



Australian Government

Department of Health and Ageing

Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 060/2005**

**Limited and controlled release of three
imported GM rose lines**

Applicant: Florigene Pty Ltd

March 2006

Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the intentional release (DIR) of genetically modified (GM) rose lines into the Australian environment, in respect of application DIR 060/2005 from Florigene Pty Ltd (Florigene).

The DIR 060/2005 licence permits the release of three GM rose lines under limited and controlled conditions.

The *Gene Technology Act 2000* (the Act) and the Gene Technology Regulations 2001 (the Regulations) govern the process undertaken by the Regulator before a decision is made on whether or not to issue a licence. The decision is based upon a risk assessment and risk management plan (RARMP) prepared by the Regulator in consultation with a wide range of experts, agencies and authorities and the public.

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

The application

Florigene applied for a licence to release three GM rose (*Rosa X hybrida*) lines on two sites over two years (March 2006 - April 2008) in the Shire of Yarra Ranges, Victoria. The first site is an enclosed, insect-proof greenhouse where the GM rose lines will be grown hydroponically. The total size of the greenhouse is 100m². The second site of approximately 25m² is nearby and will be used for shredding and composting of GM plant materials.

Three GM rose lines, originally developed in Japan, are proposed for release. They are hybrid tea and floribunda rose varieties which have been genetically modified by the insertion of genes that affect the production of blue coloured anthocyanin pigments (ie delphinidins), leading to light purple or violet coloured flowers.

Roses do not naturally generate the blue group of pigments, as they lack a key enzyme required for their production. The new genes were derived from other plants including black pansy, torenia, and iris. The GM rose plants also contain an antibiotic resistance marker gene from a common bacterium, which helped identify and select modified plants during their development in the laboratory.

About 100 plants of each GM rose line are proposed for release, along with 100 plants each of the two non-GM parental rose lines and 10 plants each of two other non-GM rose varieties. The purpose of the release is to propagate the three imported GM rose lines; evaluate the performance including the productivity, morphology and viability of the GM rose lines; biochemical analysis of flowers; and generate data to support a possible future application for a larger scale release.

Risk assessment

The risk assessment first considered what harm to the health and safety of people or the environment could arise as a result of gene technology, and how it could happen, during the proposed release of the GM rose lines into the environment (**hazard identification** refer to Chapter 2 for more information).

The hazard identification process considered the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products (eg soil used in the greenhouse, compost, cut flowers), the introduced genes, or products of the introduced genes.

A hazard (source of potential harm) may be an event, substance or organism (OGTR 2005a). A risk is identified when a hazard is considered to have some chance of causing harm. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

Sixteen events were identified and characterised that may give rise to harm to people or the environment as a result of the proposed release of the three GM rose lines.

These sixteen events included consideration of whether, or not, expression of the introduced genes could produce proteins that are toxic to people or other organisms, allergenic, result in unintended changes in biochemistry or physiology, or alter characteristics that may impact on spread and persistence of the GMOs. In addition, consideration was given to the potential for gene flow to other organisms or unauthorised activities, and whether harm could arise from these events.

None of the sixteen events are considered to give rise to an identified risk that requires further assessment. The principle reasons include:

- small scale of the trial that is limited in both area and duration;
- containment and disposal measures proposed by the applicant to limit the spread of GM plant materials;
- none of the GM plant materials will be used in food or animal feed;
- the lack of toxicity or allergenicity of enzymes and end-products from the introduced genes;
- widespread presence of the same or similar genes in the environment and lack of evidence of harm from these genes;
- limited capacity of the GM rose lines to spread and persist except by intentional horticultural techniques;
- limited opportunity and ability of the GM rose lines to transfer the introduced genes to other organisms;
- limited morphological differences between the GM rose lines compared with the parent rose lines.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM rose lines into the environment are considered to be **negligible**.

Risk management

The risk assessment process identified and characterised sixteen events whereby the proposed release of three GM rose lines might give rise to harm to people or the environment. As none of the sixteen events are considered to give rise to an identified risk that requires further assessment, the level of risk is considered to be **negligible**.

The *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation. However, containment and disposal measures have been imposed to restrict the release in location, size and duration to those requested by the applicant, as these were an important part of the context for assessing the risks.

The licence conditions, detailed in Chapter 4 of the RARMP, require the applicant to limit the duration of the release to two years at the two sites; prevent use of the GMOs, or materials from the GMOs, in food and animal feed; harvest flower buds (partly open); destroy GM plant materials by incineration or shredding and composting; and conduct monitoring of the disposal site to ensure all GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of three GM rose lines into the Shire of Yarra Ranges, Victoria poses **negligible** risks to the health and safety of people and the environment.

The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to contain the release to the proposed location, size and duration requested by the applicant.

Table of Contents

EXECUTIVE SUMMARY	I
INTRODUCTION	I
THE APPLICATION	I
RISK ASSESSMENT.....	I
RISK MANAGEMENT	II
CONCLUSIONS OF THE RARMP	III
ABBREVIATIONS	VI
TECHNICAL SUMMARY	1
INTRODUCTION	1
SECTION 1 APPLICATION	1
SECTION 2 RISK ASSESSMENT	2
SECTION 3 RISK MANAGEMENT	4
3.1 Licence conditions to manage this limited and controlled release.....	4
3.2 Other regulatory considerations.....	4
3.3 Identification of issues to be addressed for future releases.....	5
SECTION 4 CONCLUSIONS OF THE RARMP.....	5
CHAPTER 1 RISK ASSESSMENT CONTEXT	6
SECTION 1 BACKGROUND	6
SECTION 2 THE GMOs AND PROPOSED DEALINGS	7
2.1 The proposed dealings	7
2.2 The parent organism	8
2.3 The GMOs.....	10
2.4 Background information on anthocyanin biosynthetic pathway.....	11
2.5 The introduced genes and their end-products	13
2.6 Method of genetic modification	15
SECTION 3 CHARACTERISATION OF GM ROSE LINES.....	16
3.1 Stability and molecular characterisation.....	16
3.2 Morphological characterisation	16
3.3 Production of delphinidin and other anthocyanins in the GM roses.....	20
SECTION 4 THE RECEIVING ENVIRONMENT	21
4.1 Size and duration of the proposed release	21
4.2 Environmental conditions suitable for growing roses	21
4.3 Horticultural practices	21
4.4 Presence of sexually compatible rose species in the receiving environment.....	22
4.5 Presence of the introduced enzymes and their end-products in other plants in the environment .	23
SECTION 5 PREVIOUS AUSTRALIAN AND INTERNATIONAL APPROVALS.....	24
5.1 Previous Australian approvals of the same or similar GM rose lines	24
5.2 International approvals	25
CHAPTER 2 RISK ASSESSMENT	26
SECTION 1 INTRODUCTION.....	26
SECTION 2 HAZARD CHARACTERISATION	27
2.1 Production of a substance toxic to people	33
2.2 Production of a substance allergenic to people.....	37
2.3 Production of a substance toxic to organisms other than people	39
2.4 Spread and persistence of the GM rose lines in the environment.....	42
2.5 Gene flow by vertical gene transfer	47
2.6 Gene flow by horizontal gene transfer.....	49
2.7 Unintended changes in biochemistry or physiology.....	50
2.8 Unintended presence of <i>Agrobacterium</i> during release.....	52
2.9 Unauthorised activities	54
SECTION 3 CONCLUSIONS OF RISK ASSESSMENT	54

CHAPTER 3	RISK MANAGEMENT	56
SECTION 1	BACKGROUND	56
SECTION 2	OTHER AUSTRALIAN REGULATORS	56
SECTION 3	RISK TREATMENT MEASURES FOR IDENTIFIED RISKS	56
SECTION 4	GENERAL RISK MANAGEMENT	57
4.1	Licence conditions associated with managing limited and controlled releases	57
4.2	Other risk management considerations	57
SECTION 5	MONITORING AND COMPLIANCE	59
SECTION 6	ISSUES TO BE ADDRESSED FOR FUTURE RELEASES	59
SECTION 7	CONCLUSIONS OF THE RARMP	59
CHAPTER 4	LICENCE CONDITIONS	60
SECTION 1	INTERPRETATIONS AND DEFINITIONS	60
SECTION 2	GENERAL CONDITIONS	62
REFERENCES	69
APPENDIX A	DEFINITIONS OF RISK ANALYSIS TERMS	77
APPENDIX B	SUMMARY OF SUBMISSIONS RECEIVED FROM PRESCRIBED EXPERTS, AGENCIES AND AUTHORITIES ON THE APPLICATION	79
APPENDIX C	SUMMARY OF PUBLIC SUBMISSIONS RECEIVED ON THE APPLICATION	80
APPENDIX D	SUMMARY OF SUBMISSIONS RECEIVED FROM PRESCRIBED EXPERTS, AGENCIES AND AUTHORITIES ON THE CONSULTATION RARMP	81
APPENDIX E	SUMMARY OF PUBLIC SUBMISSIONS RECEIVED ON THE CONSULTATION RARMP	82

Abbreviations

5AT	Gene encoding the anthocyanin 5-acyltransferase from <i>Torenia hybrida</i>
5AT	Anthocyanin 5-acyltransferase from <i>T. hybrida</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine Inspection Service
bp	Basepair of nucleic acid
CaMV	Cauliflower mosaic virus
DFR	Gene encoding dihydroflavonol-4-reductase
DFR	Dihydroflavonol-4-reductase
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
dsRNA	Double-stranded ribonucleic acid
EFSA	European Food Safety Authority
F3'5'H	Gene encoding flavonoid 3',5'-hydroxylase from <i>Viola tricolor</i>
F3'5'H	Flavonoid 3',5'-hydroxylase from <i>V. tricolor</i>
FSANZ	Food Standards Australia New Zealand
FW	Fresh weight
g	Gram
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
IgE	Immunoglobulin E
kg	Kilogram
km	Kilometre
LD ₅₀	Amount of a substance given in a single dose that causes death in 50% of a test population of an organism
m	Metre
mg	Milligram
mas	Gene encoding mannopine synthase from <i>Agrobacterium tumefaciens</i>
mRNA	Messenger ribonucleic acid
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NLRD	Notifiable Low Risk Dealing
nos	Gene encoding nopaline synthase from <i>A. tumefaciens</i>
nptII	Gene encoding the neomycin phosphotransferase type II protein from <i>Escherichia coli</i>
NPTII	Neomycin phosphotransferase type II from <i>E. coli</i>
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase Chain Reaction
T-DNA	Transfer deoxyribonucleic acid
TGA	Therapeutic Goods Administration
US FDA	United States Food and Drug Administration

Technical summary

Introduction

The Gene Technology Regulator (the Regulator) has decided to issue a licence (DIR 060/2005) to Florigene Pty Ltd (Florigene) for dealings involving the intentional release of genetically modified roses into the Australian environment.

The DIR 060/2005 licence permits the limited and controlled release of three GM rose lines that have been altered for flower colour. The release will occur on two sites over two years (March 2006 – April 2008) in the Shire of Yarra Ranges. The first site is an enclosed, insect-proof greenhouse where the GM rose lines will be grown hydroponically. The total size of the greenhouse is 100m². The second site of approximately 25m² is nearby and will be used for shredding and composting of both GM and non-GM plant materials from the trial.

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

The Regulator's *Risk Analysis Framework* explains the approach used to evaluate licence applications and to develop the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions¹.

The RARMP for DIR 060/2005 has been finalised in accordance with the gene technology legislation. Matters raised in the consultation process regarding risks to the health and safety of people or the environment from the dealings proposed by the applicant were taken into account by the Regulator in deciding to issue a licence and the conditions that have been imposed.

Section 1 Application

Title:	Propagation and trial of imported GM rose lines
Applicant:	Florigene Pty Ltd
Common name of the parent organism:	Rose
Scientific name of the parent organism:	<i>Rosa X hybrida</i>
Modified trait(s):	Altered flower colour, selectable marker
Identity of the gene(s) responsible for the modified trait(s):	<ul style="list-style-type: none"> • flavonoid 3', 5' hydroxylase (<i>F3'5'H</i>) from black pansy (<i>Viola tricolor</i>) • anthocyanin 5-acyltransferase (<i>5AT</i>) from torenia (<i>Torenia hybrida</i>) • dihydroflavonol-4-reductase (<i>DFR</i>) from iris (<i>Iris hollandica</i>) • dihydroflavonol-4-reductase (<i>DFR</i>) from rose (<i>Rosa X hybrida</i>) with an inverted repeat • <i>nptII</i> from the bacterium <i>Escherichia coli</i> (selectable marker)
Proposed location(s):	Two sites in the Shire of Yarra Ranges, Victoria
Proposed release size:	125m ²
Proposed time of release:	March 2006 to April 2008

Florigene applied for a licence to release GM rose (*Rosa X hybrida*) lines that have been genetically modified to alter flower colour. The proposed release would be on two sites over two years (March 2006 - April 2008) in the Shire of Yarra Ranges, Victoria. The first site is an enclosed, insect-proof greenhouse where the GM rose lines will be grown hydroponically.

¹ More information on the assessment of licence applications and copies of the *Risk Analysis Framework* are available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/ir/process.htm>> and <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>> respectively.

The total size of the greenhouse is 100m². The second site of approximately 25m² is nearby and will be used for shredding and composting of both GM and non-GM plant materials from the trial, and the coco peat soil medium.

The purpose of the proposed release is to propagate three GM rose lines; evaluate their performance including productivity, morphology and viability of the GM rose lines; biochemical analysis of flowers; and generate data to support a possible future application for a larger scale release.

The GM rose lines proposed for release were originally developed in Japan. They are hybrid tea and floribunda rose varieties which have been genetically modified using one of two combinations of introduced genes affecting the synthesis of blue coloured anthocyanin pigments (ie delphinidins), leading to the production of light purple or violet flowers. Roses do not naturally make the blue group of pigments, as they lack a key enzyme required for their synthesis, flavonoid 3',5' hydroxylase (F3'5'H).

All of the GM rose lines contain an introduced gene, encoding the enzyme F3'5'H, derived from black pansy. Two of the lines (WKS82/130-4-1 and WKS82/130-9-1) also contain a gene encoding anthocyanin 5-acyltransferase (5AT) from torenia. The third GM rose line (LA/919-4-10) contains, in addition to the black pansy *F3'5'H* gene, an introduced gene encoding dihydroflavonol reductase (DFR) from iris, and an introduced rose *DFR* gene with an inverted repeat (which is intended to prevent expression of the endogenous rose *DFR* gene).

The three GM rose lines also contain a bacterial gene from *Escherichia coli* (*nptII*, conferring resistance to the antibiotics neomycin and kanamycin) that was used to select successfully modified plants during initial research and development work in the laboratory.

