples with silencing of ACC synthase via insertion of a truncated version of the pineapple ACC synthase gene in a sense orientation was authorised by the previous voluntary system under deemed licence PR-95 and an application to continue evaluation of this release has been lodged with the Regulator.

65. The second method of achieving silencing of the papaya ACC synthase activity involves a more recent technique that introduces both a sense and an antisense copy of a native gene into a plant, linked together by a short non-coding DNA sequence. Such genetic constructs are known as ‘hairpins’. Genes that are introduced in this way are more effective at silencing genes than introducing either the sense or antisense gene alone (Waterhouse et al. 1998). The *capacs 1* and *capacs 2* genes are also introduced into the papaya in this way, using a piece of non-coding DNA from the *pdK* (pyruvate orthophosphate dikinase) gene from the plant *Flavaria trinervia*, to link the sense and antisense versions of the genes. Silencing of another gene involved in ethylene synthesis, ACC oxidase (1-aminocyclopropane-1-carboxylate oxidase), by a similar technique has been demonstrated in tomato (Hamilton et al. 1998).

66. Regardless of which method initiates gene silencing, the ultimate result is decrease in expression of the target gene. Gene silencing in plants appears to be caused by mechanisms that exist naturally to control gene expression and to defend against plant viruses. Recently, many details of the functioning of these mechanisms have been revealed (Waterhouse et al. 2001; Vaucheret et al. 2001).

### **Section 3.2 The *etr1-1* gene**

67. The *etr1* gene encodes for a receptor protein that is involved in ethylene perception (Chang et al. 1993). *etr1* was identified in the plant, *Arabidopsis thaliana*, by analysing plants that are insensitive to ethylene. These plants have a non-functional version of the *etr1* gene (*etr1-1*) and lack several normal responses to ethylene, such as promotion of seed germination, inhibition of root and hypocotyl elongation and acceleration of leaf senescence (Bleecker et al. 1988). The ETR1 protein in these plants lacks a functional ethylene binding site and is, thereby, unable to perceive the presence of ethylene (Schaller & Bleecker 1995).

68. The non-functional *etr1-1* gene is a dominant gene. This means that plants carrying one copy of the functional gene (*etr1*) and one copy of the non-functional gene (*etr1-1*) are insensitive to ethylene (i.e. the effect of the non-functional gene over-rides that of the functional gene).

69. The non-functional *Arabidopsis etr1-1* gene confers ethylene insensitivity on plants other than *Arabidopsis* when it is introduced (Wilkinson et al. 1997). This effect has been demonstrated for tomato, petunia (Wilkinson et al. 1997), tobacco (Knoester et al. 1998) and carnations (Bovy et al. 1999). Plants carrying the non-functional *etr1-1* gene exhibit delayed floral senescence and delayed fruit ripening (Wilkinson et al. 1997; Knoester et al. 1998; Bovy et al. 1999).

70. Plants carrying the *etr1-1* gene do not show major differences in overall growth and development, prior to flowering, compared to plants carrying a functional copy of the gene (Bleecker et al. 1988; Knoester et al. 1998; Bovy et al. 1999). However, some characteristics other than fruit ripening and floral senescence may be altered in GM ethylene insensitive plants. *Arabidopsis* plants with a copy of the *etr1-1* gene are similar to normal *Arabidopsis* plants, except they have lower seed germination rates, altered growth patterns in germinating seeds, slightly larger and longer-lived leaves and lower peroxidase activity (Bleecker et al. 1988). These plants also produce more ethylene under certain conditions, due to lack of feedback inhibition of the ethylene production pathway. Tobacco plants carrying the *Arabidopsis etr1-1* gene also have altered growth morphology of germinating seeds, a lower rate of leaf senescence and impaired perception of neighbouring plants and produce higher levels of ethylene (Knoester et al. 1998).

#### **3.2.1 Ethylene perception and plant disease resistance**

71. In addition to its role in plant growth and development, ethylene is also involved in plant responses to stresses, such as wounding or pathogen attack (Stepanova & Ecker 2000; Thomma et al. 2001). The role of ethylene in plant resistance to pathogens appears to be complex and is not fully understood. Ethylene acts as a signal in initiating plant disease resistance responses and is also associated with symptom development (Stepanova & Ecker 2000). Ethylene insensitive plants have been observed to display both enhanced susceptibility and enhanced resistance to different pathogens.

72. The ability of plants carrying the non-functional *etr1-1* gene, and other genes conferring ethylene insensitivity, to mount resistance responses to pathogens has been investigated. *Arabidopsis* plants with loss of ability to perceive ethylene due to loss of function in the *ein2* gene may be more susceptible to disease caused by a variety of fungal and bacterial pathogens (Thomma et al. 1999; Norman-Setterblad et al. 2000; Ton et al. 2002) and are unable to mount an induced systemic resistance response normally triggered by contact with non-pathogenic soil bacteria (Knoester et al. 1999). Similarly, tobacco plants carrying the *Arabidopsis etr1-1* gene may exhibit decreased levels of some defence-related proteins, and suffer disease caused by soil-borne fungi that normally have limited ability to infect tobacco plants (Knoester et al. 1998; Geraats et al. 2002).

73. In contrast to these observations, plants with impaired ethylene perception may also suffer reduced disease symptoms when attacked by normally virulent pathogens. For example, symptom development is reduced in some ethylene-insensitive *Arabidopsis* plants infected with pathogenic bacteria (Bent et al. 1992). Tomato plants with impaired ethylene perception or ethylene synthesis also show decreased symptoms in response to some pathogenic bacteria and a fungus that are normally able to cause disease (Lund et al. 1998). Likewise, ethylene-insensitive *Arabidopsis* plants have been observed to be less susceptible to a nematode that is normally able to parasitise these plants (Wubben et al. 2001).

74. GM papaya plants with altered ethylene synthesis or ethylene perception could potentially have altered responses to both pathogenic and non-pathogenic microorganisms. Such altered responses could manifest either in non-fruit parts of plants with constitutive down-regulation of ethylene production or potentially in the fruit in *etr1-1* expressing plants. It is expected that *etr1-1* will only be expressed in GM papaya fruit tissues as the promoter controlling its expression is fruit-specific (see Section 3.5 of this Appendix). Observations made on other ethylene-insensitive plants suggest that the effect of altered ethylene metabolism on the resistance response of papaya to microorganisms encountered in the environment may not be predictable and is best elucidated by field evaluation.

75. The possibility that altering the response of GM papayas to pathogens may affect the weediness of GM papayas is considered in Appendix 3.

### Section 3.3 The *uidA* gene

76. The *uidA* gene, from the common soil bacterium *Escherichia coli*, codes for the enzyme  $\beta$ -glucuronidase (GUS). The GUS enzyme converts a colourless substrate into a blue colour in a simple laboratory assay and is used as a reporter or 'marker' to detect tissues that have been successfully genetically modified. Exposure of plant tissues containing the GUS gene to this substrate facilitates measurement of the expression of the *uidA* gene (Jefferson et al. 1986).

77. If expression of the *uidA* gene is controlled by the promoter from another gene, the strength and distribution of the blue colour in the plant tissue indicates the strength of the promoter and hence expression levels of the other gene can be inferred. In the proposed release, GUS is used to evaluate the efficiency of the transformation vector containing the CaMV 35S promoter (see Section 3.5 for details) for driving expression of the *capacs 1* and *capacs 2* genes in papaya tissues.

### Section 3.4 Antibiotic resistance genes

78. The *nptII* gene was isolated from the bacterial Tn5 transposon (Beck et al. 1982). It encodes the enzyme neomycin phosphotransferase type II (NPTII) which confers resistance to aminoglycoside antibiotics such as kanamycin and neomycin. The NPTII enzyme uses ATP to phosphorylate neomycin, and the related kanamycin, thereby inactivating the antibiotic and preventing it from killing the NPTII producing cell.

79. The *nptII* gene functions as a selectable marker in the initial laboratory stages of papaya plant cell selection following genetic modification, allowing modified cells to grow while inhibiting the growth of non-GM cells.

80. The process of the biolistic method of transformation may result in the introduction of additional genetic elements or genes from the transformation vector to the GM papaya plants. Some of the genetic elements or genes present in the vector are designed for replication of the vector in bacteria in the laboratory and will not have any function in plants. Other genes in the vector are designed for selection of bacterial cells carrying the vector in the laboratory. The GM papayas proposed for release are likely to contain either an additional copy of the kanamycin resistance gene (*nptII*) or a copy of a gene encoding resistance to the antibiotic ampicillin (*bla*). These genes are under the control of bacterial promoters and will not be expressed in the GM papaya plants. Some plants will also carry a portion of the  $\beta$ -galactosidase (*Lac Z*) gene, which produces an enzyme used for selection of *E. coli* carrying the vector in the laboratory. The *Lac Z* gene will not be functional in the GM papaya plants.

81. Future applications (which would be subject to separate applications and assessments) to release these GM papayas would require information regarding the presence in the GM papayas of other genes from the vector, before the application could be considered.

82. The potential toxicity of the introduced *ETR1-1*, antibiotic resistance and GUS proteins and ACC synthase enzymes and the potential of these genes to transfer to other organisms, are discussed in Appendices 2 and 4, respectively.

### Section 3.5 Regulatory sequences

83. Expression of the *capacs 1*, *capacs 2*, and *uidA* genes is under the control of a promoter (a region of DNA that determines whether a gene is expressed and to what extent) from the cauliflower mosaic virus 35S gene (the 35S promoter). It is likely that use of this promoter in the GM papayas will result in expression of these genes in all plant tissues. A mRNA termination region, including a polyadenylation signal, is also required for gene expression in plants, and is provided by the *nos* terminator (from the *Agrobacterium nopaline synthase* gene).

84. Expression of the *etr1-1* gene is under the control of a promoter from an apple (*Malus domestica*) polygalacturonase (*pga*) gene (Atkinson et al. 1998). In GM tomatoes, this promoter has been used to target expression of introduced genes in ripening fruit (Atkinson et al. 1998). Use of this promoter in the GM papayas proposed for release by UQ is expected to result in the *etr1-1* gene being expressed only in ripening fruit and not in other parts of the GM papaya plant.

85. Future applications to release these GM papayas would require information regarding the tissues in which genes under the control of the *pga* promoter are expressed.

86. The *nptII* gene for selection in the laboratory is under the control of the *nos*-promoter and the *nos*-terminator (both derived from the *Agrobacterium nopaline synthase* gene).

87. Although some of the regulatory sequences transferred to the GM papaya plants are derived from plant pathogens, they only represent a very small proportion of the pathogen genome. The sequences are not, in themselves, infectious or pathogenic.

#### **SECTION 4 METHOD OF GENE TRANSFER**

88. The *capacs 1*, *capacs 2*, *etr1-1*, *nptII* and *uidA* genes and their associated regulatory sequences have been introduced into papaya (variety 'Solo') by microprojectile particle bombardment. This technique is a well-established method of plant transformation that uses compressed air to 'shoot' tiny tungsten or gold particles coated with the genes to be inserted into plant cells. The introduced genes become incorporated into the genome of the bombarded plant cells. The antibiotic kanamycin is used to select for plant cells containing the introduced genes and these cells are regenerated into whole plants in the laboratory.

#### **SECTION 5 CHARACTERISATION OF THE INSERTED GENETIC MATERIAL AND STABILITY OF THE GENETIC MODIFICATION**

89. The applicant intends to gather data on key information regarding the genetic modification and successful functioning of the inserted genes from the proposed limited and controlled release. It is indicated that these data cannot be generated without a field release.

##### **Section 5.1 Characterisation by Southern blot and PCR analysis**

90. Polymerase Chain Reaction (PCR) and Southern blotting can be used to demonstrate the presence of the introduced *capacs 1*, *capacs 2*, *etr1-1*, *nptII* and *uidA* genes. PCR detects the introduced genes in the GM plant but does not indicate the number of copies of the gene that are present. In contrast, Southern blotting indicates the number of copies of each inserted gene.

91. PCR has been used to demonstrate the presence of the introduced ACC synthase genes in both sense and antisense orientation in the GM papaya plants currently growing at the proposed release site under licence number PR-128 but to date, Southern blotting has not been employed. Using PCR, UQ has indicated that four plants contain the *capacs 1* gene in the sense orientation, eight plants contain the *capacs 1* gene in the antisense orientation and eight plants contain the *capacs 2* gene in the antisense orientation. In addition, UQ has indicated that additional plants containing either sense and/or antisense copies of the *capacs 1* and *capacs 2* genes are in tissue culture. The applicant expects that these immature GM papaya plants will be ready for transplantation to the proposed release site in August 2003.

92. Neither PCR nor Southern blot data are available for the GM papaya plants containing the *capacs 1* and *capacs 2* hairpin constructs, the GUS reporter gene or the *etr1-1* gene, as these GM plants are still being developed. As with the *capacs 1* and *capacs 2* genes, the applicant expects that GM papayas containing the *capacs 1* and *capacs 2* hairpin constructs, the GUS reporter gene or the *etr1-1* gene will be ready for transplantation to the proposed release site in August 2003.

93. As a condition of licence, the applicant is required to confirm the presence of the inserted genes or hairpin constructs in all plants released into the field. More detailed characterisation of the inserted genes and how these affect the phenotype of the GM papayas is not required as a condition of the proposed licence, as the risk management measures given effect by the proposed specific licence conditions (see Appendix 5) effectively manage any risks. However, this additional information would be required by the Regulator if the same GM papayas are proposed for release in future applications (which would be subject to separate applications and assessments), before the application could be considered.