About 100 plants of each GM rose line are proposed for release, along with 100 plants each of the two non-GM parental rose varieties and 10 plants each of two other non-GM roses. The GM rose lines will be propagated through cuttings and grafting onto non-GM rootstock (either *Rosa canina* or *R. multiflora*). The applicant proposes to maintain the GM rose plants during the trial to limit diseases and pests. GM plant maintenance includes pruning, disease and insect control, and the removal and destruction of dead or diseased tissue.

In addition to conducting the trial in a greenhouse, containment measures proposed by the applicant include harvesting GM rose flowers at the bud (or partly open) stage, eliminating weeds from inside and outside the greenhouse, insect-proofing the greenhouse, and destroying GM waste material either by incineration after laboratory analysis, or by shredding and composting at the second release site.

The applicant intends to transport the GM rose lines in pots from the Florigene laboratories to the greenhouse release site. GM rose flowers and other plant materials will be transported from the greenhouse to the Florigene laboratory for analysis. Transportation will be in accordance with OGTR guidelines.

Section 2 Risk assessment

The risk assessment considered information contained in the application, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received during consultation with a wide range of prescribed experts, agencies and authorities, including the local Council where the release will take place (summarised in Appendices B and D).

Advice received from the public on the application and from consultation on the RARMP is summarised in Appendices C and E, respectively.

A reference document, *The Biology and Ecology of Roses (*Rosa X hybrida*)* was also prepared to provide baseline information on non-GM roses used in this application. This document is available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

The risk assessment first considered what harm to the health and safety of people or the environment could arise due to gene technology, and how it could happen during this release of GMOs into the environment (hazard identification), in comparison to non-GM roses and other relevant plants and in the context of the proposed release. A hazard (source of potential harm) may be an event, substance or organism (OGTR 2005a).

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

The hazard identification process considered the circumstances or events by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products (eg soil medium used in the greenhouse, compost, cut flowers), the introduced genes, or products of the introduced genes.

Sixteen events were identified and characterised whereby the proposed release of the three GM rose lines might give rise to harm to people or the environment.

These sixteen events included consideration of whether, or not, expression of the introduced genes could produce proteins that are toxic to people or other organisms, allergenic, produce unintended changes in biochemistry or physiology, or alter characteristics that may impact on spread and persistence of the GMOs. In addition, consideration was given to the potential for gene flow to other organisms or unauthorised activities, and whether harm could arise from these events.

None of the sixteen events are considered to give rise to an identified risk that requires further assessment. The principle reasons include:

- small scale of the trial that is limited in both area and duration;
- containment and disposal measures proposed by the applicant to limit the spread of GM plant materials;
- none of the GM plant materials will be used in food or animal feed;
- the lack of toxicity or allergenicity of enzymes and end-products from the introduced genes;
- widespread presence of the same or similar genes in the environment and lack of evidence of harm from these genes;
- limited capacity of the GM rose lines to spread and persist except by intentional horticultural techniques;
- limited opportunity and ability of the GM rose lines to transfer the introduced genes to other organisms;
- limited morphological differences between the GM rose lines compared with the parent rose lines.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM rose lines into the environment are considered to be **negligible**.

Section 3 Risk management

A risk management plan builds upon the risk assessment to consider whether any action is required to mitigate the identified risks, and what can be done to protect the health and safety of people and the environment.

As none of the sixteen events that were identified and characterised in the risk assessment process are considered to give rise to an identified risk that requires further assessment, the level of risk to human health and safety and the environment from the proposed release of GM rose lines is considered to be **negligible** (ie insubstantial with no present need to invoke actions for their mitigation).

However, containment and disposal measures have been imposed to restrict the release in size, duration and location to those requested by the applicant, as these were an important part of the context for assessing the risks.

3.1 Licence conditions to manage this limited and controlled release

A number of licence conditions have been imposed to limit and control the release, including requirements to:

- grow the GM roses in a greenhouse (the first site);
- grow the GM plants hydroponically in pots above the soil;
- measures to minimise insects in the greenhouse;
- control weeds on the greenhouse floor and vegetation immediately adjacent to the outside of the greenhouse;
- harvest flowers inside the greenhouse before anthers are visible to limit the possibility of pollen flow and seed set;
- destroy GM and other plant waste material (including soil medium) by incineration, or by shredding and composting at the second site; and
- measures to contain compost at the second site;
- cleaning of equipment used in the greenhouse and in shredding and composting GM plant waste material;
- monitor compost for volunteers once every 60 days, until no volunteers have been found for a period of at least 6 months.

The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, June 2001; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have also been imposed regarding transportation and storage, and to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

3.2 Other regulatory considerations

The GM rose lines proposed for release were developed in Japan and brought to Australia where they have been grown in a PC2 glasshouse under Notifiable Low Risk Dealing (NLRD) 1011/2003. Their importation into Australia also required a permit from the Australian Quarantine and Inspection Service (AQIS).

Food Standard Australia New Zealand (FSANZ) is responsible for human food safety assessment, including GM food. The proposed release involves early stage research and the applicant does not intend any material from the GM rose lines proposed for release to be used for human food. Accordingly the applicant has not applied to FSANZ for evaluation of

materials from the trial for use in human food. FSANZ approval would need to be obtained before such materials could be used for this purpose.

3.3 Identification of issues to be addressed for future releases

The risk assessment identified additional information that may be required to evaluate any future application for larger scale release of these GM rose lines, including:

- concentration levels of delphinidin in the three GM rose lines; and
- alteration of agronomic characteristics indicative of weediness as a result of the genetic modifications.

And, if the release was proposed to occur outside enclosed greenhouse facilities:

- changes in pollinator behaviour as a result of altered flower colour in the GM rose lines, that may increase gene flow under outdoor field conditions; and
- level of gene flow between the GM rose lines and compatible rose cultivars and related species under Australian field conditions.

Section 4 Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of three GM rose lines into the Shire of Yarra Ranges, Victoria poses **negligible** risks to the health and safety of people and the environment.

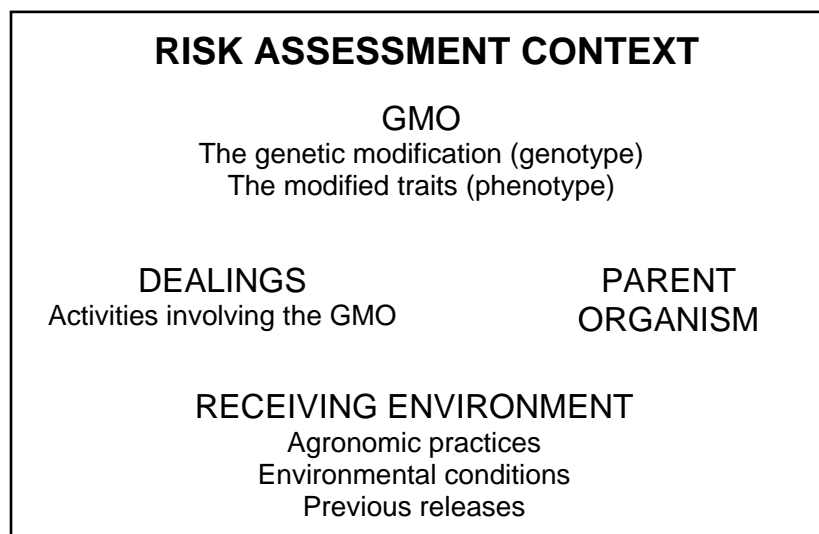
The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to contain the release to the proposed location, size and duration requested by the applicant.

Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people and the environment by the proposed release are assessed, based on scientific evidence. These include the scope and boundaries for the evaluation process required by the gene technology legislation², details of the intended dealings, the GMO(s) and parent organism(s), previous approvals and releases of the same or similar GMOs in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.1.

Figure 1.1 Components of the risk context considered during the preparation of the Risk Assessment



2. Sections 49 to 51 of the Gene Technology Act 2000 (the Act) outline the matters which the Regulator must take into account, and who she must consult with, in preparing the RARMPs that form the basis of her decision on licence applications.

3. For this application, establishing the risk assessment context includes consideration of:

- comparisons with the parent organism
- the nature and effect of the genetic modification
- relevant agricultural or horticultural practices
- the location, size and duration of the trial requested by the applicant
- the environmental conditions in the locations where the release would occur
- presence of related plants in the environment
- containment and destruction measures for the GM plant materials proposed by the applicant
- any previous releases of these or other GMOs relevant to this application.

² The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <<http://www.ogtr.gov.au/ir/process.htm>> and in the *Risk Analysis Framework* (OGTR 2005a) <<http://www.ogtr.gov.au/pdf/raffinal2.2pdf>>.

4. Initial consideration of the application under section 49 of the Act determined that public consultation was not required for the preparation of the consultation version of the RARMP.
5. In accordance with section 50 of the Act, the Gene Technology Technical Advisory Committee (GTTAC), State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and the local council where the release would take place were consulted on matters relevant to the preparation of the RARMP. This advice, and where it was taken into account in the RARMP, is summarised in Appendix B.
6. Even though public comment was not sought on the application, one submission from the public was received (summarised in Appendix C).
7. In accordance with section 52 of the Act, the Regulator notified the public when the consultation version of the RARMP had been prepared and invited written submissions. Advice on the RARMP was also sought from the same experts, agencies and authorities as before. The issues raised and how they were addressed in the RARMP are summarised in Appendices D and E, respectively.

Section 2 *The GMOs and proposed dealings*

2.1 The proposed dealings

8. Florigene proposes to release GM rose lines into the environment under limited and controlled conditions. These rose lines have been genetically modified for altered flower colour.
9. The proposed release of GM rose lines would be at two sites over two years (March 2006 - April 2008). The first site is an enclosed, insect-proof greenhouse where the GM rose lines will be grown. The total size of the greenhouse is 100m². The second site of approximately 25m² is nearby and will be used for shredding and composting of GM and other plant materials used in the trial. The proposed release sites are in the Shire of Yarra Ranges, Victoria.
10. The purpose of the proposed release is to propagate three imported GM rose lines; evaluate their performance, including productivity, morphology and viability of the GM rose lines; biochemical analysis of flowers; and generate data to support a possible future application for a larger scale release.
11. The applicant proposes to propagate the GM roses through grafting onto non-GM rootstock (either *Rosa multiflora* or *R. canina*). About 100 mature plants of each of the three GM rose lines are proposed for release, along with 100 plants each of their parental non-GM lines, and 10 plants each of two other non-GM roses. The applicant intends to cultivate the plants hydroponically using coco peat soil medium. The plants will be maintained throughout the trial, including pruning, disease and insect control, removal of dead or diseased tissue and harvesting of flowers at bud stage.
12. During the proposed release, the applicant would collect morphological and viability data, such as vegetative trait measures, flower morphology and pollen viability measurements. Harvested GM rose flowers (bud stage) will be analysed for flavonoid composition and tested for potential toxicity at the Florigene laboratory in Victoria.
13. GM and other plant materials cultivated during the trial will be destroyed by incineration after laboratory analysis, or by shredding and composting at the second release site.
14. The GM rose lines will be transported in pots from Florigene's PC2 facility to the first release site. GM plant materials will also be transported from the greenhouse to the Florigene

laboratory for analysis. Transportation of the GM plant materials will be conducted in accordance with OGTR guidelines.

15. The applicant anticipates using the plants grown under this release as source material for a possible future application for a larger scale release.

2.2 The parent organism

16. The parent organism forms part of the context that provides baseline information for comparing and assessing risks associated with GMOs. A reference document, *The Biology and Ecology of Roses (*Rosa X hybrida*)* (OGTR 2005b), was prepared to inform the risk assessment process for licence applications involving GM rose plants. This document is available at <<http://www.ogtr.gov.au>>.

17. The parent organism is rose (*Rosa X hybrida*) of the hybrid tea and floribunda varieties. *Rosa X hybrida* is not a species in the botanical sense, but is a description used for most cultivated rose cultivars. These cultivated roses have been derived over centuries through complex crosses involving a number of species of the genus *Rosa*. The *Rosa* genus is endemic to temperate regions of the northern hemisphere, including North America, Europe, Asia, and the Middle East, with the greatest diversity of species found in western China. There are no endemic or native *Rosa* species found in the southern hemisphere.

18. Roses are introduced to Australia, and have a long history of cultivation. Hybrid tea and floribunda type roses are grown by commercial flower growers for the cut flower market, and for domestic and industrial landscaping. As well as *Rosa X hybrida*, a number of *Rosa* species have been cultivated in Australia for use as ornamentals, as well as for food and the medicinal value of their hips.

2.2.1 Toxicity

19. An extensive search of the relevant scientific literature did not reveal evidence of toxicity of rose plants (including pollen) or rose products (eg rose oil, rose hips, or other rose derived foods).

2.2.2 Allergenicity

20. Allergenicity studies on roses have suggested the possibility of occupational rose allergy developing in people working in rose cultivation (Ünlü et al. 2001), rose oil extracting plants (Akkaya et al. 2004), and processing powdered rose hips (Kwaselow et al. 1990).

21. The study by Kwaselow *et al.* (1990) examined only 13 workers at a processing plant which made vitamin C tablets from ground rose hips in Detroit, Michigan. Eight of the 13 were positive for rose hip skin test and 6 of these (6/8 or 75%) had asthma symptoms. Five of the 13 workers were negative for the rose hip skin test and 3 of these (3/5 or 60%) had asthma symptoms. The number of workers examined was low and there was no statistical analysis presented for interpretation of the data. Only three workers had skin tests done with other potential causal agents (e.g. ascorbic acid, vitamin C tablets). At least one of the workers had asthma prior to working in the rose hip processing plant and data on the other workers was not presented. At most this study suggests a possible link between the allergy to powdered rose hip and occupational asthma.

22. The study by Akkaya *et al.* (2004) examined 52 workers from 4 different rose oil extracting plants in Turkey, as well as 30 control subjects. The study demonstrated a statistically significant, 8-fold increase, in *Rosa domescena* pollen hypersensitivity in workers compared to the control group. However, there was no significant difference in the pulmonary function measured, suggesting no link between hypersensitivity and occupational asthma.

23. The study by Ünlü *et al.* (2001) surveyed 600 workers employed in rose cultivation in Turkey. Of the 600, twenty (3.3%) were found to suffer from allergenic disorders such as rhinitis, rhinoconjunctivitis or both. There was no control population for comparative purposes, thus it is not known if this is significantly different from the general population. Fourteen of the twenty workers examined had increased IgE levels but seven of these workers were also positive to other allergens in the skin prick test, suggesting other allergens may have caused their increased IgE levels. These results suggest that an IgE mediated reaction to *Rosa domescena* may be responsible for the respiratory symptoms of the some of the 20 workers examined and indicates the need for further investigation using proper controls.

24. A study by (Demir *et al.* 2002) suggested that villagers in Turkey who worked in the rose industry may develop allergy to roses, but did not rule out a number of other agricultural causes of their allergy-like symptoms. Their study lacked a control population which did not have a history of exposure through working in the rose cultivation. Interestingly, their study seemed to suggest that villagers' complaints about allergy symptoms were reduced 3-fold during the rose season as compared to the rest of the year. This may indicate some beneficial effect of working with the roses during the flowering season.

25. The American Academy of Allergy Asthma and Immunology (AAAAI) website <<http://www.aaaai.org>> suggests that respiratory reactions to rose pollen must be quite rare as they could find no documented cases. This was likely due to the fact that rose pollen is heavy and sticky, designed for insect pollination rather than wind dispersal. Rose oil, which has been used in fragrance free soap and other “all natural” products, may be a more common allergen than previously recognised and further testing of this product is suggested by the AAAAI.

2.2.3 Weediness

26. Roses are long-living plants and may be found in isolation in situations where deliberately planted roses have been left unattended (eg abandoned homes or gardens, or in cemeteries). Cut flower varieties are less hardy, but if grafted onto rootstocks may still survive for decades, if left unattended.

27. Seven *Rosa* species introduced to Australia have escaped cultivation. Two of these, *R. canina* and *R. rubiginosa*, are fully naturalised and have become noxious weeds in temperate Australia. The other five have weed status. However, no cultivated complex hybrid rose types (*Rosa X hybrida*) have become naturalised, despite a long history of cultivation.

2.2.4 Potential for gene transfer

28. Roses are insect pollinated and are capable of cross- and self-pollination. Rose pollen tends to be large, sticky and heavy, and is likely to be carried by insects rather than wind (Bell 1988, see details in OGTR 2005b). Pollinating insects can include bumble bees, honey bees, wasps, and flies. Insect pollination is constrained under glasshouse conditions, particularly where there is cut flower production. Rose flowers are cut before the petals are open, thereby limiting the opportunity for insect pollination. Rose hybrids grown in gardens are more accessible to insect pollination.

29. Interspecific hybridisation is common in the *Rosa* genus. There are somewhere between 100 to 250 *Rosa* species, of which there are innumerable cultivars (Phillips & Rix 1988). Section *Caninae* of the *Rosa* genus is particularly prone to hybridisations with other *Rosa* species. *R. canina* and *R. rubiginosa*, which are weedy species in Australia, are from this section. However, there are no records of hybrids between hybrid tea or floribunda roses with *R. canina* and *R. rubiginosa* in Australia.

30. Intraspecific hybridisation among the *Rosa X hybrida* cultivars is possible, but not common. Hybrid tea and floribunda roses are generally self-pollinated. The close proximity of the anthers and stigmata, coupled with the fact that modern hybrid roses have been selected to contain more petals and for slow opening of the petals, has resulted in greatly reduced access to pollen by insects or wind. Pollen tends to shed in the unopened bloom, resulting in a high occurrence of self-pollination (Bell 1988). Furthermore, insect pollination is constrained under glasshouse conditions, particularly where there is cut flower production.

31. Hybridisation between *Rosa* species can lead to hybrids that range from fertile to completely sterile (Clapham 1987). Fertile hybrids can occur where the parents have the same or different ploidy levels. Most of the modern cultivars of *Rosa X hybrida* tend to be triploid or tetraploid (Rout et al. 1999), so crossing among them may not generate viable seed. Gudin (2000) has shown that human-assisted hybridisation among modern cut rose or garden cultivars can result in hip set of up to 25% and 18% seed germination. Therefore, there appears to be little opportunity for successful crosses to occur naturally among the *Rosa X hybrida* cultivars due to their low reproduction capacity, and a tendency to be self-pollinated (Bell 1988; Gudin 2001).

32. The level of gene flow between the GM rose lines and compatible rose cultivars and related species under Australian field conditions is not known. This information would be useful for determining the opportunity for, and possible frequency of, gene transfer between the GM rose lines and other non-GM rose lines in possible future applications involving release of the GM rose lines into the open environment. However, this information is not required for assessing the risk of this proposed release of GM rose lines as they will be grown in an enclosed, insect proof greenhouse.

2.3 The GMOs

33. Three GM rose lines (WKS82/130-4-1, WKS82/130-9-1 and LA/919-4-10), originally developed in Japan, are proposed for release. They are hybrid tea and floribunda rose varieties which have been genetically modified using one of two combinations of introduced genes affecting the synthesis of blue coloured anthocyanin pigments (ie delphinidins), leading to the production of light purple or violet flowers. Roses do not naturally make the blue group of pigments, as they lack a key enzyme required for their synthesis, flavonoid 3',5' hydroxylase (F3'5'H).

34. All of the GM rose lines contain an introduced gene (*F3'5'H*) encoding the flavonoid 3',5'-hydroxylase enzyme from *Viola tricolor* (black pansy). Two of the lines (WKS82/130-4-1 and WKS82/130-9-1) also contain a gene (*5AT*) encoding anthocyanin 5-acyltransferase from *Torenia hybrida* (torenia). The third line (LA/919-4-10), in addition to the black pansy *F3'5'H* gene, contains an introduced gene (*DFR*) encoding dihydroflavonol reductase from *Iris hollandica* (iris), and additional rose (*Rosa X hybrida*) *DFR* gene with an inverted repeat intended to suppress expression of the endogenous rose *DFR* gene.

35. The three GM rose lines also contain a selectable marker gene, *nptII*, which was derived from *Escherichia coli*, a common gut bacterium. The *nptII* gene encodes the enzyme neomycin phosphotransferase type II (NPTII), which confers resistance to aminoglycoside antibiotics (eg kanamycin or neomycin) used in the laboratory for selection of plasmids in *E. coli* and selection of transformed rose plant cells.

36. Short regulatory sequences (promoters and terminators) that control expression of the introduced genes are also present in the GM rose lines. Some of these regulatory sequences are derived from plant pathogens, including cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*. However, the sequences represent only a very small proportion

of the pathogen's total genome and are not, in themselves, infectious or pathogenic. Another regulatory sequence transferred to the GM rose lines is derived from *Petunia hybrida* (petunia).

2.4 Background information on anthocyanin biosynthetic pathway

37. Flower colour is due to the presence of three types of pigments- flavonoids, carotenoids and betalains. The flavonoids are the most common pigment and contribute to a range of colours from yellow to red to blue. Flavonoids are aromatic molecules derived from phenylalanine and malonyl-coenzyme A (Winkel-Shirley 2001).

38. The flavonoid molecules which make the major contribution to flower colour and other plant organs are the anthocyanins (water soluble pigments). These pigments are usually localised in the vacuoles of petal epidermal cells (Tanaka et al. 2005). Other factors also determine flower colour including co-pigmentation, vacuolar pH and cell shape (Mol et al. 1998).

39. There are three major types of anthocyanins that contribute to flower colour (Zucker et al. 2002):

- cyanidins, which produce the red or pink flower colour;
- pelargonidins, which produce the orange or brick red flower colour; and
- delphinidins, which produce the blue or purple flower colour.

40. Synthesis of all three types of anthocyanins follow a similar pathway until the colourless naringenin is converted to dihydrokaempferol (DHK) (Figure 1.2). DHK is either converted to the colourless leucopelargonidin by the dihydroflavonol 4-reductase (DFR) enzyme or to dihydroquercetin (DHQ) by flavonoid 3'-hydroxylase. Pelargonidin or cyanidin is produced depending on whether DHK is first converted to leucopelargonidin or DHQ respectively.

41. Delphinidin synthesis requires the conversion of DHK or DHQ to dihydromyricetin (DHM) by flavonoid 3', 5' hydroxylase (F3'5'H) (Figure 1.2). The DFR enzyme converts DHM to leucodelphinidin which is subsequently modified to delphinidin-3-glycoside through the activity of endogenous enzymes. Roses do not normally produce delphinidins, or blue pigments, because they lack this part of the anthocyanin biosynthetic pathway. It is this part of the pathway that Florigene has modified so that delphinidins are produced in roses.

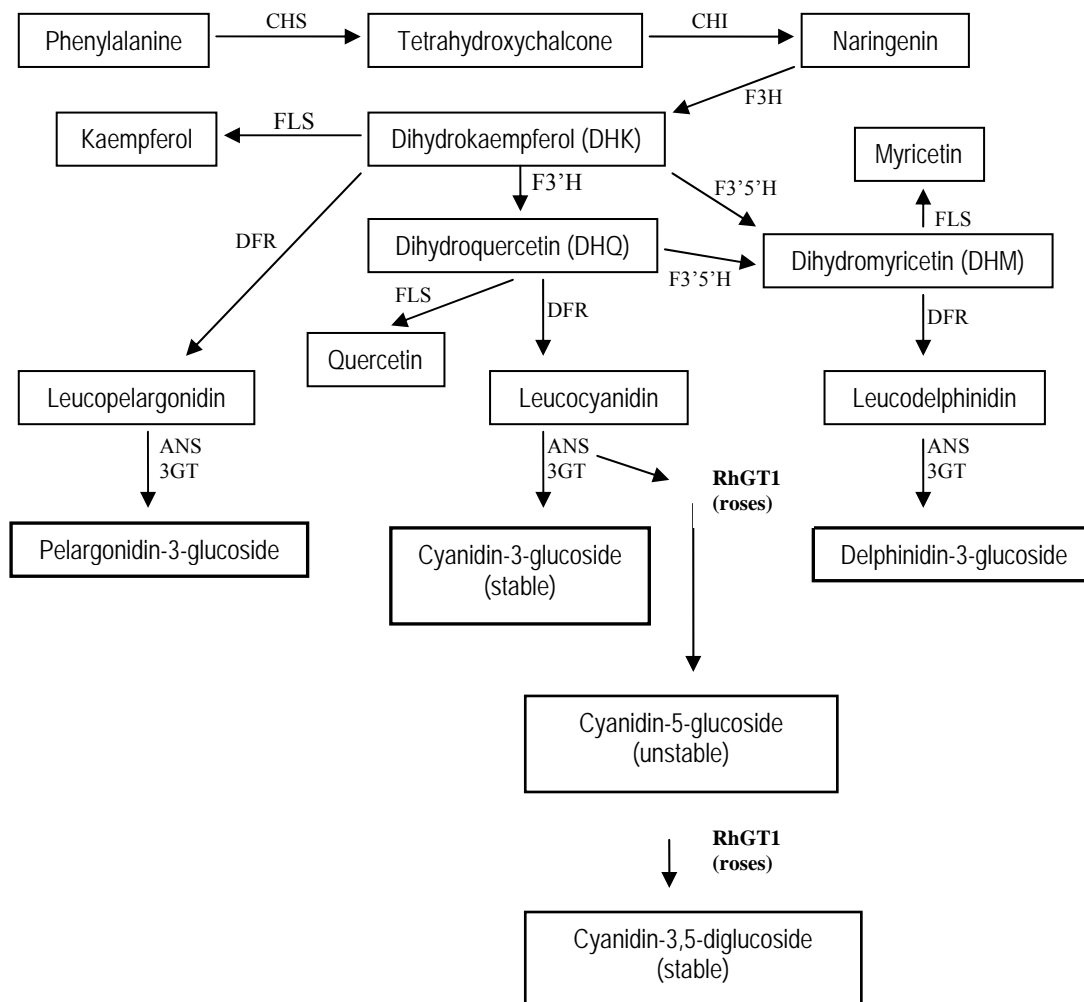
42. An intermediate compound called anthocyanidin is generated in the conversion of leucocyanidin to cyanidin 3-glucoside. Anthocyanidin is unstable and through glycosylation at the 3'-OH residue becomes the stable compound cyanidin 3-glucoside (Figure 1.2). This is the first stable anthocyanin produced in most plant species. Recently, Ogata *et al.* (2005) discovered that this part of anthocyanin biosynthesis follows a slightly different pathway in *Rosa X hybrida*. In roses, the unstable anthocyanidin is glycosylated by RhGT1 (glycosyltransferase from *Rosa X hybrida*) first at the 5'OH and then at the 3'-OH positions to form cyanidin 3,5-diglucoside, which is the first stable anthocyanin produced in roses (Figure 1.2).

43. The stable anthocyanidin 3-glucosides (eg. pelargonidin-, cyanidin-, or delphinidin-3-glucoside) often undergo further modification such as glycosylation, acylation, and methylation and these modification patterns vary among species and cultivars. It is this modification pattern, as well as vacuolar pH, metal ion complexes and co-pigmentation with flavonoids that contributes to variations in flower colour (Fujiwara et al. 1998). In many plant species, anthocyanins exist in their acylated forms (see review by Nakayama *et al.* 2003). There are two major types of acyl substituents of anthocyanins, aromatic and aliphatic acyl groups, which are usually linked to a hydroxy group of a glycosyl moiety of anthocyanins.

Aromatic acylation makes anthocyanins more stable and bluer due to intramolecular stacking of the anthocyanins with polyphenols. Aliphatic acylation of anthocyanin is important for pigment solubility in water, protecting and stabilising anthocyanins, and uptake of anthocyanins into vacuoles.

44. In *in vitro* aqueous systems anthocyanin appear to exist as several distinct forms, depending upon pH. At acidic pH (pH < 2), anthocyanin is present in its cationic flavylum form (red colour in aqueous solution). At physiological pHs (4 – 7), anthocyanins are spontaneously tautomerised to 7-quinonidal and 4-quinonoidal base isomers (bluish-purple to blue colour). At pHs higher than 5, anthocyanins also undergo hydration to produce a colourless carbinol pseudobase species. This hydration process is closely related to the instability of the anthocyanin colouration at these pHs (Nakayama et al. 2003).

Figure 1.2 Anthocyanin biosynthetic pathway (Holton & Cornish 1995) and additional rose pathway (Ogata et al. 2005)



Key to enzymes:

3GT: Flavonoid 3-glucosyltransferase
 CHS: chalcone synthase
 F3'H: flavonoid 3' hydroxylase
 RhGT1: rose glucosyltransferase

ANS: anthocyanidin synthase
 DFR: dihydroflavonol 4-reductase
 F3'5'H: flavonoid 3',5' hydroxylase

CHI: chalcone isomerase
 F3H: flavanone 3-hydroxylase
 FLS: flavonol synthase

2.5 The introduced genes and their end-products

2.5.1 The flavonoid 3',5'-hydroxylase gene (*F3'5'H*)

45. All three GM rose lines contain the introduced gene, *F3'5'H*, which was isolated from *Viola tricolor* (black pansy). The gene encodes flavonoid 3',5'-hydroxylase (F3'5'H) enzyme, which is a key enzyme in the biosynthesis of delphinidin (Tanaka et al. 2005).