94. Assessing the stability of the introduced genes in progeny requires plants to produce fruit and seed and the resulting progeny to be analysed by Southern blotting. As the GM papayas that are currently released under PR-128 have not yet produced fruit, this is yet to be done. For the same reasons, the stability of the introduced genes has not been assessed in the other GM papaya plants proposed for release.

95. UQ has indicated that plants released into the field under PR-128 are currently flowering and starting to set fruit. Determining the stability of the inserted genes and genetic constructs in progeny of the GM papayas would be required by the Regulator if an additional or larger scale release of the same types of GM papayas are proposed for release in the future (which would be subject to separate applications and assessments).

## **SECTION 6 EXPRESSION OF THE INTRODUCED PROTEINS**

96. Northern blot analysis is a technique that can be used to determine expression levels and functioning of the introduced genes and gene constructs in the GM papaya plants. Mason and Botella (1997) used Northern blot analysis to study expression levels of *capacs 1* and *capacs 2* in non-GM papaya fruit. UQ has indicated that when fruit become available, expression levels of ACC synthase will be monitored in GM papaya fruit from various stages of ripening, to verify the functioning of the inserted genes or genetic constructs.

97. As the *capacs 1* and *capacs 2* sense and antisense genes and hairpin constructs are under the control of the 35S promoter, it is expected that they will be expressed in all plant tissues. Future applications to release the same types of GM papaya (which would be subject to separate applications and assessments) would require analyses of non-fruit parts of the GM papaya plants to ascertain expression levels of the introduced genes and hairpin constructs throughout the plant, before the application could be considered. Future applications would also require analyses of plants containing the GUS gene to evaluate the probable expression pattern and expression level of the introduced genes.

98. As the *etr1-1* gene is under the control of a fruit-specific promoter, the applicant expects that *etr1-1* will only be expressed in fruit. Future applications to release the same types of GM papaya would require measurement of the expression levels of the *etr1-1* gene in fruit and non-edible plant parts such as leaves, the skin of fruit, seeds and roots, before the application could be considered.

## **SECTION 7 EVALUATION OF PLEIOTROPIC EFFECTS OF GENETIC MODIFICATION**

99. A single plant gene can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Single genes inserted into a plant by genetic modification can also be pleiotropic and it is necessary to evaluate genetically modified plants for unintended, pleiotropic effects of the inserted genes, such as changes in agronomic characteristics.

100. The applicant has indicated that because both of the ACC synthase genes targeted for down-regulation are only induced within fruit during the ripening process, and the *etr1-1* gene is controlled by a fruit specific promoter, it is not expected that any other processes will be adversely affected in the transgenic plants.

101. However, there is a potential for down-regulation of ethylene production to have other, pleiotropic effects on the plant. For example, down-regulation of ethylene production in the fruit could result in changes in the response of the fruit to pathogenic or non-pathogenic microorganisms. Down-regulation of ethylene production may also occur in non-fruit tissues of the plant, and there is a potential for effects on plant growth and development and responses to pathogenic and non-pathogenic microorganisms.

102. As a condition of licence, the applicant is required to monitor the GM papayas for any unusual responses to pathogenic or non-pathogenic microorganisms, such as increased or decreased disease occurrence or severity in comparison to what is normally associated with cultivation of non-GM papayas.

103. Evaluation of other characteristics of the GM papaya plants that may be affected by pleiotropy, including overall agronomic performance and compositional analysis of the fruit, is not proposed for this release. Future applications to release these GM papayas would require information regarding potential pleiotropic effects on agronomic performance and fruit composition.

104. Food Standards Australia New Zealand (FSANZ), is responsible for human food safety assessment. Although the applicant has not applied to FSANZ for evaluation of material from the GM papayas for use in human food, FSANZ approval would need to be obtained before GM papayas could be used as human food.

## **APPENDIX 2 TOXICITY AND ALLERGENICITY TO HUMANS AND OTHER ORGANISMS**

105. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. This part of the document considers the potential toxicity and allergenicity of the GMOs or their novel proteins for humans and for other organisms as a consequence of the proposed dealings.

### **SECTION 1 NATURE OF THE POTENTIAL TOXICITY AND ALLERGENICITY HAZARD**

106. Toxicity is the cascade of reactions resulting from exposure to a dose of chemical sufficient to cause direct cellular or tissue injury or otherwise inhibit normal physiological processes (Felsot 2000). Allergic responses are immune system reactions, resulting from stimulation of a specific group of antibodies known as IgE, or sensitisation of specific tissue bound lymphocytes (Taylor & Lehrer 1996; FAO/WHO 2000). Allergy has a well defined etiology (ie. biochemical cause) that is quite different from toxicity.

107. The potential for the GM papayas to be toxic or allergenic to humans or to be toxic to other organisms due to either expression of the novel gene products, decreased expression of the targeted naturally occurring genes or because of unforeseen, unintended effects of the genetic modification is considered. The nature of these hazards depends on the intrinsic characteristics of non-GM papaya (see The Biology and Ecology of Papaya (paw paw), *Carica papaya* L., in Australia, available at the OGTR website [<http://www.ogtr.gov.au>]), the extent to which these may be modified by the inserted genes or genetic constructs (see Appendix 1), the potential exposure of people or other organisms to the GM papayas or their by-products and on other sources of exposure to the introduced proteins.

108. If the GM papayas in the proposed release were to be toxic or allergenic to humans or toxic to other organisms, potential hazards could include adverse impacts on:

109. Human workers associated with the release;

- mammals and wildlife, including bats, rodents and possums that may feed on papaya fruit;
- invertebrates, including both beneficial insects (pollinators, parasitoids or predators of insect pests) and arthropod pests; and
- communities of microorganisms, particularly soil microorganisms.

#### **Section 1.1 Exposure of people to GM papaya**

110. Humans associated with the proposed release will be in contact with papaya plants and papaya fruit. Potentially, harm could occur if the GM papayas became toxic or allergenic than non-GM papaya to people via occupational exposure.

111. It should be noted that there will be no opportunity for humans to consume fruit or other products of the GM papayas proposed for release. However, potential toxicity and allergenicity hazards associated with consumption of GM papaya fruit with delayed fruit ripening characteristics are considered in this Appendix in view of potential future applications (which would be subject to separate applications and assessments) for larger scale release of GM papaya varieties resulting from the release currently proposed.

112. There have been no reported adverse toxic or allergenic effects on human health through occupational exposure resulting from the GM papaya plants released under PR-128.

### **Section 1.2 Exposure of other organisms to GM papaya**

113. The GM papaya plants in the proposed dealing will be grown inside an insect-proof netting enclosure (a knitted 2.0 mm<sup>2</sup> nylon grid net) that will exclude many insects and other arthropods, and all birds and mammalian herbivores and fruit eaters such as rodents, bats and possums. The potential for exposure of these organisms to the GM papaya plants and fruit is thus low. However, potential toxicity hazards to these organisms associated with GM papaya with delayed fruit ripening are considered in light of potential future applications for larger scale releases.

114. Exposure of soil microorganisms to the GM papaya plants will be limited due to the limited scale of the proposed release. Again, potential toxicity hazards to these organisms are considered in light of potential future applications for larger scale releases.

## **SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING**

115. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of the GM papayas on the health and safety of humans and other organisms, a number of factors were considered including;

- the toxicity and allergenicity of non-GM papaya;
- the toxicity and allergenicity of the purified form of the introduced proteins; and
- the toxicity and allergenicity of the GM papayas (the GMOs) proposed for release;

116. Reference to these factors are made throughout the assessment.

### **Section 2.1 Toxicity and allergenicity of non-GM papaya**

117. Numerous studies have demonstrated that leaf, fruit and/or root extracts of non-GM papaya have inherent toxicological or allergenic properties, many of which apparently relate to the complex, largely uncharacterised, chemical composition of papaya latex. Although a range of toxicological studies on papaya have been reported, many of these are associated with impacts on mammalian (including human) fertility and reproduction. In terms of allergenicity, sensitisation to papain among workers in the industry is well known (Baur et al. 1988; Iliev & Elsner 1997). More detailed information on the inherent toxicity and allergenicity of papaya can be found in a review document 'The Biology and Ecology of Papaya (paw paw), *Carica papaya* L., in Australia' that was produced in order to inform this risk assessment process. This document is available at the OGTR website (<http://www.ogtr.gov.au>)

118. The range of toxic and allergenic properties associated with unripe non-GM papaya fruit suggest that in the event of commercial production of the GM papayas in the proposed release (which would require a separate application and assessment process), the potential for the genetic modifications to alter papaya's inherent toxic and allergenic properties must be fully addressed. This consideration is especially important in light of changes in latex levels in the fruit during the fruit ripening process.

119. At this stage of evaluating the GM papayas proposed for release, these data were unavailable and will not be available until the first fruit are produced by the GM trees. As noted earlier, none of the GM papaya fruit will be available for human consumption. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment.

## **Section 2.2 Toxicity and allergenicity of the introduced proteins and of GM papaya fruit**

120. The GM papayas in the proposed release are expected to vary from non-GM papaya either in the expression of additional proteins, ETR1-1, GUS and NPTII, or in the reduction in expression of the naturally occurring ACC synthase enzymes, encoded by the capacs 1 and capacs 2 genes. It is also possible that some GM papaya plants would have increased expression of the ACC synthase enzymes, due to the presence of additional copies of the capacs 1 or capacs 2 genes in the sense orientation. Reduction in the activity of the ACC synthase enzymes could also potentially lead to accumulation of the substrate of the ACC synthase enzyme, S-adenosyl-L-methionine, because this substrate is normally utilised by ACC synthase. Reduction in ACC synthase activity may also perturb other aspects of the ethylene biosynthesis pathway.

121. None of the proteins/enzymes to be expressed, or the naturally occurring proteins to be down-regulated in the GM papaya, have any known intrinsic toxicity or allergenicity. The intrinsic toxic and allergenic properties of papaya are associated with other enzymes and compounds, which are unlikely to be affected by the genetic modifications.

### **2.2.1 Acute Toxicity Studies**

122. Due to the widespread use of both the NPTII and GUS proteins in GM plants currently approved for human consumption, data is available on the toxicology of these proteins. These data consistently demonstrate that the NPTII and GUS proteins are not toxic for mammals. For example, an acute oral toxicity study in mice, in which purified NPTII protein was fed, at doses up to 5000 mg/kg (2500 mg/kg administered twice, four hours apart), did not show any adverse effects (Berberich et al. 1993). There were no treatment-related differences in mortality, weight gain, food consumption, behaviour, clinical signs or gross pathology. Likewise, acute oral toxicity studies in mice, with purified GUS protein at doses of up to 100 mg/kg, did not show any adverse effects (Naylor 1992; ANZFA 2001). The bla gene, conferring resistance to the antibiotic ampicillin in bacteria, will not be expressed in the GM papaya plants and protein encoded by this gene will not be present.

123. At this stage of the evaluation process for the GM papayas proposed for release, acute toxicological data is not available for the ETR1-1 protein, the ACC synthase enzymes, or other components of the plant ethylene biosynthesis pathway, the levels of which in the plants could be altered by changed ACC synthase activity. Such data would be required for evaluation by the OGTR before the GM papaya could be considered for a larger scale release.

### **2.2.2 Stability of the introduced proteins in the human digestive tract**

124. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases (Astwood et al. 1996). This is because it is necessary that a protein is sufficiently stable to reach and cross the mucosal membrane for it to stimulate an allergenic response following oral ingestion (Kimber et al. 1999).

125. The NPTII protein is heat labile and degrades rapidly in simulated human gastric fluid. (Fuchs et al. 1993) reported that no NPTII was detected 10 seconds after addition of simulated gastric fluid as measured by both Western blot and enzymatic activity. Likewise, exposure of the GUS protein to simulated mammalian digestive systems resulted in its rapid degradation (ANZFA 2001). Additionally, the enzymatic activity of NPTII requires the co-factor ATP, which is unstable at the low pH of the digestive system (Flavell et al. 1992).

126. There are no data currently available on the stability of the ETR1-1 protein, ACC synthase enzymes or other components of the plant ethylene biosynthesis pathway in the human digestive tract. As with the acute toxicity studies, this information would be required before the GM papayas could be considered for larger scale releases.

### **2.2.3 Homology with known allergens**

127. The ETR1-1 protein and the ACC synthase enzymes are not derived from known allergen or allergenic organisms. ETR1-1 encodes a non-functional protein with similarity to a group of bacterial proteins involved in signal transduction known as ‘two-component receptor proteins’. The ETR1-1 protein is composed of multiple parts, each likely to have different functions. No other two-component receptor proteins, or proteins similar to the various parts of the ETR1-1 protein, are currently listed as known food or non-food allergens (The Biotechnology Information for Food Safety Database, National Centre for Food Safety and Technology, USA and The Structural Database of Allergen Proteins, The University of Texas Medical Branch).

128. ACC synthase enzymes are homologous to pyridoxal-5-phosphate (PLP)-dependent aminotransferases (Alexander & Grierson 2002). No other ACC synthase enzymes or PLP-dependent aminotransferases are currently listed as known food or non-food allergens (The Biotechnology Information for Food Safety Database, National Centre for Food Safety and Technology, USA and The Structural Database of Allergen Proteins, The University of Texas Medical Branch).

129. A full assessment of the potential allergenicity of the ETR1-1 protein and ACC synthase enzymes would require analysis of DNA or protein sequence homology to all known allergens in the Genbank, EMBL, Pir and Swiss-Prot databases. Food Standards Australia New Zealand (FSANZ), is responsible for human food safety assessment. Currently the applicant has not applied to FSANZ for evaluation of material from the GM papaya for use in human food. However, FSANZ approval for use of the GM papaya in human food would be required before commercialisation of any GM papayas.