46. F3'5'H is a member of the cytochrome P450 family (Shimada et al. 1999). F3'5'H catalyses the 3',5' hydroxylation of dihydrokaempferol (DHK) and the monohydroxylation of dihydroquercetin (DHQ) to form dihydromyricetin (DHM). It also catalyses the 3',5' hydroxylation of naringenin and eriodictyol to form pentahydroxyflavone.

47. All putative F3'5'H amino acid sequences found to date share high sequence similarity, and homologues of the *Petunia hybrida F3'5'H* gene have been found in edible plants such as *Solanum melongena* (eggplant), and flowers such as *Eustoma rusellianum* (lisianthus) and *Campanula* (bellflower) (Holton 1995).

48. The promoter controlling expression of the *F3'5'H* gene in the three GM rose lines is the enhanced (E12) 35S CaMV promoter derived from the cauliflower mosaic virus. The CaMV promoter is commonly used in GM plants to control the expression of introduced genes. The natural host range of CaMV is limited to species in the Cruciferae (eg cabbages, cauliflowers, canola, mustard, and other brassicas). However, the promoter is active in the genome of a large variety of monocotyledonous and dicotyledonous plants (Blaich 1992; Van Den Eede et al. 2004). This promoter is considered to be constitutive (ie a promoter that allows for continual transcription of its associated gene). However, some tissue specificity and cell cycle stage-dependent expression has been found (Benfey et al. 1989).

49. Also required for efficient gene expression in plants is an mRNA termination region, including a polyadenylation signal. The termination signal is provided by the 3' end of the *A. tumefaciens* nopaline synthase gene (*nos*) (Depicker et al. 1982).

2.5.2 The dihydroflavonol-4-reductase gene (*DFR*)

50. The GM rose line LA/919-4-10 contains an introduced *DFR* gene isolated from *Iris hollandica* (iris) and an introduced rose (*Rosa X hybrida*) *DFR* gene with an inverted repeat. The *DFR* gene encodes the dihydroflavonol-4-reductase (DFR) enzyme.

51. As mentioned above, the DFR enzyme generally acts on dihydroflavonols such as DHK, DHQ, and DHM to produce leucoanthocyanidins (see Figure 1.2). The leucoanthocyanidins are precursors to anthocyanin pigments. *Rosa X hybrida* contains endogenous DFR enzymes that convert DHK to leucopelargonidin and DHQ to leucocyanidin.

52. Depending on the species, the DFR enzyme may act on all three dihydroflavonol substrates, or on specific ones. For example, DFR of *Zea mays* (maize) cannot reduce DHM whereas the DFR of *Petunia hybrida* (petunia) has the highest activity with DHM as a substrate but does not reduce DHK (Meldgaard 1992).

53. The rose *DFR* gene with an inverted repeat was constructed using partial sense and antisense coding sequences from the rose (*Rosa X hybrida*) *DFR* gene. Expression of this inverted repeat results in a dsRNA (double-stranded RNA) product that acts to suppress expression of the endogenous rose *DFR* gene. Studies have shown that dsRNA-mediated gene silencing, or RNA interference (RNAi), is a potent means of suppressing gene function (Waterhouse & Helliwell 2003). Gene silencing techniques have been used to modify flower colour in torenia (*Torenia hybrida* and *T. fournieri*) by suppressing gene expression of chalcone synthase (CHS) and DFR (Aida et al. 2000; Suzuki et al. 2000; Fukusaki et al. 2004). Torenia flowers that expressed an antisense *DFR* gene were bluer than those

containing an antisense *CHS* gene because incomplete down-regulation of *DFR* lead to an accumulation of flavones and the resulting copigmentation effect with the remaining anthocyanins shifted the flower colour towards blue (Aida et al. 2000).

54. The applicant states that the iris *DFR* may have a higher affinity for DHM than the endogenous rose *DFR*. The higher affinity of iris *DFR* to DHM combined with the suppression of the endogenous rose *DFR* enzyme by the dsRNA rose *DFR*, may lead to virtually all anthocyanidin produced being delphinidin.

55. The promoter controlling expression of the *DFR* gene isolated from iris is the E12 35S CaMV promoter derived from the cauliflower mosaic virus. The same promoter was used to express the rose *DFR* gene with an inverted repeat.

56. The termination signal for the *DFR* gene isolated from *I. hollandica* is provided by the 3' region of a phospholipid transfer protein homologue gene (*D8*). The 3' region of *D8* is from petunia. The termination signal for the rose *DFR* gene with an inverted repeat is provided by the terminator region of the *A. tumefaciens* mannopine synthase gene (*mas*).

2.5.3 The anthocyanin 5-acyltransferase gene (*5AT*)

57. The GM rose lines WKS82/130-4-1 and WKS82/130-9-1 contain the introduced anthocyanin 5-acyltransferase gene (*5AT*), which was isolated from the torenia. The gene encodes the anthocyanin 5-acyltransferase (*5AT*) enzyme.

58. As mentioned above, the stable anthocyanidin 3-glucosides often undergo further modification such as acylation and methylation. Acylation makes anthocyanins more stable and bluer, and is important for pigment solubility in water, protecting and stabilising anthocyanins, and uptake of anthocyanins into vacuoles (see review by Nakayama *et al.* 2003). Acylation generally takes place on the glycosyl moieties of anthocyanins after the formation of the stable anthocyanidin 3-glucoside. This step is catalysed by anthocyanin acyltransferases, which includes the *5AT* enzyme.

59. The promoter controlling expression of the *5AT* gene is the E12 35S CaMV promoter derived from the cauliflower mosaic virus. The termination signal is provided by the 3' end of the *A. tumefaciens* nopaline synthase gene (*nos*) (Depicker et al. 1982).

2.5.4 The antibiotic resistance marker gene (*nptII*)

60. The three GM rose varieties (WKS82/130-4-1, WKS82/130-4-10, LA/919-4-10) contain the introduced *nptII* antibiotic resistance marker gene from *E. coli*, which was isolated from the bacterial Tn5 transposon (from *E. coli*) (Beck et al. 1982). The gene functioned as a selectable marker during the initial laboratory stages of development of GM rose plants.

61. The *nptII* gene is the most commonly used antibiotic resistance marker gene for the production of GM plants (Goldstein et al. 2005). It encodes an enzyme, neomycin phosphotransferase type II (NPTII), which confers resistance to the aminoglycoside antibiotics kanamycin and neomycin. NPTII uses ATP to phosphorylate kanamycin and neomycin, thereby inactivating the antibiotic and preventing it from killing the NPTII-producing cell. Modified cells containing *nptII* are able to grow in the presence of the antibiotic, while the growth of non-modified cells is inhibited. Therefore, *nptII* enabled selection of *E. coli* cells containing the introduced genes, and also selection of transformed rose plant cells following *Agrobacterium*-mediated transformation.

62. The promoter controlling the expression of the *nptII* gene is provided by the promoter region of the *A. tumefaciens* nopaline synthase gene (*nos*). The termination signal is provided by the terminator region of the *A. tumefaciens* nopaline synthase gene (*nos*).

2.6 Method of genetic modification

63. The three GM rose lines proposed for release (LA/919-4-10, WKS82/130-4-1 and WKS82/130-9-1) were generated independently by *Agrobacterium*-mediated transformation.

64. *Agrobacterium tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. Plants can be genetically modified by the transfer of DNA (transfer-DNA or T-DNA, located between specific border sequences on a resident plasmid) from *A. tumefaciens* through the mediation of genes from the virulence region of Ti plasmids.

65. Disarmed *Agrobacterium* strains were constructed specifically for plant transformation. The disarmed strains do not contain the genes responsible for the overproduction of auxin and cytokinin (*iaaM*, *iaaH* and *ipt*), which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). The binary vectors used to transfer T-DNAs contain well characterised DNA segments required for their replication and selection in bacteria, and for transfer of T-DNA from *Agrobacterium* and its integration into the plant cell genome (Bevan 1984; Wang et al. 1984). *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants without causing biosafety concerns or any adverse reactions.

66. The LA/919-4-10 rose line was generated using a binary vector, pSPB919. This vector was used to introduce the pansy *F3'5'H* gene, the *DFR* gene from iris, the rose *DFR* gene with an inverted repeat and the antibiotic resistant marker gene, *nptII*, using standard *Agrobacterium* transformation protocols. Details of the plasmid vector are shown in Table 1.1. GM rose cells were selected in the presence of kanamycin and GM plants regenerated from these cells.

Table 1.1 Transformation vector pSPB919

Promoter	origin	Gene	origin	Terminator	origin
E12 35S	CaMV (cauliflower mosaic virus)	<i>F3'5'H</i>	<i>Viola</i> <i>tricolour</i> (black pansy)	<i>nos</i>	<i>Agrobacterium</i> <i>tumefaciens</i>
E12 35S	CaMV	<i>DFR</i>	<i>Iris</i> <i>hollandica</i> (iris)	<i>D8</i>	<i>Petunia hybrida</i> (petunia)
E12 35S	CaMV	<i>DFR</i> with an inverted repeat	<i>Rosa X</i> <i>hybrida</i> (rose)	<i>mas</i>	<i>A. tumefaciens</i>
<i>nos</i>	<i>A. tumefaciens</i>	<i>nptII</i>	<i>Escherichia</i> <i>coli</i>	<i>nos</i>	<i>A. tumefaciens</i>

67. The WKS82/130-4-1 and the WKS82/130-9-1 rose lines were also generated using a binary vector, pSPB130. This vector was used to introduce the black pansy *F3'5'H* gene, the *5AT* gene from torenia, and the antibiotic resistant marker gene, *nptII*, using standard *Agrobacterium* transformation protocols. Details of the plasmid vector are shown in Table 1.2. GM rose cells were grown in culture, and were selected through their ability to grow in the presence of the antibiotic kanamycin. GM rose plants were regenerated from these cells.

Table 1.2 Transformation vector pSPB130

Promoter	origin	Gene	origin	Terminator	origin
E12 35S	CaMV	<i>F3'5'H</i>	<i>V. tricolour</i> (black pansy)	<i>nos</i>	<i>A. tumefaciens</i>
E12 35S	CaMV	<i>5AT</i>	<i>Torenia hybrida</i> (torenia)	<i>nos</i>	<i>A. tumefaciens</i>
<i>nos</i>	<i>A. tumefaciens</i>	<i>nptII</i>	<i>E. coli</i>	<i>nos</i>	<i>A. tumefaciens</i>

Section 3 Characterisation of GM rose lines

3.1 Stability and molecular characterisation

68. The GM rose lines have been grown to flowering in Japan and are being grown in a PC2 glasshouse (NLRD 1011/2003) at Florigene, Victoria. The applicant states that all three GM rose lines are genetically stable based on flower colour. Flower colour has been monitored for over one year in Japan and Australia, and there has been no reversion over a minimum of four generations.

69. The applicant has used Southern and Northern blot analyses to estimate the approximate copy number of the inserted genes for each of the GM rose lines. Preliminary results are shown in Table 1.3. The results indicate that the binary vector used in transformation experiments is not present in the LA/919-4-10 GM rose line, and only a single copy of the T-DNA, along with the right and left borders, have been inserted into the plant genome.

70. The T-DNA has also been inserted into the genome of the WKS82 /130-4-1 and WKS82/130-9-1 rose lines. However, further analysis is required to estimate the copy number of each inserted gene, and presence or absence of the right border of the T-DNA (Table 1.3).

Table 1.3 Approximate copy number of introduced genes, the selectable marker, and the right and left borders of the T-DNA

GM rose line	LB	<i>F3'5'H</i>	iris <i>DFR</i>	rose <i>DFR</i>	<i>5AT</i>	<i>nptII</i>	RB	Vector
LA/919-4-10	2	1	1	1	na*	1	1	0
WKS82 /130-4-1	1	4	na	na	nd	1	nd	nd
WKS82/130-9-1	3	2	na	na	nd	1	nd	nd

na* = not applicable

LB = left border

nd** = not done

RB= right border

3.2 Morphological characterisation

71. The applicant has provided information on the flower colour of the three GM rose lines, based on the Royal Horticultural Society colour chart <<http://www.rhs.org.uk/publications>>. This information indicates that the flower colour of the GM roses are light purple to violet, which are similar to existing non-GM (conventional) rose varieties. The number and letter codes on this chart enable specific colours to be identified and compared. The colour code of the WKS82/130-4-1 GM rose line is 84C, which corresponds to other non-GM varieties

including ‘Rhapsody in Blue’ (77A). The colour code of the WKS82/130-9-1 GM rose line is 76A, which corresponds to other non-GM varieties including ‘Delilah’ (80D). Lastly, the colour code of the LA/919-4-10 GM rose line is 85B/85C, which corresponds to other non-GM varieties including ‘Lava’ (74D).

72. The applicant has also provided data from three breeding experiments conducted in a glasshouse in Japan, using pollen from the three GM rose lines. The first breeding experiments used pollen from the LA/919-4-10 GM rose line found that viable pollen was produced from this GM rose line. Eight flowers were chosen from the parent rose line (LA, the floribunda hybrid rose) and the GM rose line (LA/919-4-10), and eight anthers were chosen from each flower. The pollen from all anthers was germinated on a pollen germination medium in the laboratory. Germination was measured after two hours at 29°C. The percentage of anthers per flower with viable pollen was 51.6% for the parent rose line and 49.2% for the GM rose line.

73. In a second experiment, crosses were carried out using a species rose (*Rosa multiflora*) as the female parent, and pollen from each of the three GM rose lines and the two non-GM parental rose lines (LA and WKS). Seeds from these crosses were collected and analysed using PCR to determine if the seed was transgenic. The results are shown in Table 1.4. The crosses using pollen from the WKS82/130-4-1 and WKS82/130-9-1 GM rose lines did not produce transgenic seed. The rose hips from the crosses using pollen from the LA/919-4-10 GM rose line did not contain seed, so these could not be analysed. The seed from crosses using pollen from the non-GM parent rose lines were not analysed.