130. The NPTII protein does not display characteristics common to known food allergen proteins (US FDA 1998; Fuchs et al. 1993). NPTII is not derived from a known allergen and shows no significant DNA or protein sequence homology to known allergens in the Genbank, EMBL, Pir and Swiss-Prot databases.

131. The GUS protein is also unlikely to be a major allergen and does not display the characteristics common to known allergen proteins (ANZFA 2001). The GUS protein does not have chemical or physical characteristics that are typical of known food allergens and does not share significant amino acid sequence similarity with known allergens.

## 2.2.4 Other sources of the introduced proteins in food

132. The ETR1-1 protein and the ACC synthase enzymes are components of ethylene perception and biosynthesis pathways that are conserved among many plant species. Proteins with a high degree of sequence homology (similarity in terms of amino acid sequence) and with similar function to ETR1-1 and the ACC synthase enzymes are present in many commonly eaten plant products, especially fresh fruits such as tomatoes, melon, cucumber and citrus (Alexander & Grierson 2002).

133. The NPTII and GUS proteins are ubiquitous in the environment and in food chains, in naturally occurring microorganisms found in soil and water and in mammalian digestive systems (Flavell et al. 1992; Gilissen et al. 1998). Both are recognised as commonly present on fresh food.

134. Humans continually ingest kanamycin-resistant microorganisms, some containing the NPTII gene. The diet, especially raw salad, is the major source: at a conservative estimate, each human ingests  $1.2 \times 10^6$  kanamycin-resistant microorganisms daily (Flavell et al. 1992). With  $10^{12}$  kanamycin- or neomycin-resistant bacteria already in the gut of each person, the addition of NPTII through GM papaya food products will be of negligible significance. All human-health analyses need to be viewed against this knowledge.

## 2.2.5 Assessment by other agencies

135. GM papaya with delayed fruit ripening has not been assessed by other regulatory agencies at this stage. However, other fruits in which similar modifications have been made have been assessed for health and safety hazards for humans and other organisms by regulatory agencies in other countries. It is likely that GM papaya with modified fruit ripening will be similar, in terms of generic biochemistry and metabolism, to other fruit crops with similar genetic modifications.

136. In the USA and Canada, tomatoes modified for delayed fruit ripening by down-regulation of ethylene biosynthesis have been approved for human food since the mid 1990s (Chapter 1, Section 2.4). Health Canada assessed these tomatoes as being as safe and nutritious as currently available commercial tomato cultivars (FD/OFB-095-306-A, <http://www.hc-sc.gc.ca>). Health Canada considered that, other than reduced ACC synthase activity, the disease, pest and other agronomic characteristics of the transgenic line was comparable to the unmodified parent line. They also concluded that analysis of nutrients did not reveal any significant differences in the levels of macro- and micronutrients between the GM line and its unmodified parent line. Additionally, the introduction of the truncated ACC synthase gene was not judged to have any potential for additional human toxicity or allergenicity. The same GM tomato line is approved for unregulated commercial use by the USDA Animal and Plant Health Inspection Service (APHIS) (Payne 1995) and is considered to be a safe human food by the US FDA. The USDA/APHIS also concluded that this tomato line exhibits no plant pathogenic properties and is unlikely to harm beneficial organisms, such as bees.

137. In other lines of GM tomatoes down-regulation of ethylene biosynthesis has been achieved in a slightly different way to the GM papayas proposed for release, by insertion of genes encoding enzymes that degrade essential components of ethylene biosynthesis in the plant. USDA/APHIS also concludes that these GM tomatoes are as safe to grow as traditionally bred tomato lines. The US FDA also considers these GM tomato lines to be a safe human food.

138. The use of the NPTII and GUS proteins in food crops has previously been assessed by regulatory agencies both in Australia and in other countries. Use of the NPTII enzyme in tomatoes, canola and cotton has been previously evaluated by the US FDA. The FDA concluded that this enzyme does not have any of the recognised characteristics of food allergens or any attributes that would distinguish it toxicologically from other phosphorylating enzymes in the food supply (FDA 1994), cited in (ANZFA 1999).

139. In their draft risk analysis report for application A378 'Food derived from glyphosate-tolerant sugarbeet line 77 (GTSB77)' ANZFA concluded that food derived from this plant, which expresses the GUS protein, was safe for human consumption. The US Environmental Protection Agency (US EPA) does not consider GUS to be toxic for mammals and has approved its exemption from the requirement to establish tolerance levels.

140. Food Standards Australia New Zealand (FSANZ), is responsible for human food safety assessment. Currently the applicant has not applied to FSANZ for evaluation of material from the GM papaya for use in human food. FSANZ approval would need to be obtained before it could be used in human food.

### **2.2.6 Toxicity to invertebrates and microorganisms**

141. The potential exists for the GM papayas in the proposed release to have altered responses to pathogenic or non-pathogenic microorganisms (see Appendix 1). This impact is unlikely to occur as a direct result of toxic effects of the introduced proteins or the GM papaya to microorganisms but rather, as a result of increased or decreased resistance in the GM papaya plants to certain microorganisms.

142. The applicant is required to report any observed alterations in the responses of the GM papaya plants to pathogenic or non-pathogenic microorganisms as a condition of the licence, if it is issued.

143. ETR1-1 and the ACC synthase enzymes are the only novel proteins expressed in the GM papayas, other than GUS and NPTII, both of which has been extensively studied in the context of other GMOs containing these proteins. The potential for persistence and/or accumulation in the environment of ETR1-1, the ACC synthase enzymes or other components of ethylene biosynthesis in the GM papayas is unknown. However, the ETR1-1 protein, the ACC synthase enzymes and other components of ethylene biosynthesis are naturally occurring plant proteins and are not inherently toxic, the release is on a limited scale and all fruit from the release will be harvested. Thus, any potential persistence and/or accumulation of the novel proteins is unlikely to have an adverse impact on the environment, should this occur.

### SECTION 3 CONCLUSIONS REGARDING TOXICITY AND ALLERGENICITY

144. It is considered that the risk of the GM papayas in the proposed release being more toxic or allergenic to humans or other organisms than non-GM papayas is low because:

- the GM papayas are not likely to become more toxic or allergenic than non-GM papayas as a result of the introduced proteins or the down-regulation of ethylene biosynthesis or altered ethylene perception;
- the introduced proteins, or naturally occurring proteins that may have altered expression levels, are not likely to be toxic or allergenic in themselves;
- available data to date suggest that the risk of toxicity and allergenicity is low but detailed toxicity and allergenicity studies have not yet been conducted;
- exposure of humans and other organisms to the GM papayas will be minimal due to the limited scale and enclosure of the trial within an insect-proof enclosure that also prevents wildlife that may feed on papaya fruit and other animals from entering the release site;
- none of the GM material from the release will be used for human or animal consumption; and
- fruit produced during the release will be removed from the site to prevent fruit proteins from entering the soil;

145. As a condition of licence, the University of Queensland must report any adverse effects on human health and safety or the environment (for example allergic reactions as a result of occupational exposure to the papaya).

146. Further data on the toxicity and allergenicity of the introduced proteins and the GM papaya would be required for any potential future approval for release of GM papaya with delayed fruit ripening (which would be subject to separate applications and assessments) intended for human or animal consumption.

## **APPENDIX 3 ENVIRONMENTAL SAFETY – WEEDINESS**

147. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. Appendices 2-4 consider potential hazards that may be posed to the environment. The potential weediness of the GMO is considered in this Appendix.

148. There are numerous definitions of weeds including ‘a plant growing where it should not be’. Weeds become a problem to the community when their presence or abundance interferes with the intended use of the land they occupy. Weed impacts on biodiversity may occur directly, via direct competitive displacement of more desirable species or indirectly, on various trophic levels within the community, by altering the structure of food webs.

### **SECTION 1 NATURE OF THE WEEDINESS HAZARD**

149. The possibility was considered that the GM papayas might have the potential to be harmful to the environment because of increased potential for weediness, either as a direct result of the genetic modification, or as a result of pleiotropic effects. This could result in increased fitness due to increased seed production, establishment, growth rate, persistence or potential for dispersal, or enhanced pathogen or herbivore resistance. If the GM papayas were to persist and spread in the environment as a weed, this could result in impacts such as loss of native biodiversity or adverse effects on agricultural systems.

### **SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING**

#### **Section 2.1 Weediness of non-GM papaya**

150. In Australia, *C. papaya* is not considered to be a problematic weed of either agriculture (Groves et al. 2002) or of the natural environment (Groves et al. 2000). Although this species has naturalised in some tropical and sub-tropical areas of the continent (Australia’s Virtual Herbarium 2003; Randall 2002), it does not appear to impact significantly on native biodiversity (Groves et al. 2000).

151. *Vasconcella pubescens* (formerly *C. pubescens*) is the only close relative of papaya that has been recorded as a weed (Randall 2002). These reports indicate that weedy infestations are apparently limited to certain tropical islands and localised areas of New Zealand (Randall 2002) and that at worst, the species is only considered to be ‘moderately invasive’ (see references in Randall 2002).

152. There are no records of *V. pubescens* (or other *Vasconcella* species) in any Australian herbaria (Australia’s Virtual Herbarium 2003) and no evidence from key Australian horticultural literature (see, for example, Elliot and Jones 1980) that these species are being cultivated in Australia. While it is possible that locally, *V. pubescens* (and related species) may be grown in Australia, there is no evidence that the species is widespread or is a problematic weed of either agriculture or the natural environment (Groves et al. 2000; 2002).

153. More detailed information on the weediness of papaya can be found in a review document ‘The Biology and Ecology of Papaya (paw paw), *Carica papaya* L., in Australia’ that was produced in order to inform this risk assessment process. This document is available at the OGTR website (<http://www.ogtr.gov.au>).

## **Section 2.2 Potential for enhanced weediness of the GM papayas**

154. Potentially, the GM papayas proposed for release could be at a selective advantage, which may enhance their weediness, if traits such as competitive ability, growth rate, seed production, germination or seed dormancy are affected by the genetic modifications. Similarly, if resistance to pathogens or tolerance of herbivores is affected by the genetic modifications, the papayas may have increased potential for weediness.

### **2.2.1 Effects of modified ethylene biosynthesis on weediness**

155. The applicant has indicated that, other than delayed fruit-ripening, it is not expected that down-regulation of ethylene biosynthesis would affect other ethylene-related processes in the GM papayas modified with the ACC synthase genes. As summarised in Appendix 1, however, ethylene influences a number of processes in plants and these may also be affected in the GM papayas, particularly because the introduced ACC synthase genes are under the control of the 35S promoter (see Appendix 1, Section 3.5). Although down-regulation of ethylene production in fruit or other plant tissues is unlikely to affect attributes of the GM papayas that affect their weedy potential, the Regulator has been unable to determine this conclusively in the absence of evidence regarding the functioning and impact of the introduced ACC synthase genes in different plant tissues.

### **2.2.2 Effects of modified ethylene perception on weediness**

156. Genetic modifications to the ability of some of the GM papayas to perceive ethylene may also modify other processes in the GM papayas, with potential to affect weediness. For example, *Arabidopsis thaliana* plants with altered ethylene perception due to expression of a non-functional version of the *etr1* gene germinated at very low rates compared to 'normal' *A. thaliana* seeds (Bleecker et al. 1988). Decreased germination rates imply a potential for seeds to accumulate in the soil seed bank and persistent soil seed banks are an attribute of many weed species (Baker 1965; Noble 1989; Williamson & Fitter 1996).

157. As papaya only reproduces by seed (Nakasone & Paull 1998), potential development of a persistent soil seed bank may affect the weedy potential of the GM papayas proposed for release. Accordingly, future applications to release the same types of GM papayas (which would be subject to separate applications and assessments) would require investigation of the germinability of GM papaya seeds and determination of the impact of altered ethylene perception and biosynthesis on key ethylene-related processes within the plant, before any such application could be considered. The Regulator would also require information regarding the extent and persistence of a *C. papaya* soil seed bank associated with the known naturalised populations of papaya in Queensland, in the event of any future application to release the GM papayas on a larger scale. There appears to have been few published investigations of papaya soil seed banks, but the potential for their development is clear (Perez-Nasser & Vazquez-Yanes 1986) and they are an attribute of papayas that enable establishment in some disturbed habitats (Kwit et al. 2000).

158. Disrupted ethylene perception in ethylene-insensitive *A. thaliana* led to a 25% increase in the size of some leaves, delayed senescence of some leaves and delayed production of mature flowers (Bleecker et al. 1988). Potentially, modifications of this type in the ethylene-insensitive papayas proposed for release may affect their capacity to tolerate herbivory. For example, if leaf area is increased significantly, the area of leaves damaged by herbivores may be a relatively low proportion of the total leaf area in the GM plants. Alternatively, disruption of ethylene perception may alter plant defence responses to herbivores, thereby affecting tolerance of herbivory. Nonetheless, it should be noted that, compared with fungal and viral pathogens, arthropod herbivores are relatively minor pests of commercial papaya plantations (see OGTR 2003) and are unlikely to significantly affect the persistence and spread of potentially weedy papaya plants.

159. Increased leaf area associated with disrupted ethylene perception may also modify competitive ability and, thereby, affect potential weediness. It is well-known, for example, that increased leaf area affects competition between neighbouring plants (Garrity et al. 1992; Van Delden et al. 2002).

160. Ethylene also plays a role in the defence response of plants challenged by microbial pathogens. As detailed in Appendix 1, ethylene insensitivity has led to increased susceptibility to some pathogens (Thomma et al. 1999; Norman-Setterblad et al. 2000; Ton et al. 2002) and to some normally non-pathogenic microorganisms (Knoester et al. 1998; Knoester et al. 1999; Geraats et al. 2002). Conversely, ethylene insensitive plants, and plants with down-regulated ethylene production, have reduced symptoms in response to some normally pathogenic organisms (Wubben et al. 2001; Lund et al. 1998; Bent et al. 1992). If pathogens are a major limitation to the spread and persistence of *C. papaya* in natural Australian ecosystems, the ethylene insensitivity conferred by the *etr1-1* gene may confer a selective advantage leading to enhanced weediness. Future applications to release the same types of GM papayas would require data regarding the impact of limiting ethylene perception (and of down-regulating ethylene production) on the response of the GM papayas to susceptibility to key pathogens, before the application could be considered.

161. The applicant has indicated that the *etr1-1* gene introduced into the GM papayas proposed for release will be controlled by a fruit-specific promoter (*pga*; see Appendix 1, Section 3.5). Accordingly, *etr1-1* is only expected to be expressed in tissues associated with papaya fruit and it is unlikely that ethylene-related processes in other papaya tissues would be affected by its introduction. While leaf area, for example, may not be affected by ethylene-insensitivity because of the fruit-specific promoter driving expression of *etr1-1*, characteristics of the seeds, which are born by fruit, may be affected. In the absence of evidence regarding either the introduction and functioning of the *etr1-1* gene, or the effectiveness of the *pga* promoter in limiting its expression to fruit tissues, the Regulator is unable to determine conclusively that the GM papayas with altered ethylene perception do not have potential for enhanced weediness.

### **2.2.3 Effect of antibiotic resistance and GUS expression on weediness**

162. The antibiotic resistance *nptII* gene will not confer a selective advantage on the GM papayas, since antibiotics are not applied to papayas and there is no reason to expect that expression of the protein would alter any of the characteristic attributes of papaya that would be important for weediness. Similarly, the GUS protein is extremely unlikely to confer any selective advantage on the GM papayas that might result in weediness (Gilissen et al. 1998).

## **Section 2.3 Spread of GM papayas in the environment**

### **2.3.1 Spread of pollen from the GM papayas**

163. In Queensland, most *C. papaya* fruit are produced following cross-pollination by hawkmoths (Lepidoptera: Sphingidae) (Garrett 1995; Morrisen et al. 2003) (see Appendix 4). To prevent pollen flow from the release site the applicant has proposed to contain the whole release area in an insect-proof enclosure designed to eliminate access to the GMOs by key pollinating insects. The genetic modifications to the papaya plants proposed for release are unlikely to alter the dispersal of papaya pollen or other aspects of its pollination biology. Licence conditions require that this enclosure is monitored regularly and that any damage is repaired immediately.

### **2.3.2 Spread of fruit and seeds from the GM papayas**

164. Other than human transportation of papaya fruit, the most likely means of *C. papaya* being dispersed in the environment is by flying foxes (*Ptilinopus* spp.). The applicant has indicated that flying foxes are the chief mammalian predators of papaya in Australia and, therefore, the most likely non-human dispersers of papaya seeds. Potentially, however, a range of other species could aid papaya fruit and seed dispersal including other bats, various other mammals including rodents and possums, and some birds.

165. The genetic modifications to the papaya plants proposed for release are unlikely to alter the dispersal of papaya fruit and seeds. However, if fruit ripening is delayed and fruits are retained on trees for longer, they may be available to frugivores that disperse the fruit for longer periods of time. Delayed fruit ripening traits may also make the fruit a less attractive food resource and, potentially, may decrease the natural dispersal patterns of papaya fruit and seed.

166. The insect-proof enclosure which was proposed by the applicant to prevent dispersal of GM papaya pollen from the release site will have the additional advantage of preventing animals from accessing the release site. This will significantly limit the potential for GM papaya fruit or seeds to be dispersed, particularly as a licence condition requires that the enclosure is sealed at ground level. Additional licence conditions require the applicant to implement an accounting procedure for all fruit that are produced by the GM papaya trees and to monitor the insect-proof enclosure every day to ensure that its integrity is not compromised. If this occurred to the extent that immediate repairs could not be made, the applicant is required to remove and destroy all flowers and fruit to prevent the inadvertent dispersal of fruit and seed.

167. The licence also requires the applicant to transport any GM material, including fruit and seeds for analyses, in accordance with OGTR transportation guidelines. These require that GMOs and GM materials must not be transported unless contained within a primary, sealed container that is packed in a secondary, unbreakable container. In combination, these measures would significantly limit the likelihood of spread in the environment.

## **Section 2.4 Persistence of the GM papayas at the release site**

168. Fruit will be harvested during the trial for analyses of physiological, nutritional and quality attributes. Although some of the fruits will be allowed to ripen on the trees, many will be harvested while still green and immature, to enable analysis of the fruit-ripening process at different stages of maturity. As fruit bare the seeds, this research objective will significantly limit the potential for seeds of the GM papayas to become incorporated into a soil seed bank within the trial site and, thereby, will limit the potential for persistence of the GMOs at the site. As noted above, however, the applicant is required to account for each fruit produced on each papaya tree planted in the insect-proof enclosure, to regularly monitor the development of such fruit and to prevent ripe papaya fruit from dropping to the ground. This accounting and monitoring procedure would further limit the potential for GM papaya seeds to persist at the release site.

169. Although it is difficult to reproduce papaya vegetatively (e.g. by ‘cuttings’) the applicant is also required to destroy the GM papayas at the trial’s conclusion, by cutting the trees to ground level and spraying the stumps with an appropriate herbicide. This will limit the potential for persistence of the GM papayas at the site. Further, licence conditions require that all plant material derived from the felled trees and not required for further research is destroyed by incineration.

170. In addition, post harvest monitoring of the release site is required as a condition of the licence to ensure that any papayas that may germinate or re-grow in the release area after the trial has concluded are destroyed before flowering and that the GM papayas are unable to persist in the environment.

## **SECTION 3 CONCLUSIONS REGARDING WEEDINESS**

171. It is concluded that the down-regulation of ethylene biosynthesis, the modification to ethylene perception and the other introduced genes are unlikely to affect attributes of the GM papayas proposed for release that may alter potential weediness.

172. Aspects of the proposed release supporting this consideration are that:

- papaya is not a problematic weed in Australia; and
- no closely related species are problematic weeds in Australia.

173. However, further information on the impact of the genetic modifications on attributes of GM papayas that may affect their weediness (eg. dormancy of seeds, competitive ability, susceptibility to natural enemies and spread within the environment) would be required before the Regulator could determine this conclusively. This information would be required before a larger scale or less stringently controlled release could be considered (see Chapter 2, section 3).

174. In addition, it is further concluded that the low risk of weediness could be managed to an acceptable level by implementing various strategies to minimise the spread and persistence of GM papayas in the environment. As proposed by the applicant, these strategies include:

- restricting the proposed release to one hectare; and
- preventing dispersal of the GM papaya seeds by containing the release in an insect-proof enclosure that also prevents larger animals accessing the GM plants and their fruit.

175. Details of these and other risk management conditions relating to the potential weediness of the GM papayas proposed for release are provided in Appendix 6.

## **APPENDIX 4 ENVIRONMENTAL SAFETY - TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS**

176. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. Appendices 2 - 4 consider potential hazards that may be posed to the environment. The potential for gene transfer from the GM papayas to other organisms is considered in this Appendix.

177. Gene transfer is the movement of genes between individuals. Within a species, genes are routinely exchanged between individuals of successive generations through sexual reproduction in animals, cross pollination in plants and conjugation in bacteria. Hybrids can be produced between closely related species. In plants, for example cross pollination of wheat and rye produces triticale, in animals sexual reproduction of a horse and a donkey produces a mule. Hybrid progeny may be fertile or sterile, meaning that hybridisation may or may not lead to the introgression of a gene or genes into a population. Gene transfer is not readily observed between distantly related species. However, gene transfer between sexually incompatible organisms can occur. Reconstruction of 'family trees' based on DNA sequence similarities reveals that ancestral plants have occasionally exchanged small DNA fragments with distantly related organisms. In general, there seems to have been only very limited transfer of genes from plants to other types of organisms.

178. For ease of reference, the assessment of gene transfer to other organisms is presented in four main sections:

- Section 1 details the nature and likelihood of genes introduced to the GM papayas transferring to other plants, including to other papayas;
- Section 2 details the nature and likelihood of genes introduced to the GM papayas transferring to microorganisms;
- Section 3 details the nature and likelihood of genes introduced to the GM papayas transferring to animals, including humans; and
- Section 4 provides conclusions regarding the gene transfer hazard with respect to the issues considered in each of the sections 1-3.

179. In general terms, the types of hazards that might result from transfer of the genes introduced into the GM papayas to other organisms include the production of plants with delayed fruit ripening or other attributes associated with ethylene production or perception such as altered responses to soil microbes, modified germination characteristics or increased leaf area. Such plants may then develop an advantage in the natural environment with potential to reduce native biodiversity or disrupt ecosystem structure and function. Another potential hazard is the transfer of the antibiotic resistance genes to pathogens which may generate antibiotic-resistant pathogens with potential to harm human or animal health.

## SECTION 1 TRANSFER OF INTRODUCED GENES TO OTHER PLANTS

### Section 1.1 Nature of the gene transfer hazard

#### 1.1.1 Transfer of genes to other papayas

180. Transfer of the introduced genes or regulatory sequences to other papaya plants would present the same hazards and have the same potential impacts as the presence of the genes in the GM papayas proposed for release (see Appendices 2 and 3). Gene transfer to non-GM papaya by cross-pollination is very likely to produce seeds containing the inserted genes and, if these germinate and establish, would most probably result in papaya plants with similar traits to the GM papayas proposed for release.

181. If transfer occurred to papaya plantations, domestically cultivated papaya trees or naturalised papaya populations, this would increase the possibility that the genes would persist in the environment. The flow-on impacts of such transfer would depend on whether the inserted genes confer a selective advantage, potentially enhancing weediness of papaya in Australia (see Appendix 3), or whether the inserted genes affect the toxicity or allergenicity of papaya which may affect the safety of humans or other organisms that may consume or handle the fruit or other tissues derived from the GM papayas.

#### 1.1.2 Transfer of genes to other plant species

182. Transfer of the introduced genes or regulatory sequences into other plant species, in particular to native flora, may have adverse effects on biodiversity. Other potential hazards specific to the transferred gene sequences are as follows:

➤ ACC synthase genes and related constructs:

All plant species have ACC synthase genes. It is thus theoretically possible, that if gene transfer of the *capacs 1* or *capacs 2* gene silencing constructs to other plant species occurred, these constructs could initiate silencing of endogenous ACC synthase genes in other plant species. The likelihood of this occurring depends on the degree of DNA sequence homology between the *capacs 1* or *capacs 2* genes and the ACC synthase genes in other plant species.

Plants could thus have down-regulation of endogenous ethylene production, which may delay ethylene-related processes, particularly floral senescence and fruit ripening. In the unlikely event of this occurring, there is a very low possibility that it may decrease the attractiveness of such plants to natural enemies (herbivores and pathogens) or enhance their ability to compete with other plants, with potential consequences for weediness.

➤ *etr1-1* gene:

Plants could become insensitive to ethylene. This may delay or prevent maturation of fruit or affect other ethylene-related processes in the plant including, for example, responses to pathogens or promotion of seed germination, as summarised in Appendix 1, Section 3.2. These aspects of plant biology have potential to affect weediness.

- Antibiotic resistance and reporter genes:  
Plants could become resistant to the antibiotics or produce the GUS protein. Antibiotic resistance would only have an impact if the antibiotics were used for control of the plants. The antibiotics in question (*nptII* and *bla*) were used in the laboratory solely to select for genetically modified cells or plants and are not applied to plants outside the laboratory. Note that although *nptII* is expressed in the GM papayas proposed for release, *bla* is under the control of a bacterial promoter and is not expressed in the GM plants because the promoter that is required for its expression is not active in plants. Note, also, that GUS expression is unlikely to be toxic or allergenic (Appendix 2) and is unlikely to affect the weediness (Appendix 3).

- CaMV 35S promoters and other regulatory sequences:  
If gene transfer did occur, there could be unintended or unexpected effects if the introduced regulatory sequences altered the expression of endogenous plant genes. If such perturbation of normal plant gene expression occurred, the impact would depend on the phenotype.

One of these regulatory sequences is derived from a plant pathogen (*Agrobacterium tumefaciens*). The possibility has been considered that it may have pathogenic properties.

## **Section 1.2 Likelihood of gene transfer to other papayas, or other plant species**

183. The most likely means by which the inserted genes could be transferred to other plants is by cross-pollination (out-crossing). It has been well-demonstrated that hawkmoths (family Sphingidae) are the primary pollinators of papaya in Australia and that other agents, including bees and wind, are of little, if any, importance (Garrett 1995; Morrisen et al. 2003; OGTR 2003). Hawkmoths potentially provide, therefore, the most likely means by which pollen could be transferred to non-GM papayas or to other plant species. The likelihood of transfer to non-GM papayas would be affected by several variables including the relationship between pollinator foraging ranges and the distance between papaya trees. There appear to be no published data on the typical foraging ranges of sphingids, but their foraging behaviour does not appear to be affected by wind direction (Garrett 1995).