Table 1.4 Results of crosses between *Rosa multiflora* (female) and pollen collected from the three GM rose lines, and the two non-GM parental rose lines

	LA non-GM parent	LA/919-4-10	WKS non-GM parent	WKS82/130-4-1	WKS82/130-9-1
Number of <i>R. multiflora</i> flowers pollinated	162	177	152	159	134
Number of flowers forming hips	27	5	6	12	9
Percentage of crosses leading to hips	16.7	2.8	3.9	7.5	6.7
Number of seed analysed	0	0*	0	19	11
Number of transgenic seed	na	na	na	0	0
Percentage of seed that were transgenic	na	na	na	0	0

na = not applicable

* = hips did not contain seed

74. In a third breeding experiment, crosses were carried out using two different non-GM parental rose varieties (‘Queen Elizabeth’, a hybrid tea cultivar, and ‘Gold Barbie’, a

Floribunda cultivar) as the female parent, and pollen from each of the three GM rose lines, and each of the two non-GM parental rose lines (LA and WKS). Again, the seeds from these crosses were collected and analysed using PCR to determine if the seed was transgenic. The results are shown in Tables 1.5 and 1.6. The crosses using pollen from the LA and WKS non-GM parent rose lines produced a high percentage of rose hips, but the seed from these rose hips were not analysed. None of the crosses using pollen from the WKS82/130-4-1 and WKS82/130-9-1 GM rose lines produced transgenic seed. However, the crosses using pollen from the LA/919-4-10 GM rose line produced 37.1% of transgenic seed when crossed with the ‘Queen Elizabeth cultivar, and 48.6% of transgenic seed when crossed with the ‘Gold Barbie’ cultivar (Tables 1.5 and 1.6).

Table 1.5 Results of crosses between ‘Queen Elizabeth’ (female) and pollen collected from the three GM rose lines, and the two non-GM parental rose lines

	LA non-GM parent	LA/919-4-10	WKS non-GM parent	WKS82/130-4-1	WKS82/130-9-1
Number of flowers pollinated	20	20	20	20	20
Number of flowers forming hips	20	20	19	19	20
Percentage of crosses leading to hips	100	100	95	95	100
Number of seed analysed	—	62	—	94	94
Number of transgenic seed	—	23	—	0	0
Percentage of seed that were transgenic	—	37.1	—	0	0

Table 1.6 Results of crosses between ‘Gold Barbie’ (female) and pollen collected from the three GM rose lines, and the two non-GM parental rose lines

	LA non-GM parent	LA/919-4-10	WKS non-GM parent	WKS82/130-4-1	WKS82/130-9-1
Number of flowers pollinated	20	20	20	20	20
Number of flowers forming hips	20	20	16	14	14
Percentage of crosses leading to hips	100	100	80	70	70
Number of seed analysed	—	35	—	94	94
Number of transgenic seed	—	17	—	0	0
Percentage of seed that were transgenic	—	48.6	—	0	0

75. The applicant provided results from an *in situ* hybridisation experiment also conducted in Japan, where petal tissue from the WKS82/130-4-1 and WKS82/130-9-1 GM rose lines was hybridised with a probe to F3'5'H. Petal tissue from the WKS82/130-4-1 and WKS82/130-9-1 GM rose lines and their non-GM parent (WKS) was hybridised with a digoxigenin-labelled F3'5'H probe. The presence of the transcript, visualised by stained cells, was only detected in the WKS/82 GM rose lines, and not in the non-GM parent. Furthermore, the transcript was only visualised in the epidermal cells of the petal, the L1 layer, suggesting that the WKS82/130-4-1 and WKS82/130-9-1 GM rose lines are periclinal chimeras for the introduced genes.

76. Chimera plants are composed of two or more genetically distinct tissues growing adjacent to each other (Hartmann & Kester 1975). In periclinal chimera plants, tissues of one genetic composition occur as a relatively thin layer, one or several layers in thickness, over a genetically different core of other plant tissues. Periclinal chimera plants are relatively stable and can be vegetatively propagated (Hartmann & Kester 1975). Data from the applicant suggests that it is the outer layer (L1) of the WKS/82 GM plant tissue that contains the introduced genes. The L1 layer usually produces the epidermis, and is not involved in the production of reproductive cells in the anthers and ovules (Hartmann & Kester 1975). Therefore, the introduced genes are only expressed in the outer epidermal layer of the WKS/82 GM rose lines (which forms a continuous layer over all tissues of the leaf, stem, flower petals, etc.), and are not in the pollen or anthers of these GM rose lines. Gene flow via pollen dispersal from these two WKS/82 GM rose lines will not result in transfer of the introduced genes. However, the WKS/82 GM rose lines can still be vegetatively propagated. In contrast, the breeding experiments show that the LA/919 GM rose line does produce transgenic seed, so this GM rose line is unlikely to be a chimeric plant (Table 1.5).

77. The applicant has provided preliminary data on changes in phenotype between the parent rose lines and the GM rose plants. The GM rose lines have been grown to flowering in Japan (10 plants per line), and are undergoing field trials in California, USA (20 plants per line). Preliminary results from these trials indicate that there are no apparent morphological differences between the WKS82/130-4-1 and the WKS82/130-9-1 GM lines and their parent rose line (hybrid tea rose). Some morphological differences were detected between the LA/919-4-10 GM line and its parent rose line (floribunda rose) (Table 1.7). Plant height and petal number are lower in the GM line compared to the parent line, while petal height, petal width and flower width are greater. The only significant difference was detected for the lower petal number in the GM line. The applicant states that these results are indicative of suppressed vigour in the LA/919-4-10 rose line compared to its parent line.

Table 1.7 Field trial data for rose plant morphological characteristics (data is based on 15 replicates for each morphological characteristic)

	LA	LA/919-4-10	Statistical significance
Plant height (mm)	542	481	not sd*
Petal number (mm)	22	11	highly sd
Petal height (mm)	2.5	3.4	not sd
Petal width (mm)	3.3	4.4	not sd
Flower height (mm)	10	10	not sd
Flower width (mm)	8.8	10.8	not sd

*sd = significant difference

78. The applicant has provided preliminary data on adventitious root formation in the LA/919-4-10 GM line compared to its parent rose line. Table 1.8 provides data on adventitious root formation in cuttings. The same experiment was repeated three times, with 20 cuttings per test. In each experiment, the GM rose line showed a lower percentage of rooting and fewer roots per rooted cutting when compared to the parent rose line.

Table 1.8 Field trial data for adventitious root formation for the parent (LA) and transgenic (LA/919-4-10) rose lines

	Experiment 1		Experiment 2		Experiment 3	
	LA	LA/919-4-10	LA	LA/919-4-10	LA	LA/919-4-10
Percentage rooting	90	80	90	40	100	60
Number of roots per rooted cutting	4.5	3.4	2.4	1.8	4.5	1.6

3.3 Production of delphinidin and other anthocyanins in the GM roses

79. Delphinidin is produced as a result of the combined expression of the introduced genes (*F3'5'H*, *DFR* and *5AT*) together with endogenous genes in the anthocyanin biosynthetic pathway. The *35S* CaMV promoter controlling expression of these genes is a constitutive promoter and is therefore expected to lead to the production of delphinidin in most of the GM rose plant tissues.

80. The applicant has measured the production of delphinidin and other anthocyanins in the flowers from the GM rose plants. Preliminary data on the production of key flavonoid and anthocyanin in the flowers of the GM rose plants compared to their non-GM parent rose lines is shown in Table 1.9. The amount of delphinidin ranges from 0.06-0.11 mg/g FW (fresh weight) petal for the three GM rose lines.

Table 1.9 Concentration of key flavonoids in GM rose flowers

Rose line	Anthocyanidin (mg/g FW)			Flavonol (mg/g FW)		
	% delphinidin	delphinidin	cyanidin	myricetin	quercetin	kaempferol
WKS82 non-GM parent	0	0	0.11	0*	1.60*	0.08*
WKS82/130-4-1	90	0.10	0.01	1.34	0.09	0.08
WKS82/130-9-1	93	0.11	0.01	1.93*	0.44*	0.04*
LA non-GM parent	0	0	0.08	0	0.72	0.11
LA/919-4-10	94	0.06	0.01	0.32	0.52	0.02

*value from one rose line

81. The applicant also measured the production of delphinidin in leaves and roots of the GM rose plants. Delphinidin was detected in the leaves of the GM rose plants, but not in the roots.

82. Detailed information on the concentration levels of delphinidin would be required for any future application involving a larger scale release of these GM rose lines.

Section 4 The receiving environment

83. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This may include the size, duration and regions of the dealings, intended agronomic and horticultural practices, including those that may be altered in relation to normal practices; other relevant plants in the environment and same or similar GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2005a).

4.1 Size and duration of the proposed release

84. The size and duration of the proposed release is outlined in Section 2.1. The proposed release would be on two sites over two years (March 2006 - April 2008). The first site is an enclosed, insect-proof greenhouse where the GM roses will be grown. The total size of the greenhouse is 100m². The second site of approximately 25m² is nearby and will be used for shredding and composting of GM plant materials. The proposed release sites are in the Shire of Yarra Ranges, Victoria.

4.2 Environmental conditions suitable for growing roses

85. Roses can grow in all parts of Australia, but are more difficult to manage in hot tropical regions, due to diseases such as black spot which are more prevalent in hot humid areas. Nearly all rose species are able to tolerate hot summers, and are hardy down to around -15°C. Hybrid tea roses are susceptible to cold climates (Phillips & Rix 1988).

86. The proposed release is to occur in the Shire of Yarra Ranges, Victoria. Table 1.10 shows the average daily temperatures and average monthly rainfall in summer and winter for areas surrounding the Yarra Ranges. The climate in this region indicates that it is suitable for growing roses.

Table 1.10 Climatic data for areas surrounding the Yarra Ranges

	Healesville (Badger Creek Sanctuary)	Mount Dandenong (GTV9)	Scoresby Research Institute
Average daily max/min temperature (summer)	25.4°C/11.2°C	21.6°C/11.3°C	25.3°C /12.9°C
Average daily max/min temperature (winter)	12.6°C/4.6°C	8.9°C/4.1°C	13.5°C /6.1°C
Average monthly rainfall (summer)	70.4 mm	73.1 mm	61.4 mm
Average monthly rainfall (winter)	80.8 mm	104.6 mm	77.8 mm

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

4.3 Horticultural practices

87. Commercial greenhouse production of roses as cut or potted flowers generally involves culture in sterile soil, sterile soil-less media, or by hydroponics.

88. A number of environmental factors influence greenhouse production of cut roses, including light levels (natural and artificial), plant architecture, temperature, CO₂ levels, pest management systems, timing of fertilizer application, growth media and choice of cultivar. The greenhouse rose is self-inductive, meaning that flowers are initiated autonomously in extending shoots. Under Australian cultivation conditions, the first flowers are produced

about four months after planting, and flowering is continuous, unless the rose plants are pruned to encourage vegetative growth.

89. Propagation of roses by seed is only used in breeding new cultivars or in the production of rootstock plants of some species such as *R. canina*. Once a new hybrid rose has been selected, it is propagated asexually, with T-budding of the hybrid rose onto a vigorous rootstock the most common method. Asexual propagation of the hybrid rose is used because the rootstock tends to be more winter-hardy and disease-resistant than the hybrid rose (Hartmann & Kester 1975). More importantly, the asexually propagated hybrid rose will be a clone of the original selection, preserving all the characteristics for which it was selected. In contrast, any seed derived progeny will segregate and have characteristics that differ from the original hybrid rose parent.

90. Different rootstock plants are utilized depending upon the utility of the hybrid rose. For garden roses, rootstocks such as *R. multiflora* and *R. canina* have been commonly used, whereas for greenhouse rose flower production, *R. x noisettiana* and *R. odorata* are used. *R. multiflora* is popular because it is thornless and not prone to suckering. *R. canina* has been used as the rootstock for standard roses and *R. fortuneana* has been a suitable choice for sandy soils (Hartmann & Kester 1975; Bell 1988).

91. The applicant intends to propagate and manage the GM rose lines using horticultural practices similar to those used for non-GM roses cultivated for cut-flower production. The GM rose lines will be grown in a greenhouse and propagated through grafting onto non-GM rootstock (either *Rosa multiflora* or *Rosa canina*).

92. The roses will be grown hydroponically in pots above soil level. Coco peat will be used as the soil medium within the pots. Excess irrigation solution, containing liquid nutrient, will drain into the ground. A Rock Wool slow filter system will be installed to reduce the amount of run off of the liquid waste.

93. The applicant proposes to limit the presence of insects in the greenhouse by ensuring that the greenhouse is insect-proof. The applicant also proposes to limit the presence of weeds and plant material on the greenhouse floor.

94. The maintenance of the GM rose plants will include pruning, disease and insect control, removal and destruction of dead or diseased tissue and the harvesting of flowers at bud stage.

95. The applicant proposes to shred and compost GM plant waste material from the greenhouse by:

- placing the compost approximately 10 metres from the greenhouse; and
- roughly shredding waste material and then heaping onto the compost (no additives are used in the composting process).

Composted plant material is usually used on garden beds within two months (information supplied by the applicant).

4.4 Presence of sexually compatible rose species in the receiving environment

96. There are a number of sexually compatible rose species present in Australia that could receive genes from the GM rose lines, including naturalised rose species and other cultivated rose species. The applicant proposes to grow sexually compatible non-GM rose plants alongside GM rose plants, including the parent rose lines as controls for comparison. Furthermore, the proposed planting will take place in an enclosed insect-proof greenhouse (site 1) within a commercial rose growing facility.

97. Two rose species are listed as noxious weeds in Australia, *R. canina* and *R. rubiginosa* (Parsons & Cuthbertson 2001). *R. canina* is not listed as a noxious weed in Victoria. However, *R. rubiginosa* is listed as a Regionally Controlled Weed in Victoria, meaning that it occurs in the region, and is capable of spreading further. Control measures are required to prevent the spread of this weed (information from the Weeds Australia website <<http://www.weeds.org.au>>).

4.5 Presence of the introduced enzymes and their end-products in other plants in the environment

98. The F3'5'H, DFR, 5AT enzymes and their end-products (ie delphinidin), as well as the NPTII enzyme, are widespread in the environment. This forms the baseline data for assessing the risks from exposure to these enzymes as a result of the proposed release of the GM rose lines.

99. Delphinidin is produced as a result of the combined expression of the introduced genes (*F3'5'H*, *DFR* and *5AT*) together with endogenous genes in the anthocyanin biosynthetic pathway. Delphinidin and other anthocyanins are naturally produced in purple coloured fruits and vegetables which are commonly consumed and have a long history of safe use. Examples of plant foods containing these proteins include blueberries, cherries, grapes, and red onions (Table 1.11). Delphinidin is also produced by a number of Australian native plants, including Eucalypt species (Asenstorfer et al 2003), Acacia species (Sharma et al 1974) and a native West Australian waxplant, *Chamelaucium uncinatum* (Klyne et al 2001). Furthermore, anthocyanins extracted from red cabbage (*Brassica oleracea*) and sweet potato (*Ipomea batatas*) have been used as natural food colourants, and have had no adverse effects on human health (Nakayama et al. 2003). Delphinidins are also found in flowering plants such as agapanthas, wisteria, rhododendrum, and pansies.