184. For a detailed consideration of the likelihood of gene transfer from papayas, including an overview of the pollination biology of papaya, see the document, 'The Biology and Ecology of Papaya (paw paw), *Carica papaya* L., in Australia' that was produced in order to inform this risk assessment process. This document is available at the OGTR website (<http://www.ogtr.gov.au>).

185. In summary, the likelihood of gene transfer to other species, including papaya's closest relatives in the genus *Vasconcella*, is negligible because of substantial genetic incompatibility. Moreover, although these close relatives may be available horticulturally, key literature (e.g. Elliot and Jones 1980) does not reference them or recommend their cultivation, and there is no evidence that they occur widely in Australia. Well-demonstrated genetic differences also limit gene transfer to more distantly related plant genera.

### 1.2.1 Proximity to other papayas

186. Other than six non-GM papaya trees grown about 200 m from the proposed release site elsewhere on the Queensland Department of Primary Industries research station at Redlands, Queensland, the closest papaya trees occur in domestic gardens, about 500 m from the release area. The closest commercial papaya plantation is located 12.5 km from the proposed release site (information provided by the applicant). There are 50-60 km between the proposed release area and the nearest known naturalised papaya population (Australia's Virtual Herbarium 2003), which was recorded from the southern slopes of Mt Beerburrum, near Caloundra, in south-east Queensland. Both the commercial plantation and the naturalised population are unlikely to be within foraging range of any pollinators that may access the GM papayas proposed for release.

187. Irrespective of the distances between the proposed release site and other non-GM papayas, the licence requires the applicant to enclose the entire release site in a self-supporting insect-proof enclosure secured at ground level, that prevents the movement of known papaya pollinators into, and from, the enclosure. In addition, the licence requires that the enclosure is monitored every day for breaches of its integrity and that any such breaches are repaired immediately. The licence also requires that any male flowers produced by the GM papayas are removed prior to opening. These measures would limit the likelihood of gene transfer to other papayas to negligible levels.

188. The consultation version of the risk assessment and risk management plan included a risk management measure to bag hermaphrodite flowers to minimise the unlikely potential for gene transfer from the GM papayas. Advice received from the applicant indicated that bagging hermaphrodite flowers was likely to affect the experimental objectives of the release by damaging developing flowers and fruit. In addition, the applicant submitted that the bagging was unnecessary and not warranted, given the requirement to contain the trial in an insect-proof enclosure that prevents pollen movement out of the release area by pollinators, and the assessment of a low level of risk posed by the proposed dealing in the unlikely event of such pollen movement occurring.

189. In relation to the applicant's submission, it is significant that available evidence clearly demonstrates that in Queensland, papaya flowers are rarely, if ever, successfully pollinated by wind-dispersed pollen (Garrett 1995; OGTR 2003). The insect-proof enclosure is likely to further lower the likelihood of successful wind pollination, particularly because papaya pollen is sticky or powdery (see Garrett 1995) and, thereby, unlikely to be blown through the insect-proof netting.

190. In consultation with GTTAC members it was determined that the risk of gene transfer from the GM papayas could be managed effectively without bagging hermaphrodite flowers, so long as the frequency with which the insect-proof enclosure is inspected for damage is increased from twice per week to daily, that any damage is repaired immediately and that if damage cannot be repaired immediately, flowers and fruits from the GM papayas are removed and destroyed to prevent the potential for pollen or seed movement from the release site.

191. Accordingly, the proposed condition to bag hermaphrodite flowers has been removed and the licence now requires the UQ to inspect the insect-proof enclosure for damage every day and to repair any damage immediately. If the damage cannot be repaired immediately, the licence then requires that flowers and fruits from the GM papayas are immediately removed and destroyed.

## SECTION 2 TRANSFER OF INTRODUCED GENES TO MICROORGANISMS

192. The transfer of genes from plants to other types of organisms cannot occur through cross pollination. The most likely means by which this could occur is via horizontal gene transfer — the transfer of genetic material from one organism (the donor) to another organism (the recipient) which is not sexually compatible with the donor (Conner et al. 2003). Horizontal gene transfer is not an abstract theoretical process (Jain et al. 1999). There is growing evidence that horizontal gene transfer has been a principal force in the evolution of genomes, particularly in bacterial genome evolution (Ochman et al. 2000; Jain et al. 1999; Smalla et al. 2000; Stanhope et al. 2001).

### Section 2.1 Nature of the gene transfer hazard

193. Potential hazards, with respect to the specific gene sequences, are as follows:

- ACC synthase genes and related constructs:  
This is unlikely to present a risk to human health or the environment, in the extremely unlikely event that it occurred, because it is highly unlikely that the genes would function or produce functioning proteins.
- Antibiotic resistance genes:  
Microorganisms could become resistant to the antibiotics. The consequences of this for human health and safety and the environment would depend on:
  - the pathogenicity of the microorganism;
  - the use and significance of the antibiotic in clinical and/or veterinary practice;
  - whether resistance to the antibiotic is already widespread in the microbial population.

The antibiotic resistance genes occur within naturally occurring genetic elements (transposons and plasmids) that are readily transferable between bacterial species (US FDA 1998; Flavell et al. 1992; Langridge 1997; Pittard 1997). Gene transfer between bacteria via these elements, through well documented mechanisms for horizontal transfer (Dobhoff-Dier et al. 2000; Nielsen et al. 1998; Nielsen 1998), is far more likely than transfer of the same gene from GM papaya.

- GUS reporter gene (*uidA*):  
This would not present a risk to human health or the environment, in the extremely unlikely event that it occurred. The GUS gene occurs naturally in some soil bacteria and transfer from these bacteria to other bacteria is much more likely than from the GM papayas proposed for release.
- CaMV 35S promoter and other regulatory sequences:  
The expression of endogenous genes in the recipient microorganism could be altered. If a change in normal gene expression did occur, the hazard to the recipient microorganism and to the environment would depend on the specifics of the resultant phenotypic change.

Some of these sequences are derived from plant pathogens (cauliflower mosaic virus, figwort mosaic virus, *Agrobacterium tumefaciens*). The possibility was considered that the sequences might have pathogenic properties.

The possibility that the regulatory sequences could recombine with the genome of another virus infecting the plants to create a novel recombinant virus has also been considered.

(a)

The CaMV 35S promoter is already ubiquitous in the environment and in the human diet (Hodgson, 2000). This promoter and the other bacterial regulatory sequences could be transferred to other microorganisms by their native bacterial hosts.

## **Section 2.2 Likelihood of gene transfer from the GM papayas to microorganisms**

194. Horizontal Gene transfer can occur between sexually incompatible organisms. Most gene transfers have been identified through analyses of gene sequences (Ochman et al. 2000; Worobey & Holmes 1999). In general, gene transfers are detected over evolutionary time scales of millions of years (Lawrence & Ochman 1998). Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman et al. 2000).

195. In contrast, transfers of plant genes to other organisms such as bacteria, fungi or viruses is exceedingly rare (Mayo & Jolly 1991; Nielsen et al. 1998; Nielsen et al. 2000; Harper et al. 1999; Schoelz & Wintermantel 1993; Greene & Allison 1994; Pittard 1997; Aoki & Syono 1999; Worobey & Holmes 1999). The transfer of plant genes to bacteria and viruses has been observed in laboratory and glasshouse experiments (Nielsen et al. 1998; Nielsen et al. 2000; Schoelz & Wintermantel 1993; Greene & Allison 1994; Pittard 1997; Worobey & Holmes 1999). However, in all cases this was achieved only under controlled conditions with the presence of related gene sequences (homologous recombination), and using highly sensitive or powerful selection methods to detect rare gene transfer events.

### **2.2.1 Bacteria**

196. Natural transformation is a mechanism by which transfer of DNA from plants to microorganisms could have occurred during evolution (Bertolla & Simonet 1999) and is the mechanism that is most likely to contribute to a horizontal gene transfer from transgenic plants to bacteria (Smalla et al. 2000). Natural transformation enables competent bacteria to generate genetic variability by taking up and integrating free DNA that is present in their surroundings. This uptake of DNA does not necessarily depend on DNA sequence, thus indicating the potential of gene transfer from divergent donor organisms (Nielsen, 1998).

197. Bertolla and Simonet (1999) identified several steps that would be required for natural transformation to occur:

- Release of the DNA molecules from plant cells into the environment;
- Protection of the free DNA from enzymatic activities;
- Presence of bacterial genotypes capable of developing competence for natural transformation;
- Appropriate biotic and abiotic conditions for the development of the competent stage.
- Efficient adsorption of the DNA to the bacterial cell surface.

- Efficient DNA uptake.
- Chromosomal integration via recombination or autonomous replication of the transforming DNA.
- Expression of the genes by the recipient bacterium.

198. Competence in bacteria is not usually constitutively expressed and bacterial cells that are transformable need to enter a physiologically regulated state of competence for the uptake of exogenous DNA (Lorenz & Wackernagel 1994). The major limiting factor for natural transformation remains the presence of competent bacteria and the development of competence (Smalla et al, 2000). Few bacteria induced to express competence in the laboratory have subsequently been shown able to express competence under natural conditions (Nielsen, 1998).

199. All of the steps identified by Bertolla & Simonet (1999) would need to occur simultaneously in the same place to enable gene transfer to occur via this mechanism. Barriers such as the development of competence make this scenario highly unlikely. It is yet to be demonstrated that plant-bacterium transfer occurs under natural conditions.

200. Several studies have demonstrated the persistence of plant DNA in the soil (Gebhard & Smalla 1999; Paget & Simonet 1994; Widmer et al. 1996; Paget & Simonet 1997; Widmer et al. 1997). Bacteria residing on the plant surface can access nutrients leaking from the leaf or exuded from the root and they often aggregate in biofilms that can facilitate cell-to-cell contact and may, thereby, possibly transfer DNA. Several studies have also demonstrated the persistence of plant DNA in the gastrointestinal tract of animals (see Section 2.3.1), in contact with the microorganisms that colonise the whole length of the gastrointestinal tract and aid in the digestive process. However, the proportion of DNA which may derive from the introduced genes of GM plants in the animal diet is extremely low (see Section 2.3.1).

201. Horizontal gene transfer from plants to bacteria has not been demonstrated under natural conditions (Syvanen 1999) and deliberate attempts to induce such transfers have so far failed (eg Schlüter et al. 1995; Coghlan 2000). Transfer of plant DNA to bacteria has been demonstrated only under highly artificial laboratory conditions, between homologous sequences and under conditions of selective pressure (Mercer et al. 1999; Gebhard & Smalla 1998; De Vries & Wackernagel 1998; De Vries et al. 2001) and even then only, at a very low frequency.

202. Using antibiotic selection to detect extremely rare events, *Acinobacter* sp. cells containing a defective copy of the neomycin resistance (*nptII*) gene (with 10 bp or 317 bp of DNA deleted) were observed to incorporate DNA from GM plants (sugarbeet, tomato, potato or oilseed rape) carrying the intact *nptII* gene, leading to restoration of neomycin resistance. Without the artificially introduced homology in the recipient strain, no uptake of DNA could be detected in *Acinobacter* sp. (Nielsen et al. 2000; De Vries et al. 2001) or in *Pseudomonas stutzeri* (De Vries et al. 2001).

203. Electrical fields and current are also known to be capable of permeabilising bacterial cell membranes under laboratory conditions, facilitating experimental transformation. Given that the environment is subjected to regular thunderstorms and lightning discharges that induce enormous electrical perturbations, the possibility of natural electro-transformation of bacteria has been investigated. Bacteria added to soil have been transformed via simulated lightning in the laboratory (Demaneche et al. 2001).

204. Integration of genes into the genome of recipient bacteria is known to be dependent on sequence homology between the captured DNA and that of the recipient bacteria. It seems that heterology between these sequences is the main barrier to the stable introduction of diverged DNA in bacteria (Baron et al. 1968; Rayssiguier et al. 1989; Matic et al. 1995; Vulic et al. 1997). There is a decreasing exponential relationship between recombination frequencies in enterobacteria and increasing sequence divergence of the introduced DNA (Vulic et al. 1997). Although there is a higher probability of recombination when the sequences become more similar, the risks of adverse effects resulting from such recombination is reduced because the likelihood of novel and hazardous recombinants being generated is less.

205. Even if transfer and establishment barriers were overcome, there are also barriers to expression of the exogenous genes. Gene promoters have to be compatible with expression in prokaryotes. Probably the single most important factor in determining whether the exogenous DNA would be integrated into bacteria is the strength of selection pressure. Prokaryotes have efficient genomes and generally do not contain extraneous sequences. If the genes are not useful to the organism then there will be no selective advantage in either integrating the genes or maintaining them in the genome.

### **2.2.2 Viruses**

206. There is a theoretical possibility of recombination between sequences that have been introduced into the genome of GM plants and the genome of viruses that infect the plants (Hodgson 2000a; Ho et al. 2000; Hodgson 2000b). Recombination between viral genomes and plant DNA has only been observed at very low levels, and only between homologous sequences under conditions of selective pressure, eg regeneration of infectious virus by complementation of a defective virus by viral sequences introduced into a GM plant genome (Greene & Allison 1994; Teycheney & Tepfer 1999).

### **2.2.3 Fungi**

207. Fungi are known to be transformable, and horizontal gene transfer from plants to plant-associated fungi has been claimed. Uptake of DNA from the host plant by *Plasmodiophora brassicae* (Bryngelsson et al. 1988; Buhariwalla & Mithen 1995) and uptake of the hygromycin gene from a GM plant by *Aspergillus niger* (Hoffman et al. 1994) have been reported. However, stable integration and inheritance of the plant DNA in the genome of these fungi has not been substantiated by experimental evidence (Nielsen 1998).

## **SECTION 3 TRANSFER OF INTRODUCED GENES TO ANIMALS**

### **Section 3.1 Nature of the gene transfer hazard**

208. The potential hazards associated with the genes introduced in the GM papayas transferring to animals, including humans, could be highly variable, broadly depending upon the phenotype of the recipient and any changes to the survival or reproductive capacity of it or its progeny. The potential hazards posed by the specific gene sequences, are as follows:

- ACC synthase genes and related constructs:  
This would not present a risk to human health or the environment, in the extremely unlikely event that it occurred.

- Antibiotic resistance genes:  
Animals could become resistant to the antibiotics. If the transfer occurred to humans or other animals treated with these antibiotics, the antibiotics may be inactivated before they are able to control the targeted bacterial pathogen. The possibility of the transfer of these genes reducing the efficacy of antibiotic treatments has been considered.
- GUS reporter gene:  
This would not present a risk to human health or the environment, in the extremely unlikely event that it occurred.
- CaMV 35S promoter and other regulatory sequences:  
The expression of endogenous genes in the recipient animal could be altered. If a change in normal gene expression did occur, the hazard to the recipient animal and to the environment would depend on the specifics of the resultant phenotypic change.

## **Section 3.2 Likelihood of gene transfer from the GM papayas to animals**

### **3.2.1 Humans**

209. The most significant route for entry of foreign DNA into humans is through food, as it passes through the gastrointestinal tract. The epithelial lining of the gastrointestinal tract is routinely exposed to foreign DNA released from food. The likelihood of DNA from the GM papayas proposed for release transferring to humans directly, following consumption is extremely unlikely.

210. In addition, it should be noted that Food Standards Australia New Zealand (FSANZ), is responsible for human food safety assessment. Currently, the applicant has not applied to FSANZ for evaluation of material from the GM papayas for use in human food. FSANZ approval would need to be obtained before it could be used in human food.

#### **TRANSFER TO HUMANS VIA BACTERIA**

211. It has been hypothesised that the genes introduced to GM plants could be transferred to humans (and other animals) indirectly, via bacteria that occur in the gut. Several studies have investigated the likelihood of this potential hazard, since the gastrointestinal tract may be exposed to DNA from ingested food for many hours on a daily basis and microorganisms colonise the length of the gastrointestinal tract, aiding the digestive process.

212. Netherwood et al. (2002) investigated whether DNA in food was able to survive the human gastrointestinal tract. Seven ileostomists (people with a colostomy bag) were recruited for the trial in which they were fed GM (Roundup Ready<sup>®</sup>) soya products. The survival of both the Roundup Ready<sup>®</sup> gene and an endogenous soya gene were traced. Surprisingly, a large portion of the transgene from the GM soya was observed to survive the passage of the small intestine. The level of persistence of the endogenous soya gene was similar to that of the Roundup Ready<sup>®</sup> gene, indicating that the transgene was degraded at a similar rate to the bulk soya DNA. In this study, GM soya was used as a model experimental system and the results have broad applicability to many other GM plant foods, including GM papaya.

213. To determine whether the transgene in the GM soya survived passage through the complete gastrointestinal tract, a further 12 human volunteers were fed the GM soya-containing meal. The transgene was not detectable in faeces from any of the subjects. These data indicate that GM soya, although surviving passage through the small intestine, is completely digested in the large intestine and the colon. It is possible that the small intestine of ileostomists differ from that in people with an intact gastrointestinal tract. For example ileostomists could secrete lower levels of DNAase. Alternatively, the rate of passage of the digesta could differ, or the structure of the microbial ecosystem in the small intestine may be quite different.

214. Microorganisms present in the digesta samples from the ileostomists were tested for evidence of gene transfer from the GM soya. Genetic tests for the presence of the DNA to bacteria confirmed that gene transfer had occurred at a very low levels. However, individual bacteria harbouring the transferred DNA could not be isolated, indicating that the bacteria containing the GM soya transgene represented an extremely minor component of the intestinal microflora of these subjects.

215. Studies on intestinal colonising bacteria such as lactobacilli, and *Salmonella typhimurium* (an intracellular pathogen) indicate that transfer of plant genes in food, specifically transgenes in soya foods, to the intestinal epithelium, is unlikely to occur because gene transfer could not be induced under highly selective experimental conditions.

### **3.2.2 Animals**

216. Potentially, tissues from the GM papayas proposed for release, including the fruit, may be fed to farm animals, exposing their gastrointestinal tract to the introduced genes. The fate of DNA in the digestive tract of various animals has been studied. A review of the safety issues associated with the DNA in animal feed derived from GM crops (Beever & Kemp 2000) indicated exposure to introduced DNA from GM crop material is negligible compared with normal exposure to non-GM DNA. They calculated that in a diet containing 40% GM maize, the introduced genes would represent 0.00042% of total dietary DNA intake.

217. Using GM glyphosate-tolerant canola as a model experimental system, Alexander et al. (2002) investigated the digestive fate of DNA from GM plants. They used PCR to detect the presence of two genes in various canola feed fractions following *in vitro* incubated in bovine ruminal fluid. The genes analysed were the CP4 EPSPS gene introduced by genetic modification and an endogenous nuclear-encoded *rbcS* gene (encoding the small subunit of the photosynthetic enzyme Rubisco).

218. Whole seed, cracked seed, canola meal or a prepared diet (containing 6.5% canola meal) were examined. Processing of canola seed to meal was found to significantly reduce the amount of DNA detected. There were no significant differences in the detection of the introduced or endogenous gene. These feeds were incubated in batch cultures of ruminal fluid. Both genes could be detected in the cultures of whole and cracked seed for up to 48 hours, but only up to eight hours for meal and four hours for the prepared diet. The genes were detected in the plant debris but not in the aqueous phase of the ruminal cultures. The authors concluded that the plant DNA was rapidly degraded by rumen fluid and that the persistence of DNA was inversely related to plant cell digestion (Alexander et al. 2002). These results support the conclusion that the rapid degradation of DNA following release from plant cells during ruminant digestion represents a considerable barrier to transfer of plant DNA, GM and non-GM, to rumen bacteria or to ruminant animals.

219. Einspanier et al. (2001) investigated the fate of DNA from GM maize fed to cattle and chickens, using PCR to detect the introduced cryIA(b) gene (which confers resistance to insects) and an endogenous plant chloroplast gene. Since multiple chloroplasts are present in plant cells, more copies of the chloroplast gene are in the GM maize than of the cryIA(b) gene.

220. For cattle fed GM maize silage, both the cryIA(b) gene and the chloroplast marker were detected in chyme (duodenal juice). The chloroplast marker was detected in lymphocytes and faint signals were occasionally detected in milk, but it was not detected in faeces, whole blood, muscle, liver or spleen. The cryIA(b) gene was not detected in any of these samples (Einspanier et al. 2001).

221. In chickens fed a diet containing GM maize, the chloroplast marker was detected in muscle, liver, spleen and kidney, but not in faeces or eggs. In contrast, the cryIA(b) gene was not detected in any tissue sample or eggs (Einspanier et al. 2001).

222. The possibility of DNA transfer in the gut has also been investigated by feeding mice large quantities of purified bacteriophage DNA (Schubbert et al. 1997). Bacteriophage DNA was detected in the faeces and the livers of mice as well as in rarely in newborn mice (Schubbert et al. 1997). However the relevance of this work to gene transfer from GM plants was questioned by Beever and Kemp (2000), who concluded that the bacteriophage DNA was in a form which would stimulate a response by cells of the immune system, and that the cells containing this DNA in various organs and newborns were macrophages involved in scavenging and removing foreign DNA.

223. In the rare event of plant DNA uptake by animals cells, a further step of chromosomal integration has not been demonstrated. Furthermore, any uptake of plant DNA is likely to occur in non-reproductive (somatic) cells such as immune system or gut epithelium cells, and the introduced gene would not be transmitted to progeny.

## **SECTION 4 CONCLUSIONS REGARDING GENE TRANSFER TO OTHER ORGANISMS**

### **Section 4.1 Conclusions regarding gene transfer to other plants**

224. It is considered that the risk of gene transfer from the GM papayas to papaya plantations, domestically cultivated papayas and naturalised papaya populations is negligible because:

- pollen movement will be very limited because the release will be contained within an insect-proof enclosure; and
- male flowers will be removed, thereby reducing the availability of pollen.

225. In addition, potential gene flow to commercial plantations and naturalised papaya populations will be limited by geographic isolation.

226. It is considered that the risk of gene transfer from the GM papayas to other plant species is negligible because, in addition to being contained within an insect-proof enclosure:

- genetic incompatibility with papaya's closest relatives effectively prevents the formation of hybrids and limits potential for back-crossing to the parental species; and
- strong and well-demonstrated genetic differences significantly limit gene transfer to more distantly related plant genera.

## **Section 4.2 Conclusions regarding gene transfer to microorganisms**

227. It is considered that the risk of the introduced genes transferring from the GM papayas to microorganisms is negligible, because:

- horizontal gene transfer is the only possible mechanism for such transfer, yet this has not been demonstrated from plants to microorganisms under natural conditions.

Note that if the gene transfer were to occur it is unlikely that it would have adverse effects on human health and safety or the environment, as these genes are naturally present in microorganisms.

## **Section 4.3 Conclusions regarding gene transfer to animals, including humans**

228. The most significant route of entry of foreign DNA into animals and humans is through food. The gastrointestinal tract may be exposed to free DNA during digestion. It is considered that the risk of the introduced genes transferring from the GM papayas to animals, including humans, is negligible. This is because:

- simulated ruminant digestion studies with model experimental systems indicate that introduced genes and endogenous plant genes are rapidly degraded, representing a considerable barrier to gene transfer;
- vertebrate animals will not be exposed to the GM papaya fruits; and
- FSANZ approval would need to be obtained before tissues from the GM papayas, including fruits, could be used in human food, yet the applicant has not applied to FSANZ for evaluation of the GM material in this regard.

It should be noted that in the extremely unlikely event of such a transfer occurring, human health and safety and the environment are unlikely to be adversely effected.

## **APPENDIX 5 LICENCE CONDITIONS**

### **Note in relation to the approval of genetically modified foods for human consumption**

*Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority, ANZFA), is responsible for human food safety assessment. Currently, the University of Queensland has not applied to FSANZ for evaluation of material from the GM papayas for use in human food. FSANZ approval would need to be obtained before any parts of the GM papayas, including the fruits, could be used as human food.*

### **PART 1**

#### **Duration of Licence**

- 1 This licence remains in force until it is suspended, cancelled or surrendered. No dealings with GMOs are authorised during any period of suspension.

#### **Holder of Licence**

- 2 The holder of this licence ('the licence holder') is the University of Queensland.

#### **Details of Project Supervisor**

- 3.1 The Project Supervisor in respect of this Licence is identified at Attachment A.
- 3.2 The licence holder must immediately notify the Regulator in writing if any of the contact details of the Project Supervisor change.

#### **No dealings with GMO except as authorised by this licence**

- 4 Persons authorised by this licence must not deal with the GMO except as expressly authorised or contemplated by this licence.

#### **Permitted dealings**

- 5.1 The GMOs are described at Attachment B.
- 5.2 The permitted dealings with the GMOs are to, plant, grow and conduct experiments with the GMOs, and the possession, supply, use, transport and disposal of the GMOs for the purpose of any of the permitted dealings, or in the course of any of these dealings.

#### **Persons covered by this GMO licence**

- 6.1 The persons authorised by this licence to conduct dealings with the GMOs are set out in a list at Attachment C. The licence holder may vary the list by notice in writing to the Regulator.
- 6.2 The licence holder must not allow a person to deal with the GMO unless the person is listed.

***Explanatory Note:*** People named in the list are persons covered by a licence for purposes of the *Gene Technology Act 2000*. It may be an offence, or a breach of this licence, if a person not on the list deals with a GMO covered by this licence.

### **Informing people of their obligations**

- 7.1 The licence holder must inform each person covered by this licence of the obligations imposed on them as a result of the conditions in this licence.
- 7.2 The licence holder must provide the Regulator, on the Regulator's written request, with a signed statement from each person covered by this licence that the licence holder has informed the person of the conditions of this licence that apply to that person.

### **Applicant to notify of circumstances that might affect suitability**

- 8 The licence holder must immediately, by notice in writing, inform the Regulator of:
- (a) any relevant conviction of the licence holder occurring after the commencement of this licence;
  - (b) any revocation or suspension of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country, being a law relating to the health and safety of people or the environment; or
  - (c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the licence holder to meet the conditions in it.

### **Additional information to be given to the Regulator**

- 9 The licence holder must inform the Regulator if he or she:
- (a) becomes aware of additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or
  - (b) becomes aware of any contraventions of the licence by a person covered by the licence; or
  - (c) becomes aware of any unintended effects of the dealings authorised by the licence.

### **People dealing with GMO must allow auditing and monitoring of the dealing**

- 10 If a person is authorised by this licence to deal with a GMO and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

### **Remaining an accredited organisation**

- 11 The licence holder must at all times remain an accredited organisation and comply with conditions of accreditation set out in the OGTR guidelines for accreditation of organisations.

## PART 2

### *Interpretation and Definitions*

Words and phrases used in this licence have the same meanings as they do in the *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001*.

Words importing a gender include any other gender.

Words in the singular include the plural and words in the plural include the singular.

Words importing persons include a partnership and a body whether corporate or otherwise.

References to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time unless the contrary intention appears.

Where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word or phrase has a corresponding meaning.

In this licence:

**‘Cage’** means a self-supporting enclosure designed to eliminate key pollinating insects, comprising transparent nylon netting no greater than 2.0 mm<sup>2</sup> pore size, sealed to the ground.