Table 1.11 Anthocyanin content of some common fruits and vegetables

Source	Anthocyanin content (mg/g FW)
Grapes	0.1 – 6.0
Black chokeberries	5.6
Blueberries	0.3 – 5.0
Elderberry	4.5
Cherries	0.1 – 4.5
Black raspberries	3.0 – 4.0
Black currants	1.3 – 4.0
Bilberries	3.0 – 3.2
Blackberries	0.8 – 3.2
Cranberries	0.6 – 2.0
Kiwi	1.0
Red raspberries	0.2 – 0.6
Red radishes	0.1 – 0.6
Strawberries	0.2 – 0.4
Plum	0.02 – 0.3
Red cabbage	0.25
Red onions	0.1 – 0.2
Apples	0.1

Source: <http://www.does.org/images/TabF1_2_3.jpg>

100. None of the introduced enzymes are known toxins. The introduced enzymes are derived from black pansy (F3'5'H), iris (DFR), rose (DFR) and torenia (5AT). There are no reports of toxicity of black pansy, rose or torenia. However, rhizomes (thickened roots) and rootstocks from the iris (*Iris* spp.) produce a toxin which can cause low toxicity if ingested. Contact with seeds, rootstock and cell sap from the iris can cause minor skin irritation (<http://www.ces.ncsu.edu/depts/hort/consumer/poison/Iris_sp.htm>). Ingestion of iris bulbs can also cause mild gastrointestinal signs in humans, and in cats and dogs. Severe vomiting and diarrhoea can occur if a significant amount of the bulb is ingested (Plumlee 2002). The toxins produced in iris (irisin, iridin and irisine) are not anthocyanins, or anthocyanin related compounds, or involved in the anthocyanin biosynthetic pathway.

101. The NPTII protein is widespread in the environment since it is naturally produced by the common gut bacterium *Escherichia coli*. *E. coli* is widespread in human and animal digestive systems (Jefferson et al. 1986) as well as in the environment.

102. Humans (and, by implication, other animals) continually ingest kanamycin-resistant microorganisms, some containing the NPTII enzyme. The diet, especially raw salad, is the major source: estimated conservatively, each human ingests 1.2×10^6 kanamycin-resistant microorganisms daily (Flavell et al. 1992). Large numbers of kanamycin- or neomycin-resistant bacteria already inhabit the human digestive system (Levy et al. 1998), with Flavell et al. (1992) estimating about 10^{12} per person. Kanamycin-resistant bacteria have been isolated from soil, river water and sewage (Smalla et al. 1993).

Section 5 Previous Australian and international approvals

5.1 Previous Australian approvals of the same or similar GM rose lines

5.1.1 Previous releases approved by GMAC or the Regulator

103. There have been no previous releases of the GMOs proposed for release in this trial. The three GM rose lines were developed in Japan and, after importation, have been grown in a PC2 glasshouse under NLRD 1011/2003. However, two field trials of GM roses by Calgene Pacific Pty Limited (now Florigene) were approved under the former voluntary system that was overseen by the Genetic Manipulation Advisory Committee (GMAC): PR30 and PR35. These proposed releases of GM roses were assessed by GMAC as posing negligible risks to human health and safety or to the environment. However, these trials were not conducted, and the approvals were allowed to lapse.

5.1.2 Approvals by other Australian government agencies

104. The *Gene Technology Act 2000* is designed to operate in a cooperative legislative framework with other regulatory authorities that have complementary responsibilities and specialist expertise. As well as enhancing coordinated decision making, this arrangement avoids duplication.

105. While the Regulator is responsible for identifying, assessing and managing risks to the health and safety of people and the environment associated with the development and use of gene technology, other government regulatory requirements may also have to be met in respect of release of GMOs, and the use of GMO products.

106. The importation of the three GM rose lines proposed for release required approval from the Australian Quarantine Inspection Service (AQIS).

107. Food Standard Australia New Zealand (FSANZ) is responsible for human food safety assessment, including GM food. The proposed release involves early stage research and the applicant does not intend any material from the GM rose lines proposed for release to be used

for human food. Accordingly the applicant has not applied to FSANZ for evaluation of materials from the trial for use in human food. However, FSANZ approval would need to be obtained before such materials could be used for this purpose.

5.2 International approvals

108. In the United States of America, four notifications (JP 001, JP 002, JP 004 and JP 005) of the same GM rose lines have been submitted. The GM rose plants are currently being trialled under these notifications in the state of California.

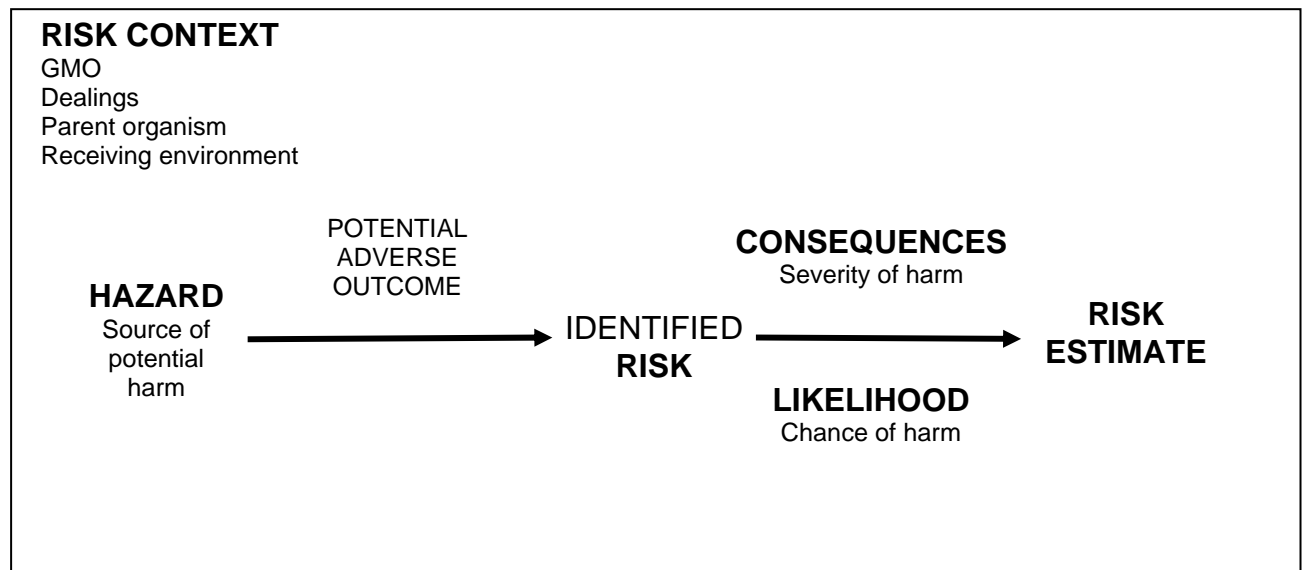
109. In Japan, greenhouse trials of the same GM rose lines have been approved. The GM rose plants are held at the Suntory greenhouses, located in Osaka. A contained trial has been approved and carried out in Okayama and an application has been submitted for an additional outdoor field trial at this location (information supplied by the applicant).

Chapter 2 Risk assessment

Section 1 Introduction

110. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 2.1) considers risks from the proposed dealings with the GMO that could result in harm to the health and safety of people or the environment posed by or as a result of gene technology.

Figure 2.1 The risk assessment process



Note: words in **bold** are defined in Appendix A.

111. Once the risk assessment context has been established (see Chapter 1) the next step is hazard identification to examine what harm could arise and how it could happen during this release of GMOs into the environment.

112. It is important to note that the word ‘hazard’ is used in a technical rather than a colloquial sense in this document. The hazard is a source of *potential* harm. There is no implication that the hazard will *necessarily* lead to harm. A hazard can be an event, a substance or an organism (OGTR 2005a).

113. Hazard identification involves consideration of events (including causal pathways) that may lead to harm. These events are particular sets of circumstances that might occur through interactions between the GMO and the receiving environment as a result of the proposed dealings.

114. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2005a). In conjunction with these techniques, hazards identified from previous RARMPs prepared for releases of the same and similar GMOs are also considered.

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

Section 2 Hazard characterisation

116. The list of events compiled during hazard identification are characterised to determine which events represent a risk to the health and safety of people or the environment posed by or as a result of gene technology.

117. A risk is identified only when there is some chance that harm will occur. Those events that do not lead to an adverse outcome or could not reasonably occur do not represent an identified risk and will not advance in the risk assessment process. Risks associated with the remaining events are assessed further to determine the seriousness of harm (consequence) and chance of harm (likelihood). The identified risks must be posed by or result from gene technology.

118. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2005a). Harm is assessed in comparison to the parent organism and in the context of the proposed dealing and the receiving environment. The risk assessment process focuses on measurable criteria for determining harm.

119. The following factors are taken into account during the assessment of events that may give rise to harm:

- the proposed dealings, which may include experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- the characteristics of the non-GM parent (OGTR 2005b)
- routes of exposure to the GMOs, the introduced genes and their products
- potential effects of the introduced genes and its products expressed in the GMOs
- potential exposure to the introduced genes and its products from other sources in the environment
- properties of the biotic and abiotic environment at the sites of release
- agronomic or horticultural management practices for the GMOs
- the size, duration and regions of the release.

120. Sixteen events that are discussed in detail later in this Section are summarised in Table 2.1. Events that share a number of common features are grouped together in broader hazard categories as indicated in the table. None of the events that were characterised are considered to lead to an identified risk that required further assessment.

Table 2.1 Summary of events that may give rise to adverse outcomes

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
SECTION 2.1 Production of a substance toxic to people	1. Ingestion of GM plant materials containing the introduced enzymes [flavonoid 3',5'-hydroxylase (F3'5'H), dihydroflavonol-4-reductase (DFR), anthocyanin 5-acyltransferase (5AT)] and their end-products (ie delphinidin and other anthocyanins and the suppression of rose <i>DFR</i> by dsRNA), as a result of the release.	Toxicity for people	No	<ul style="list-style-type: none"> No products from the release will be used in food. The introduced enzymes and their end-products are naturally present in a wide variety of plants, and have a long history of safe use in food. The introduced enzymes and their end-products are not known to be toxic to people. Toxicity studies show that the introduced enzymes and their end-products have no oral toxicity for mammals.
	2. Ingestion of GM plant materials containing the antibiotic resistance marker, neomycin phosphotransferase (NPTII) enzyme as a result of the release.	Toxicity for people	No	<ul style="list-style-type: none"> The NPTII is widely present in the environment and is not known to be toxic.
	3. Contact with, inhalation of, GM plant materials containing the introduced enzymes (including NPTII) and their end-products as a result of the release.	Toxicity for people	No	<ul style="list-style-type: none"> People are exposed to the introduced anthocyanin biosynthesis enzymes and their end-products via contact with a wide variety of foods and plants that already contain these enzymes and their end-products. The NPTII enzyme is widely spread in the environment and is not known to be toxic. Although neither of the WKS82 GM rose lines express the introduced genes, anthocyanins may be produced in the pollen of LA/919-4-10 GM rose line. Contact with the GM plant materials including the LA/919-4-10 pollen, will be limited because the proposed trial will be conducted in an enclosed insect-proof greenhouse for a short duration of two years and the flowers will be harvested before fully open. There will be some contact via shredding of GM plant materials before composting. However, the introduced enzymes and their end-products are not toxic to people.
SECTION 2.2 Production of a substance allergenic to people	1. Use of GM plant materials in food (eg honey, rose water, rose hips)	Allergic reactions in people	No	<ul style="list-style-type: none"> None of the GM plant materials will be used in food.
	2. Contact with GM plant materials containing the introduced enzymes (including NPTII) and their end-products, including contact with GM plant by-products (eg soil medium used in greenhouse, compost, cut	Allergic reactions in people	No	<ul style="list-style-type: none"> The introduced anthocyanin biosynthesis enzymes (F3'5'H, DFR, 5AT) and their end-products are naturally present in a wide variety of plants, and have a long history of safe use in food.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	flowers) as a result of the release.			<ul style="list-style-type: none"> • There are no reports of allergic reactions caused by the introduced enzymes and their end-products. • The NPTII enzyme is widely present in the environment and is not known to cause allergic reactions. • Contact with the introduced enzymes and their end-products will be limited because the proposed trial will be conducted in an enclosed insect-proof greenhouse for a short duration of two years. • There will be some contact via shredding of GM plant materials before composting. However, the introduced enzymes and their end-products are not known to cause allergic reactions in people.
SECTION 2.3 Production of a substance toxic to organisms other than people	1. Direct or indirect ingestion of GM plant materials containing the introduced anthocyanin biosynthesis enzymes and their end-products by vertebrates or invertebrates as a result of the release.	Toxicity for vertebrates or invertebrates	No	<ul style="list-style-type: none"> • Vertebrates and invertebrates are already exposed to the introduced enzymes and their end-products via a wide variety of plants that naturally contain them. • The introduced enzymes and their end-products are not known to be toxic to vertebrates and invertebrates. • Contact with the introduced enzymes and their end-products will be limited because the proposed trial will be conducted in an enclosed, insect-proof greenhouse for a period of two years. • Exposure of invertebrates to the GM roses will be further limited because the applicant proposes to spray the roses to keep them free of insect pests. • There will be some contact via composted GM plant material from the trial. However, the introduced enzymes and their end-products are not known to be toxic to vertebrates or invertebrates.
	2. Direct or indirect ingestion of GM plant materials containing the introduced NPTII enzyme by vertebrates or invertebrates as a result of the release.	Toxicity for vertebrates or invertebrates	No	<ul style="list-style-type: none"> • NPTII is widely present in the environment and is not known to be toxic to vertebrates or invertebrates.
	3. Contact between microorganisms (including soil biota) and GM plant materials containing the introduced enzymes (including NPTII) and their end-products, as a result of the release.	Toxicity for microorganisms	No	<ul style="list-style-type: none"> • Microorganisms are already exposed to the introduced anthocyanin biosynthesis enzymes and their end-products via a wide variety of plants that naturally contain them. • The introduced enzymes and their end-products are not known to be

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
				<p>toxic to microorganisms.</p> <ul style="list-style-type: none"> • The NPTII protein is widely present in the environment and is not known to be toxic to microorganisms. • The applicant proposes to grow the GM roses hydroponically in pots. • Contact between soil medium biota and the GM roses will be limited as delphinidins are not produced in the roots of the GM roses and in addition, the GM roses have non-GM rootstock. Also, the proposed release will be for a short duration of two years. • There will be some contact between microorganisms and composted GM material. However, the introduced enzymes and their end-products are not known to be toxic to microorganisms.
SECTION 2.4 Spread and persistence of the GM roses in the environment	<p>1. Expression of F3'5'H, DFR, 5AT and nptII genes, including the rose DFR with an inverted repeat, in the GM rose plants increasing the spread and persistence of the GM roses.</p>	<p>Weediness, allergic reactions, toxicity</p>	<p>No</p>	<ul style="list-style-type: none"> • The introduced enzymes and their end-products are not involved in plant growth and development and do not provide a selective advantage over non-GM roses. • The introduced anthocyanin biosynthesis enzymes and their end-products are naturally present in a wide variety of plants, and have a long history of safe use in food. • The NPTII enzyme is widely present in the environment. • The introduced enzymes and their end-products are not known to be toxic, or cause allergic reactions in people. • The dispersal of the GM rose lines proposed for release will be limited because the applicant proposes to grow the GM rose plants hydroponically in pots within an enclosed, insect-proof greenhouse. • The applicant proposes to shred the GM plant materials before composting.
	<p>2. Dispersal of GM plant materials, pollen or seed by various means, including:</p> <p>(a) by mammals, birds and invertebrates;</p> <p>(b) by germination of seeds from compost or asexual reproduction;</p> <p>(c) by spillage during transport;</p> <p>(d) by flooding or movement of compost containing any viable</p>	<p>Weediness</p>	<p>No</p>	<ul style="list-style-type: none"> • Mammals, birds and invertebrates will have limited access to the GM roses because the proposed trial will be conducted in an enclosed, insect-proof greenhouse. • Exposure of invertebrates to the GM roses will be further limited because the applicant proposes to spray the roses to keep them free of insect pests. • Seed germination and asexual

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	GM plant materials from the release sites.			<p>reproduction of complex hybrid roses requires controlled horticultural conditions.</p> <ul style="list-style-type: none"> • The applicant proposes to harvest the GM rose flowers, thereby limiting rosehip production and seed set. The flowers will be harvested at bud stage, further limiting pollen dispersal. • The applicant proposes to transport GM plant materials according to OGTR guidelines. • Limited scope for dispersal of viable GM rose materials as the proposed trial will be in an enclosed, insect-proof greenhouse. • The applicant proposes to grow the GM roses hydroponically in pots on benches. • The applicant proposes to shred and compost GM plant waste material.
<p>SECTION 2.5</p> <p>Gene flow by vertical gene transfer</p>	<p>1. Production of introduced enzymes and their end-products, in other rose species or cultivars as a result of gene transfer.</p>	<p>Weediness, allergic reactions, toxicity</p>	<p>No</p>	<ul style="list-style-type: none"> • Roses are insect pollinated and are capable of cross- and self-pollination. • The WKS82/130-4-1 and WKS82/130-9-1 GM rose lines appear to be periclinal chimeras, whereby the introduced genes are not expressed in the pollen, and crosses involving these two GM rose lines do not produce transgenic seed. • The introduced enzymes and their end-products do not provide a selective advantage over non-GM roses. • The introduced enzymes and their end-products are not known to be toxic, or cause allergic reactions. • Mammals, birds, invertebrates and microorganisms are already exposed to the introduced enzymes and their end-products are widespread in the environment. • There is limited scope for gene transfer to other rose species or cultivars, or for exposure of people and other organisms to the introduced enzymes and their end-products as a result of gene transfer, because the trial will be conducted in an enclosed, insect-proof greenhouse. • Furthermore, the applicant proposes to harvest the GM rose flowers when they are at the bud stage, limiting any pollen flow.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	2. Presence of the introduced regulatory sequences in other rose species or cultivars as a result of gene transfer.	Unpredictable effects	No	<ul style="list-style-type: none"> There is limited scope for gene transfer to other rose species or cultivars. However, the introduced regulatory sequences do not behave any differently than endogenous regulatory sequences in plants.
SECTION 2.6 Gene flow by horizontal gene transfer	1. Presence of the introduced genes or regulatory sequences in other plants, animals or microorganisms.	Weediness, toxicity, allergic reactions in people, increased pathogenicity or decreased effectiveness of antibiotic medicines	No	<ul style="list-style-type: none"> Horizontal gene transfer of the introduced sequences to other sexually incompatible organisms is not expected to result in any adverse outcomes during this release. Horizontal gene transfer events are expected to be rare and offer no selective advantage. No evidence of outcrossing between roses and unrelated plant species.
SECTION 2.7 Unintended changes in biochemistry or physiology	1. Altered biochemistry or physiology of the GM roses resulting from the introduction or expression of the introduced genes, including the rose DFR gene with an inverted repeat.	Toxicity for people or other organisms, weediness	No	<ul style="list-style-type: none"> The introduced enzymes and their end-products function in the same way as the native enzymes and end-products. Mismatches between the rose <i>DFR</i> dsRNA fragments and other genes (including the introduced iris <i>DFR</i>) may occur, leading to the suppression of non-targeted genes. However, the LA/919-4-10 GM rose line produces mauve rose flowers, suggesting that the introduced iris <i>DFR</i> is not suppressed by the rose <i>DFR</i> dsRNA fragments. The introduced enzymes and their end-products are secondary metabolites, are not involved in plant growth, development or reproduction, and do not provide any selective advantage over non-GM roses. Preliminary data show that there are no significant morphological differences between the two WKS/82 GM rose lines and their non-GM parent rose line. The LA/919 GM rose line shows lower petal number compared to its non-GM parent rose line. This is not likely to pose any harm to people or other organisms, or provide a selective environmental advantage.
SECTION 2.8 Unintended presence of <i>Agrobacterium</i> during release	1. Carry over of <i>Agrobacterium</i> with gene constructs from genetic modification experiments on GM rose plants (ie cuttings)	Potential impact on biodiversity, increased pathogenicity	No	<ul style="list-style-type: none"> Studies have shown that <i>Agrobacterium</i> is unlikely to persist in rose shoots/plants in a field trial environment. The GM rose lines were imported as tissue cultures from Japan under AQIS permit in October 2003. One of the conditions of their entry into

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
				<p>Australia was that the tissue culture growth media must not contain antibiotics or other microbial suppressants. The GM tissue cultures must also be free of bacterial contamination.</p> <ul style="list-style-type: none"> • <i>Agrobacterium</i> was not detected in similar GM rose lines held in Japan. • Dispersal of the three GM rose lines from the release sites will be limited because the proposed trial will be conducted in an enclosed, insect-proof greenhouse. In addition, the GM roses will be grown hydroponically in pots, and sprayed to keep them free of diseases. • Although the conditions for <i>Agrobacterium</i> infection and gene transfer may exist in nature, the creation of a GM plant would be highly unlikely.
SECTION 2.9 Unauthorised activities	1. Use of GMOs outside the proposed licence conditions (ie non-compliance)	Weediness, allergic reactions, toxicity	No	<ul style="list-style-type: none"> • The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires a test of the suitability of the applicant to hold a licence by the Regulator.

2.1 Production of a substance toxic to people

121. Toxicity is the cascade of reactions resulting from exposure to a dose of a chemical that is sufficient to cause direct cellular or tissue injury, or otherwise inhibit normal physiological processes (Felsot 2000). Toxic proteins are known to act via acute mechanisms rather than through chronic exposure (Sjoblad et al. 1992). Toxicity may occur through ingestion, contact or inhalation. The level of toxicity is often expressed as the LD₅₀. This is the amount of a substance given in a single dose that causes death in 50% of a test population of an organism.

122. Toxicity assays generally use the purified toxin of interest rather than the product that expresses the protein (eg GM plant material). This is necessary because the aim of the assays is to determine the concentration of toxin at which an adverse effect is seen. The level of expression in the product is used to determine the level of exposure to the toxin and comparison to the results of the toxicity assay indicate whether or not this is a safe level of exposure (OECD 1998; Konig et al. 2004). The use of purified toxin also increases the reproducibility of the assays.

2.1.1 *Ingestion of GM plant materials containing the introduced enzymes (F3'5'H, DFR, 5AT) and their end-products (delphinidin and other anthocyanins, and the suppression of the rose DFR by dsRNA), as a result of the release*

123. Exposure to the introduced enzymes and their end-products could occur as a result of ingestion of materials from the GM rose plants. However, this is considered unlikely as the applicant does not intend to use the GM plant materials from the proposed release for use in human food. Food Standards Australia and New Zealand (FSANZ) is responsible for human

food safety assessment, and FSANZ approval would be required before products from GM rose lines could be used in human food.

124. Delphinidin and other anthocyanins are produced as a result of the combined expression of the introduced genes *F3'5'H*, *DFR* and *5AT* together with endogenous genes in the anthocyanin biosynthetic pathway. As mentioned in Section 4.5 (Chapter 1), delphinidin and other anthocyanins are found in many fruits and vegetables which are commonly consumed and have a long history of safe use (Bridle & Timberlake 1997). Examples of plant foods containing these proteins include blueberries, cherries, grapes, and red onions (refer to Table 1.11, Chapter 1). The introduced enzymes and their end-products are also found in other common flowering plant species (eg wisteria, agapanthus and irises), and Australian native plants (eg eucalypts, acacias and wax plants).

125. None of the introduced enzymes are known toxins. The introduced enzymes are derived from black pansy (*F3'5'H*), iris (*DFR*), rose (*DFR*) and torenia (*5AT*). There are no reports of toxicity of black pansy, rose or torenia. However, rhizomes (thickened roots) and rootstocks from the iris (*Iris* spp.) produce a toxin which can cause low toxicity if ingested. However, the toxins produced in iris (irisin, iridin and irisine) are not anthocyanins, or anthocyanin related compounds, or involved in the anthocyanin biosynthetic pathway.

126. The applicant has provided preliminary data on the concentration of key flavonoid and anthocyanin in the flowers of the GM rose plants (refer to Table 1.9 in Chapter 1). The amount of delphinidin ranges from 0.06-0.11 mg/g FW (fresh weight) petal for the three GM rose lines. These values are less than the level in bilberry (*Vaccinium myrtillus*), which may contain up to 3.70 mg/g FW anthocyanin content of which approximately 43% is delphinidin (Kalt et al. 1999).

127. Anthocyanins are antioxidants and dietary antioxidants are believed to play a role in reducing the risks of various human degenerative diseases (Wang et al. 1997; Prior & Cao 2000). Anthocyanins from the European bilberry have been well studied with regard to human health (Morazzoni & Bombardelli 1996), and bilberry anthocyanin extract is currently marketed in a variety of pharmaceutical and food supplement products (Kalt & Dufour 1997).

128. Anthocyanins are approved for use as a food additive in Australia by FSANZ, in the European Union (E number 163) by the European Food Safety Authority (EFSA), and in the USA by the Food and Drug Administration (US FDA). The Therapeutic Goods Administration (TGA) has also approved the use of anthocyanins as colourants in orally administered medicines (www.tga.gov.au/meds/colourings.pdf). Anthocyanins extracted from red cabbage (*Brassica oleracea*) and sweet potato (*Ipomea batatas*) have been used as natural food colourants, and have had no adverse effects on human health (Nakayama et al. 2003).

129. Delphinidin is not known to be toxic when consumed or handled. As mentioned in Section 4.5 (Chapter 1), delphinidin and other anthocyanins are found in many raw foods (refer to Table 1.11 in Chapter 1). As a result, humans are naturally exposed to anthocyanins through ingestion of these fruit and vegetables. There are no toxicity data in the Merk Index for the aglycone (ie the non-sugar group of a glycoside), the mono-glucoside or the 3'5' di-glucoside of delphinidin.

130. Toxicological studies on delphinidins and anthocyanins are limited and have been carried out with mixtures extracted from a variety of fruits. The available data indicate that such extracts are of a very low order of toxicity. Diets containing 7.5% or 15% of a grape-skin extract preparation (approximately 3% anthocyanin) had no effect on the reproductive performance of rats in a 2-generation reproductive study. No compound-related effects were observed in a short-term study in which dogs were fed diets containing 7.5% or 15% of the

grape-skin extract preparation. The estimate of acceptable daily intake for humans is up to 2.5mg/kg body weight (IPCS 2003).

131. The phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L (also called roselle, red sorrel and karkade) was reviewed by Ali et al. (2005) (Ali et al. 2005). The calices (ie collective term for sepals of a flower) from this species are used in many parts of the world to make cold and hot drinks. The anthocyanin content is in the range of 1.7 to 2.5% of the calyx dry weight. The LD₅₀ of *H. sabdariffa* calyx extract in rats was found to be above 5000 mg/kg (Onyenekwe et al. 1999), indicating that the extract has very low oral toxicity.

132. A study on pharmacology reported short-term improvements in visual acuity and darkness adaptation in humans after receiving oral doses of up to 700 mg of anthocyanins (Pourrat et al. 1967 cited in IPCS 2003).

133. Studies on mutagenicity have shown that delphinidin is inactive in the Ames assay system using five different strains of *Salmonella typhimurium* (Brown & Dietric 1979). The Ames test identifies potential carcinogens by screening chemical compounds for their ability to cause mutations in genes, resulting in damage to the cell's DNA. Certain mutants of the *Salmonella* bacteria are used that are unable to produce the amino acid histidine that is essential for growth. The bacteria are then exposed to the compounds to be tested. If the bacteria are genetically altered by this exposure, they may regain the ability to make histidine, and start to grow. The Ames test is the world's most widely used test for identifying food additives and other substances likely to cause cancer.

134. Teratogens are agents that raise the incidence of congenital malformations. Studies on teratogenicity have shown that anthocyanin glycosides extracted from currants, blueberries and elderberries were not teratogenic in rats, mice or rabbits when given at dose levels of 1.5, 3 or 9 g/kg over 3 successive generations (Pourrat et al. 1967 cited in IPCS 2003). LD₅₀ values for oral administration of this extract were reported to be 25 and 20 g/kg of body weight for mice and rats respectively (IPCS 2003).

135. There is some evidence that anthocyanins may have beneficial health properties, including anti-oxidant, anti-inflammatory and anti-mutagenic activity (Harborne & Williams 2000; Nakayama et al. 2003).

136. Any ingestion, or use in food, of material from the GM rose lines containing the introduced enzymes and their end-products is not expected to occur from the proposed release. Furthermore, oral toxicity studies in mammals have not shown any adverse effects. Therefore, **no risk is identified** and the potential for toxicity for people as a result of ingestion of the introduced enzymes and their end-products will not be assessed further.