**‘Clean’** (or **‘Cleaned’** or **‘Cleaning’**) means, as the case requires:

- (a) in relation to a Location or an area, the Destruction of the GMOs or Material from the GMOs to the reasonable satisfaction of the Regulator; or
- (b) in relation to Equipment, the removal and Destruction of the GMOs, and Material from the GMOs, to the reasonable satisfaction of the Regulator.

**‘Destroy’**, (or **‘Destroyed’**, or **‘Destruction’**) means, as the case requires, killed by autoclaving or incineration.

**‘Equipment’** includes planting equipment, harvesting equipment, storage equipment, transport equipment (eg. bags, containers, trucks), clothing and tools.

**‘GM’** means genetically modified.

**‘GMOs’** mean the genetically modified organisms covered by this licence.

**‘Location’** means the location referred to in Clause 1.1 of Part 3 of this licence.

**‘Material from the GMOs’** means stem sections, leaves, pollen or any other genetically modified material (including parts of GMOs) that are derived from or produced by the GMOs.

**‘OGTR’** means the Office of the Gene Technology Regulator.

**‘QDPIRRS’** means Queensland Department of Primary Industry Redlands Research Station.

**‘Regulator’** means the Gene Technology Regulator.

**‘Waterways’** includes streams, rivers and open irrigation channels.

## PART 3 CONDITIONS OF LICENCE

### Location and size of release

- 1.1 Planting of GMOs must be confined to a single planting area ('the Location') within the QDPIRRS in the Shire of Redlands.
- 1.2 The QDPIRRS must be secured against public access by fencing with a lockable gate.
- 1.3 The Location must have appropriate signage to indicate that GM papayas are being grown in it and that the GMOs and Material from the GMOs (eg. fruit) must not be removed from the Location except as expressly authorised by the Licence.
- 1.4 The total area of the Location must not exceed one hectare.
- 1.5 No more than 300 GMOs in total may be planted under this Licence.
- 1.6 The perimeter of the Location must be at least 100 metres from the nearest Waterway and the licence holder must not allow Material from the GMOs to enter any Waterway.

### Cage

- 2.1 The GMOs must be grown under a Cage that prevents key pollinating insects and vertebrate animals (other than humans) from accessing the GMOs.
- 2.2 The integrity of the Cage must be inspected every day for the duration of the licence. Any damage to the Cage must be repaired immediately.
- 2.3 If the Cage cannot be repaired immediately, all inflorescences and fruit must be removed from the GMOs immediately and Destroyed.
- 2.4 Within 30 days of issuing this Licence, the licence holder must install in the Cage at least two insect light traps able to collect hawkmoths in the family Sphingidae (Lepidoptera). The light traps must be located in opposite corners of the Cage and must be inspected at least once every week until the Location is cleaned for the presence of sphingid moths.
- 2.5 The presence (or absence) of sphingid hawkmoths in the light traps must be determined by someone able to recognise moths in the family Sphingidae (Lepidoptera).
- 2.6 A log book must be maintained recording each date and time of inspection of the Cage, the results of the inspection, the nature and location of any repairs to the Cage that may have been required under Part 3, condition 2.2, and the GMOs from which inflorescences or fruit may have been removed, as required under Part 3, condition 2.3. The log book must also record each date on which the insect light traps were inspected and the total number sphingid hawkmoths present at each inspection. The log book must be available on request for examination or photocopying by OGTR inspectors or authorised officers.

***Explanatory note:*** As insects often accumulate in corners of insect-proof enclosures, it is advisable to concentrate inspections for damage to the cage in the uppermost corners of the Cage. Regarding the insect light traps, it would be advisable to verify the effectiveness with which the traps collect hawkmoths, by positioning at least one additional trap outside, and about 50 m from, the Cage. These considerations are not required by the Licence but may assist the licence holder to fulfil its obligations under the Licence.

### **Access and control of the Location**

- 3 The licence holder must be able to access and control the Location to the extent necessary to comply with this licence, for the duration of the life of the licence.

### **GMOs must not be eaten**

- 4 The licence holder must ensure that any fruit or other Material from the GMOs is not used as food for humans or other vertebrate animals.

### **Notification of commencement of planting**

- 5 The licence holder must notify the OGTR at least 7 days, but not more than 20 days, in advance of each day on which the GMO is planted.

### **Notification of commencement of flowering**

- 6 The licence holder must notify the OGTR at least 7 days, but not more than 20 days, in advance of the expected commencement of each flowering season of the GMOs that is to occur.

### **Management of Flowers**

- 7 All male flowers must be removed prior to anthesis and Destroyed.

*Explanatory Note: Conditions 2.3 and 2.4 of Part 3 of this Licence describe further actions that must be taken to manage flowers and fruit, including their removal and destruction, in the event that the Cage is damaged and cannot be repaired immediately.*

### **Notification of commencement of fruiting and harvesting**

- 8.1 The licence holder must notify the OGTR at least 30 days, but not more than 60 days, in advance of the expected commencement of harvest of fruit in each fruiting season.
- 8.2 All fruit must be harvested before they fall to the ground, removed from the Location and either transported to a laboratory certified by the Regulator to at least physical containment level 2 (PC2) for analysis, or Destroyed.
- 8.3 If harvested, the GMOs or Material from the GMOs must be harvested and stored separately from any other papaya fruit or material from papaya plants.
- 8.4 A log book must be kept by the licence holder recording, for each season, the following information:
  - (a) the number of fruit set on each tree;
  - (b) the number of fruit on each tree that sustain damage consistent with being eaten by fruit-eating vertebrate animals;
  - (c) the number of fruit removed from each tree and transported to a laboratory for further experiments;
  - (d) the number of fruit removed from the tree and destroyed; and
  - (e) the number of fruit missing without explanation.
- 8.5 The log book must be available on request for examination or photocopying by OGTR inspectors or authorised officers.

- 8.6 The findings recorded in the log book must be included in the licence holder's annual report to the OGTR.

### **Reporting of toxic and allergenic responses**

- 9.1 All persons exposed to the GMOs must be notified by the licence holder (eg. by signage at doors into the Location and laboratories) that they should report to the licence holder any unusual allergenic responses that could be attributed to contact with the GMOs.
- 9.2 Any such reports (to the Licence holder) must be provided to the OGTR immediately.

### **Reporting of plant disease incidence**

- 10.1 GMOs must be inspected at least every month for disease. A record of all inspections must be entered in a log book. The log book must be available on request for examination or photocopying by OGTR inspectors or authorised officers. The log book must record the date of inspections, reports of any disease symptoms, (eg. foliar chlorosis or necrosis, dieback, root rot, loss of fruit set), severity of symptoms, disease control measures, if any, carried out and which GMO shows which disease symptoms.
- 10.2 The results of findings with respect to disease and disease control measures must be included in the licence holder's annual report to the OGTR.

### **Cleaning**

- 11 All Equipment used in the planting, growing and harvesting of GMOs must be Cleaned immediately after use, within the Cage.

### **Storage and Transportation**

- 12.1 Transportation of GMOs outside the Cage must be in accordance with the Guidelines for the Transport of GMOs June 2001 issued by the Regulator.
- 12.2 Material from the GMOs (including fruit) may only be stored in a facility that is certified by the Regulator.

### **Clean-up of location**

- 13 During the period 1-15 November 2006:
- (a) all flowers and fruit of the GMOs must be removed from the GMOs and destroyed.
  - (b) after flowers and fruit from the GMOs have been removed, the GMO must be cut off at ground level and raked into piles together with any other Material from the GMOs.
  - (c) cut stumps must be immediately treated with herbicide to kill them.
  - (d) the raked piles must be left to dry for at least a month, after which they must be burned. The raked piles may be taken out of the Cage to be burned, but must be burned within the confines of the QDPIRRS.
  - (e) the raked piles must be burned no later than 31 December 2006.

## **Inspection**

- 14.1 During the 12 months following the clean up of the location, the Location must be inspected to establish whether any GMOs or Materials from the GMOs have survived at the Location (whether by regrowth of herbicide-treated stumps or some other regrowth).
- 14.2 Inspection must take place at least every 3 months during the 12 month period.
- 14.3 Any GMOs or Material from the GMOs found to have survived at the Location during the 12 month period must be killed by herbicide treatment, incineration, hand weeding or autoclaving.
- 14.4 A log must be kept of all inspections carried out recording the date of inspection and findings. The log must be available on request for examination or photocopying by OGTR inspectors or authorised officers.
- 14.5 The findings of the log book kept under the above clause must be included in the licence holder's annual report to the OGTR.
- 14.6 The Cage must remain in place during the 12 month period.
- 14.7 Inspections must be carried out by a person qualified to identify GMO seedlings, the GMO and Material from the GMOs.
- 14.8 The Location must not be planted with any other papaya plants while the inspections are continuing. The Location may be planted to a legume crop while the inspections are continuing.

## **Testing Methodology**

- 15 The licence holder must provide a written instrument to the Regulator describing an experimental method that is capable of reliably detecting the presence of GMOs and the presence of the genetic modifications described in Attachment B in a recipient organism. The instrument must be provided within 24 months of the issuing of the licence.

## **Contingency Plan**

- 16.1 Within 30 days of the date of the commencement of this licence, a written Contingency Plan must be submitted to the Regulator detailing measures to be taken in the event of:
  - (a) damage to the enclosure that cannot be repaired immediately;
  - (b) the unintended presence of the GMO or Material from the GMO at places not contemplated by this licence
- 16.2 The Contingency Plan must include details of procedures to:
  - (a) ensure the Regulator is notified immediately if the licence holder becomes aware of an event;
  - (b) remove flowers and fruits from the GMOs and prevent potential gene transfer from the Location; and
  - (c) eradicate the GMOs or Material from the GMOs from places not contemplated by the licence.
- 16.3 The Contingency Plan must be implemented in the event that the unintended presence of the GMO, or Material from the GMOs is discovered.

## **Compliance**

- 17 A written Compliance Management Plan must be provided to the Regulator within 30 days of the date of commencement of this licence. The Compliance Management Plan must describe in detail how the licence holder intends to ensure compliance with these conditions and document that compliance.

## **Research Requirements**

- 18.1 The licence holder must, in consultation with the OGTR, develop an agreed research program that includes, but need not be limited to, confirming the genes that have been introduced into the GMOs.
- 18.2 The results of the research program must be included in the licence holder's annual report to the OGTR.

## **Annual Report**

- 19 The licence holder must provide an annual report within 90 days of each anniversary of this licence to the OGTR containing all the information required by these conditions to be reported.

## REASONS FOR LICENCE CONDITIONS

The reasons for inclusion of the specific licence conditions follow (with reference to the numbering of the conditions in the licence).

### Location and size of release

**Conditions 1.1- 1.5** are to limit the size and location of the release and to ensure the location is secure. Limiting the scale of the release and securing the location decreases the likelihood of exposure of humans and other organisms to the GMOs. **Condition 1.3** requires appropriate signage at the location to decrease the likelihood of unauthorised access to the location and removal of GMO material from the location.

**Condition 1.6** is to prevent the entry of Material from the GMOs into waterways. This condition decreases the likelihood of spread of GMOs and Materials from the GMOs.

### Cage

**Conditions 2.1 – 2.6** require the GMOs to be grown under a Cage to limit access to the GMOs for pollinators and other animals. Limiting access for pollinators prevents movement of pollen beyond the release site and limits the possibility of gene transfer from the GM papayas to other papayas in the local area. Limiting the ability of other animals to access the GMOs prevents inadvertent movement of viable Material from the GMOs beyond the release site and also decreases the likelihood of exposure of animals to the GMOs.

**Conditions 2.2, 2.4 and 2.5** require monitoring of the Cage to ensure that the ability of the Cage to limit access for pollinators and other animals is maintained at all times. **Condition 2.3** ensures that if the Cage is damaged to the extent that it cannot be immediately repaired, movement of pollen and fruit from the location by pollinators and other animals will be prevented. **Condition 2.6** ensures that compliance with the licence conditions can be monitored by the OGTR.

### Access and control of the Location

**Condition 3** ensures that the licence holder or persons covered by the licence have access to the release site and enables them to monitor the release site so as to comply with the licence conditions.

### GMOs must not be eaten

**Condition 4** prevents fruit and other Material from the GMOs being used as food for humans or other animals. This is because there is insufficient data to evaluate the safety of the GMOs for use in human or animal food at this stage in the experimental program.

### Notification of commencement of planting, flowering, fruiting and harvesting

**Conditions 5, 6 and 8.1** ensure that minimum advance information about the likely higher risk times of the trials (namely the likely planting, flowering and harvest dates) is provided to the OGTR to ensure proper planning of the OGTR monitoring of the release sites.

## **Management of flowers**

**Condition 7** requires that all male flowers must be removed prior to anthesis to limit the likelihood of pollen movement beyond the release site. As with Conditions 2.1 – 2.6, this condition limits gene transfer from the GM papayas to other papayas in the local area.

## **Management of fruiting and harvesting**

**Condition 8.2** prevents fruit from falling to the ground and decreases the likelihood of exposing soil organisms to the introduced proteins.

**Condition 8.3** prevents Material from the GMOs becoming mixed with non-GM Material and decreases the likelihood of exposing humans and other organisms to the GMOs.

**Conditions 8.4 – 8.6** decreases the likelihood of spread of viable Material from the GMOs beyond the release site and allows the OGTR to monitor compliance with the licence conditions.

## **Reporting of toxic and allergenic responses**

**Conditions 9.1** requires appropriate signage to ensure that people working with the GMOs are aware of possible allergenicity or toxicity of the GMOs and **Condition 9.2** ensures that reporting of any allergic or toxic responses will allow the Regulator to adapt risk management measures to protect human health and safety, if necessary.

## **Reporting of plant disease incidence**

**Conditions 10.1 – 10.2** ensure that any unexpected alteration in the response of the GM papayas to pathogenic or non-pathogenic microorganisms will be recorded. This data will allow the Regulator to evaluate risks to the environment for future larger scale releases of the GMOs, subject to separate assessments and approvals.

## **Cleaning**

**Condition 11** requires cleaning of all equipment used in conjunction with the GMOs to prevent spread of Material from the GMOs beyond the release site.

## **Storage and Transportation**

**Conditions 12.1 – 12.2** describe conditions for transport of the GMOs or Material from the GMOs to prevent escape and the spread or persistence of the GMOs outside the release site or certified laboratory in which research is conducted with the GMOs.

## **Clean-up of Location and Inspection**

**Conditions 13** and **14.1 – 14.8** require the release site to be cleaned of all Material from the GMOs and prevents persistence of the GMOs at the release site after completion of the trials. **Conditions 14.1, 14.2, 14.3, 14.7** and **14.8** ensure that any GMOs surviving at the release site after completion of the trial will be identified and Destroyed. **Conditions 14.4** and **14.5** ensure that the OGTR will be able to determine whether the licence conditions have been complied with and whether further licence conditions need to be imposed if clean-up of the release site is inadequate. **Condition 14.6** requires the Cage to remain in place during the post-release monitoring period to ensure that the GMOs do not spread beyond the release site during this period.

## Testing Methodology

**Condition 15** requires that the licence holder develop a method capable of detecting the GMOs and provide this method to the Regulator in writing. This method will allow detection of the GMOs in a situation where the GMO or Material from the GMOs may be present outside of the release site.

## Contingency Plan

**Conditions 16.1 - 16.3** describe requirements of the applicant in the event of an unintentional release of the GMOs. **Condition 16.1** requires the licence holder to develop a contingency plan to deal with damage to the Cage that cannot be immediately repaired or the unintended presence of the GMOs or GM material outside the release site and to provide the Regulator with a written copy of this plan. This is so that the Regulator is aware of the contingency plan and can, if necessary, revise it or impose licence conditions to require any other measures that might be necessary to prevent the continued spread or persistence of the GMOs outside the release site and protect the health and safety of people and the environment. **Condition 16.2** requires that the plan must have procedures to ensure immediate notification of the Regulator, so that the Regulator can take any actions necessary to protect the health and safety of people and the environment. The contingency plan must also provide for the destruction of flowers and fruit from the GMOs in the event of damage to the Cage that cannot be repaired and destruction of any GMOs or GMO Material from places not contemplated by the licence, to prevent the continued spread and persistence of the GM cotton in the environment. **Condition 16.3** obliges the licence holder to implement the plan.

## Compliance

**Condition 17** requires the licence holder to provide a compliance management plan to the Regulator. This is so that the Regulator is aware of the procedures that the licence holder has in place to ensure and document compliance with the licence conditions so that the Regulator can, if necessary, impose additional licence conditions to amend these.

## Research Requirements

**Condition 18.1** requires the licence holder to develop a research program that will allow the Regulator to evaluate risks to human health and safety and the environment that might be posed by future larger scale releases of the GMOs, which would be subject to separate assessments and approvals. **Condition 18.2** ensures that the results of the research program are communicated to the Regulator.

## Annual Report

**Condition 19** requires the licence holder to provide an annual report to the Regulator for administrative and auditing purposes.

## **APPENDIX 6 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES**

### **SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA**

229. The Gene Technology Act 2000 (the Act) took effect on 21 June 2001. The Act, supported by the Gene Technology Regulations 2001, an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, underpins Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs). The regulatory system replaces the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC).

230. The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation.

231. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), a Commonwealth regulatory agency located within the Health and Ageing portfolio.

232. The Act prohibits persons from dealing with GMOs unless the dealing is exempt, a Notifiable Low Risk Dealing, on the Register of GMOs, or licensed by the Regulator (see Section 31 of the Act).

233. The requirements under the legislation for consultation and for considering and assessing licence applications and preparing risk assessment and risk management plans are discussed in detail in Division 4, Part 5 of the Act and summarised below.

234. Detailed information about the national regulatory system and the gene technology legislation is also available from the OGTR website ([www.ogtr.gov.au](http://www.ogtr.gov.au))

### **SECTION 2 THE LICENCE APPLICATION**

235. Applications for DIR licence must be submitted in accordance with the requirements of Section 40 of the Act. As required by Schedule 4, Part 2 of the Regulations, the application must include information about:

- the parent organism;
- the GMOs;
- the proposed dealing with the GMOs;
- interaction between the GMOs and the environment;
- risks the GMOs may pose to the health and safety of people;
- risk management;
- previous assessments of approvals; and
- the suitability of the applicant.

236. The application must also contain:

- additional information required for a GMO that is:
  - a plant;
  - a microorganism (not living in or on animals and not a live vaccine);
  - a microorganism that lives in or on animals;
  - a live vaccine for use in animals;
  - a vertebrate animal;
  - an aquatic organism;
  - an invertebrate animal;
  - to be used for biological control;
  - to be used for bioremediation; and
  - intended to be used as food for human or vertebrate animal consumption;
- supporting information from the Institutional Biosafety Committee.

### **SECTION 3 THE INITIAL CONSULTATION PROCESSES**

237. In accordance with Section 50 of the Act, the Regulator must seek advice in preparing a risk assessment and risk management plan (RARMP) from prescribed agencies:

- State and Territory Governments;
- the Gene Technology Technical Advisory Committee (GTTAC);
- prescribed Commonwealth agencies (Regulation 9 of the *Gene Technology Regulations 2001* refers);
- the Environment Minister; and
- relevant local council(s) where the release is proposed..

238. Section 49 of the Act requires that if the Regulator is satisfied that at least one of the dealings proposed to be authorised by the licence may pose significant risks to the health and safety of people or to the environment, the Regulator must publish a notice in respect of the application inviting written submissions on whether the licence should be issued.

239. As a measure over and above those required under the Act, in order to promote the openness and transparency of the regulatory system, the Regulator may take other steps. For example, receipt of applications is notified to the public by posting a notice of each application's receipt on the OGTR website and directly advising those on the OGTR mailing list. A copy of applications is available on request from the OGTR.

## SECTION 4 THE EVALUATION PROCESSES

240. The risk assessment process is carried out in accordance with the Act and Regulations, using the Risk Analysis Framework (the Framework) developed by the Regulator (available on the OGTR website). It also takes into account the guidelines and risk assessment strategies used by related agencies both in Australia and overseas. The Framework was developed in consultation with the States and Territories, Commonwealth government agencies, GTTAC and the public. Its purpose is to provide general guidance to applicants and evaluators and other stakeholders in identifying and assessing the risks posed by GMOs and in determining the measures necessary to manage any such risks.

241. In undertaking a risk assessment, the following are considered and analysed:

- the data presented in the proponent's application;
- data provided previously to GMAC, the interim OGTR or the OGTR in respect of previous releases of relevant GMOs;
- submissions or advice from States and Territories, Commonwealth agencies and the Environment Minister and the public;
- advice from GTTAC;
- information from other national regulatory agencies; and
- current scientific knowledge and the scientific literature.

242. In considering this information and preparing the risk assessment and risk management plan, the following specific matters are taken into account, as set out in Section 49 and required by Section 51 of the Act:

- the risks posed to human health and safety or risks to the environment;
- the properties of the organism to which the dealings relate before it became, or will become, a GMO;
- the effect, or the expected effect, of the genetic modification that has occurred, or will occur, on the properties of the organism;
- provisions for limiting the dissemination or persistence of the GMO or its genetic material in the environment;
- the potential for spread or persistence of the GMO or its genetic material in the environment;
- the extent or scale of the proposed dealings;
- any likely impacts of the proposed dealings on the health and safety of people.

243. In accordance with Regulation 10 of the Regulations, the following are also taken into account:

- any previous assessment, in Australia or overseas, in relation to allowing or approving dealings with the GMO;
- the potential of the GMO concerned to:
  - be harmful to other organisms;
  - adversely affect any ecosystems;

- transfer genetic material to another organism;
  - spread, or persist, in the environment;
  - have, in comparison to related organisms, a selective advantage in the environment; and
  - be toxic, allergenic or pathogenic to other organisms.
- the short and long term when taking these factors into account.

## SECTION 5 FURTHER CONSULTATION

244. Having prepared a RARMP, the Regulator must, under Section 52 of the Act, seek comment from stakeholders, including those outlined in Section 3 and the public.

245. All issues relating to the protection of human health and safety and the environment raised in written submissions on an application or RARMP are considered carefully, and weighed against the body of current scientific information, in reaching the conclusions set out in a final RARMP. Section 56 of the Act requires that these be taken into account in making a decision on whether or not to issue a licence for the proposed release.

246. Comments received in written submissions on this risk assessment and risk management plan are very important in shaping the final risk assessment and risk management plan and in informing the Regulator's final decision on an application. A summary of public submissions and an indication of where such issues have been taken into account are provided in an Appendix to the RARMP.

247. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings are able to be managed so as to **protect human health and safety and the environment**. Matters in submissions that do not address these issues and/or concern broader issues outside the objective of the legislation will not be considered in the assessment process. In most instances, as determined in the extensive consultation process that led to the development of the legislation, they fall within the responsibilities of other authorities.

## SECTION 6 DECISION ON LICENCE

248. Having taken the required steps for assessment of a licence application, the Regulator must decide whether to issue or refuse a licence (Section 55 of the Act). The Regulator must not issue the licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in such a way as to protect the health and safety of people and the environment.

249. The Regulator must also be satisfied, under section 57 of the Act, that the applicant is a suitable person to hold the licence. Section 58 outlines matters the Regulator must consider in deciding whether a person or company is suitable to hold a licence eg.:

- any relevant convictions;
- any relevant revocations or suspensions of a licences or permits; and
- the capacity of the person or company to meet the conditions of the licence.

250. The Regulator carefully considers all of this information which is supplied in a declaration signed by licence applicants.

251. The Monitoring and Compliance Section of the OGTR compiles compliance histories of applicants, considering all previous approvals to deal with GMOs under the Act and the previous voluntary system. These histories as well as other information such as follow-up actions from audits may be taken into account. The ability of an organisation to provide resources to adequately meet monitoring and compliance requirements may also be taken into account.

252. If a licence is issued, the Regulator may impose licence conditions (Section 62 of the Act). Conditions may be imposed to:

- limit the scope of the dealings;
- require documentation and record-keeping;
- require a level of containment;
- specify waste disposal methods;
- manage risks posed to the health and safety of people, or to the environment;
- require data collection, including studies to be conducted;
- limit the geographic area in which the dealings may occur;
- require contingency planning in respect of unintended effects of the dealings; and
- limiting the dissemination or persistence of the GMO or its genetic material in the environment.

253. It is also required as a condition of a licence that the licence holder inform any person covered by the licence of any condition of the licence which applies to them (Section 63). Access to the site of a dealing must also be provided to persons authorised by the regulator for the purpose of auditing and monitoring the dealing and compliance with other licence conditions (Section 64). It is a condition of any licence that the licence holder inform the Regulator of:

- any new information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence;
- any contraventions of the licence by a person covered by the licence; and
- any unintended effects of the dealings authorised by the licence.

## APPENDIX 7 SUMMARY OF PUBLIC SUBMISSIONS ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

### Abbreviations:

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

**Issues raised:** **A:** allergenicity; **APVMA:** issues dealt with by APVMA; **D:** insufficient data/evidence; **EN:** environmental risks; **ET:** ethical concerns; **FC:** food chain; **FSANZ:** food safety and labelling; **G:** gene transfer; **GTR:** the Gene Technology Regulator; **H:** human health and safety; **HB:** herbicide resistance; **IR:** insecticide resistance; **LC:** licence conditions; **MA:** markets; **PU:** pesticide use; **RA:** risk assessment; **RARMP:** risk assessment and risk management plan; **RM:** risk management; **SE:** socioeconomic impacts; **SEG:** segregation; **T:** toxicity; **U:** unknown risks; **W:** weediness.

**OSA:** outside scope of the assessment; **NR:** No specific response

<sup>a</sup> Submission from: **A:** agricultural organisation; **I:** individual; **E:** environmental organisation; **F:** food interest organisation; **C:** consumer/public interest organisation.

Sub. no:	Type <sup>a</sup>	Approval	Summary of issues raised	Issue	Consideration of issue
01	C	n	<p>The following are concerns about the above Risk Framework:</p> <ul style="list-style-type: none"> <li>• The term Risk Management is ... used out of context ... This definition is in clear conflict with AS/NZS 4360 [risk assessment standard] and refers more to the process of Risk Control than Risk Management...</li> <li>• There is no definition of each risk level... [and]... no explanation on how to allocate a particular risk level!</li> <li>• There is no reference to risk assessment matrices or calculators.</li> <li>• There are no examples of quantitative and qualitative risk assessment methods!</li> </ul>	RARMP	OSA
02	I	n	<ul style="list-style-type: none"> <li>• I object to the proposed release [DIR 026/2002]</li> <li>• All these genetically engineered organisms pose unknown ...risks.... However slight you think these risks are you have no right to take them. I am outraged at this abuse of power.</li> </ul>	None U	Noted Noted
03	E	n	<ul style="list-style-type: none"> <li>• ...no comment</li> </ul>		Noted

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