2.1.2 Ingestion of GM plant materials containing the antibiotic resistance marker neomycin phosphotransferase (NPTII) enzyme as a result of the release

137. The NPTII enzyme is widespread in the environment and in food chains, in naturally occurring kanamycin-resistant microorganisms that are found in soil and in mammalian digestive systems, without any suggested toxicity for humans. Proteins conferring resistance to kanamycin are naturally present in human foods, particularly raw vegetables (Flavell et al. 1992) (see Section 4.5, Chapter 1, for details).

138. NPTII is a phosphorylating enzyme, which does not possess any properties that distinguish it toxicologically from other phosphorylating enzymes present in microorganisms, plants and animals (FDA 1994). The function of this enzyme is the phosphorylation (inactivation) of the antibiotic neomycin (and the related kanamycin).

139. NPTII was the most commonly used marker gene recorded in the US field trial database for 2001 and 2002 (Mike & McHugh 2004). The insertion of the *nptII* gene into a wide range of GMOs has not resulted in any adverse effects (Flavell et al. 1992).

140. Oral toxicity studies in mammals have not shown any adverse effects (Calgene 1990, Berberich et al. 1993, Fuchs et al. 1993b). The toxicity of the NPTII protein has been examined in detail in a number of other RARMPs (most recently DIR 055/2004 and DIR 056/2004), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office.

141. FSANZ has concluded that food derived from GM corn, which expresses the NPTII protein, is safe for human consumption (FSANZ 2003). Regulatory agencies in other countries have also approved the use of the *nptII* gene as a selectable marker in food crops and approved the use of the gene and its encoded protein in human food and animal feed.

142. Currently kanamycin is not in human clinical use, while neomycin has limited and very specific clinical uses (TGA, MIMS Online database). Even if plant material containing the NPTII protein were ingested, this enzyme is not likely to be active outside of living cells, as it requires specific chemical conditions for activity, including the availability of specific co-factors (essential for the normal catalytic activity of an enzyme). Neomycin is registered with the APVMA for limited veterinary uses, however resistance to neomycin and kanamycin are widespread and other antibiotics are preferred (Flavell et al. 1992).

143. Therefore, **no risk is identified** and the potential for toxicity for people as a result of ingestion of the GM rose lines containing the NPTII enzyme will not be assessed further.

2.1.3 Contact with, or inhalation of, GM plant materials containing the introduced enzymes (including NPTII) and their end-products, as a result of the release

144. Human contact with plant materials from the GM roses includes handling of GM plant materials, cuts caused by thorns or inhalation of pollen. Contact may occur in the greenhouse where the GM roses will be grown (eg through harvesting flowers or pruning) or via the compost (eg shredding GM plant material prior to composting). This contact may lead to toxic effects for people as compared to non-GM rose plants and other plants that naturally contain similar enzymes and their end-products.

145. Generally, anthocyanins are predominantly produced in fruits or flowers (Seigler 1998), but have also been detected in vegetative (ie leaves, stems) and reproductive (ie anthers, pollen) plant tissues. Ten flavonoid biosynthesis-related genes, including *F3'5'H*, *DFR* and *5AT*, were found to be preferentially expressed in gentian (*Gentiana triflora*) petals compared with leaves and stems (Nakatsuka et al. 2005). The principle anthocyanin detected in the gentian petal tissues is delphinidin. Leaf and stem tissues contain little or no anthocyanin (Nakatsuka et al. 2005). An earlier study on the production of 5AT in gentian flowers also found that the enzyme appears to be confined to petals (Fujiwara et al. 1998). Similarly, a review by Nakayama et al. (2003) found that the 5AT enzyme occurs mainly in flowers and in the leaves of a few plant species, but rarely in roots. Anthers and pollen were not analysed in these studies. In gerbera (*Gerbera hybrida*), anthocyanins are produced in leaves, floral stems and flowers, with floral production limited to epidermal cell layers of the petals. In GM tobacco, anthocyanins are detected in vegetative parts and anthers (Elomaa et al. 2003).

146. There are few reports in the literature on the production of specific anthocyanins or the enzymes involved in anthocyanin biosynthesis in pollen. The anthers and pollen of some tulip cultivars were shown to contain delphinidins, but not pelargonidins or cyanidins (Nakayama et al. 2004). Anthocyanins are also produced in the pollen of poppy (*Papaver orientale*), petunia (*Petunia hybrida*), six pear species (*Pyrus*) and five pear cultivars (*Pyrus communis*)

(Roshchina & Mel'nikova 2001; Sharifani & Jackson 2004). The first *DFR* gene sequence identified from anthers is from rice (*Oryza sativa* L.) (Zhuang et al. 1999).

147. As mentioned in Section 2.5 (Chapter 1), the *F3'5'H*, *DFR* and *5AT* genes introduced into the GM roses are controlled by an enhanced cauliflower mosaic virus promoter (CaMV). This promoter is considered to be constitutive (ie a promoter that allows for continual transcription of its associated gene). However some tissue specificity and cell cycle stage-dependent expression has been found (Benfey et al. 1989). Preliminary experiments by the applicant have detected delphinidin in the flowers and leaves of the three GM rose lines, but not in the roots (refer to Section 3.3 of Chapter 1 for details). This suggests that the expression of the introduced enzymes and their end-products is restricted to specific plant tissues.

148. Breeding and *in situ* hybridisation experiments conducted in Japan show that the WKS/82-130-4-1 and WKS82/130-9-1 GM rose lines appear to be periclinal chimeras, where the introduced genes are only expressed in the outer layer of the rose plant tissue, and not in the pollen (refer to Section 3.2 of Chapter 1 for details).

149. Since the introduced enzymes and their end-products are retained within the GM plant cells, dermal and inhalation contact may occur only when the plant cells have been damaged or broken. Therefore, only people who work with the GM roses may come into contact with the introduced enzymes and their end-products during handling or processing the GM roses. There will also be some exposure to people via the compost (eg shredding the GM plant material prior to composting). However, as discussed in Section 2.1.1, people are already exposed to the introduced enzymes and their end-products via contact with a wide variety of foods and plants that naturally contain them. Toxicity studies of delphinidins and anthocyanins using mammalian models indicate very low levels of toxicity. Similarly, oral toxicity studies in mammals using NPTII have not shown any adverse effects (refer to Section 2.1.2).

150. On the basis that *F3'5'H*, *DFR*, *5AT* and their end-products, and NPTII, are not known to be toxic, they are also expected to be of very low acute dermal and inhalation toxicity. Furthermore, contact with introduced enzymes and their end-products will be limited because the proposed trial will be conducted in an enclosed greenhouse for a limited duration of two years. Therefore, **no risk is identified** and the potential for toxicity for people as a result of contact with, or inhalation of, the introduced enzymes and their end-products will not be assessed further.

2.2 Production of a substance allergenic to people

151. The possibility that exposure of people to proteins expressed from the introduced genes or their enzymatic products in the GM rose plants may result in an allergic reaction is considered. Routes of exposure to the introduced anthocyanin biosynthesis enzymes, their end-products and the NPTII enzyme could include consumption of food containing rose products, accidental ingestion of material from the rose plants, inhalation of pollen, contact with the compost or contact with material from rose plants, either as a result of occupational exposure or from living near the proposed release.

2.2.1 Use of GM plant material in food

152. None of the GM plant material from the proposed release will be used in food. Therefore, **no risk is identified** and the potential for allergic reactions in people resulting from exposure through food will not be assessed further.

2.2.2 Contact with GM plant materials containing the introduced enzymes (including NPTII) and their end-products, including contact with GM plant by-products as a result of the release

153. The possibility that exposure of people to the introduced enzymes (including NPTII) and their end-products in the GM rose plants may result in an allergic reaction is considered. In other plants, anthocyanins are predominantly produced in flower petals, but have also been detected in leaves, stems, anthers and pollen (refer to Section 2.1.3). Similarly, anthocyanins may be produced in these tissues in the GM roses because of the constitutive promoter controlling expression of the *F3'5'H*, *DFR* and *5AT* genes (refer to Section 2.5, Chapter 1). Consequently, human contact with these GM plant materials, and any GM rose by-products, may cause an allergic reaction as compared to non GM rose plants and other plants that naturally contain similar enzymes and their end-products. GM rose by-products include compost, harvested flowers and soil medium used to grow the GM roses.

154. As discussed in Section 2.1.1, the introduced enzymes and their end-products (ie delphinidin and other anthocyanidins) are widespread in the environment and have a long history of safe use.

155. The introduced enzymes are derived from black pansy (*F3'5'H*), iris (*DFR*), rose (*DFR*) and torenia (*5AT*). There are no reports of allergic reactions caused by black pansy, iris or torenia. There are some reports of humans developing allergies to roses (refer to Section 2.2, Chapter 1). However, none of these studies could rule out other factors that may be the cause of the allergy-like symptoms. Further details of these studies are available in the *Biology and Ecology of Roses (Rosa X hybrida)* (OGTR 2005b), which was produced to inform the risk assessment process for licence applications involving GM rose plants. This document is available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. The American Academy of Allergy Asthma and Immunology (AAAAI) website <<http://www.aaaai.org>> suggests that respiratory reactions to rose pollen must be quite rare as they could find no documented cases. This was likely due to the fact that rose pollen is heavy and sticky, designed for insect pollination rather than wind dispersal.

156. Literature searches have not found any reports of allergic reactions caused by the introduced enzymes and their end-products. Instead, there is some evidence that anthocyanins may have beneficial health properties, including anti-oxidant, anti-inflammatory and anti-mutagenic activity (Harborne & Williams 2000; Nakayama et al. 2003).

157. Anthocyanins are used as natural food colour additives in Australia, the European Union and the USA. Natural colouring compounds are of small molecular weight and are non-protein chemicals that would not be expected to elicit food allergies, either IgE or cell-mediated. Reports of allergenic reactions to natural colour additives are rare and have been attributed to the presence of protein residues in the additives rather than to anthocyanins (Taylor & Dormedy 1998). Furthermore, natural colour additives are not included among foods and food groups which are major causes of food allergy (information from the Codex Alimentarius Commission).

158. Grape skin extract or grape colour extract (also called enocianina) is a commonly used natural food colour derived from the skin of concord grapes. It contains anthocyanin pigments such as 3- and 3,5-diglucosides of malvin, delphinidin and cyanidin, and their acylated derivatives. There have been no reported allergic reactions to grape skin extract or grape colour extract (Lucas et al. 2001). Similarly, anthocyanin extracts from red cabbage and sweet potato have been used as natural food colourants with no adverse effects on human health (Nakayama et al. 2003).

159. As discussed in Section 2.1.3, breeding and *in situ* hybridisation experiments conducted in Japan show that the WKS/82-130-4-1 and WKS82/130-9-1 GM rose lines appear to be periclinal chimeras, where the introduced genes are only expressed in the outer layer of the rose plant tissue, and not in the pollen (refer to Section 3.2 of Chapter 1 for details).

160. The NPTII protein is approximately 29 kDa in size, which is within the typical size range of allergenic proteins. However, it does not possess glycosylation sites, is not stable in the mammalian digestive system and is heat labile, decreasing the probability that it is allergenic (US FDA 1998; Fuchs et al. 1993a; FDA 1994; ANZFA 2001e; ANZFA 2001f). Fuchs et al. (1993b) reported that no NPTII was detected 10 seconds after addition of simulated gastric fluid as measured by both Western blot and enzymatic activity. Protein sequence comparisons using sequences from four separate protein databases (EMBL, Genbank, PIR29 and Swiss-Prot) indicated that NPTII does not have significant sequence identity to any known protein food allergens (Fuchs & Astwood 1996).

161. Other regulatory agencies, in Australia and in other countries, have previously assessed the use of the *nptII* gene in food crops (eg ANZFA 2001a; ANZFA 2001b; ANZFA 2001c; ANZFA 2001d; FSANZ 2003). The FDA has evaluated data submitted for deliberate releases of GMOs expressing the NPTII protein and concluded that NPTII does not have any of the characteristics associated with allergenic proteins (US FDA 1998).

162. A number of genetically modified food crops containing the *nptII* gene have been approved for commercial release both in Australia (DIRs 012/2002, 021/2002 and 022/2002) and overseas. No adverse effects on humans, animals or the environment have been reported from these releases (US FDA 1998; Flavell et al. 1992; EFB 2001).

163. The UK Royal Society have concluded that there is at present no evidence that available GM foods cause allergic reactions, and that the risks posed by GM plants are in principle no greater than those posed by conventional breeding or by plants introduced from other areas of the world (The Royal Society 2002).

164. Human contact with introduced enzymes and their end-products will be limited because the GM roses will be grown in an enclosed insect-proof greenhouse for a limited duration of two years. However, there will be some contact between people and the compost where GM plant material will be destroyed (eg shredding of GM plant materials before composting). There are no reports of the introduced enzymes and their end-products causing allergic reactions in people. As a result, it is unlikely that contact with the GM plant materials will cause allergic reactions in people. Therefore, **no risk is identified** and the potential for allergic reactions in people resulting from contact with GM plant materials containing the introduced enzymes and their end-products will not be assessed further.

2.3 Production of a substance toxic to organisms other than people

165. A range of organisms may be exposed directly, through feeding on the GM rose plants or GM plant materials from the compost, or indirectly through eating organisms that have fed on the GM rose plants or the compost. These organisms include vertebrates, invertebrates and microorganisms.

2.3.1 *Direct or indirect ingestion of the GM plant materials containing the introduced anthocyanin biosynthesis enzymes and their end-products, by vertebrates or invertebrates as a result of the release*

166. Vertebrates and invertebrates may be exposed to the GM rose plants in a number of ways. For example, mammals may feed on the GM rose flowers, birds may ingest GM rose hips or seeds, and invertebrates may act as pollinators (eg bees) or insect pests (eg aphids). Direct or indirect ingestion may occur where the GM roses are grown (ie the greenhouse) or

where the GM materials are destroyed (ie the compost). Ingestion of these GM rose plant materials, containing the introduced enzymes and their end-products, may lead to toxic effects for vertebrates and invertebrates.

167. Vertebrates, including mammals, and birds, are already exposed to the introduced enzymes and their end-products via the wide variety of foods that contain them and plants that naturally contain them (refer to Section 2.1.1 for details). Studies testing the toxicity of delphinidins and other anthocyanins for mammals have been performed (refer to Section 2.1.1 for details). No adverse effects of anthocyanins have been detected in studies testing for mutagenicity (*Salmonella*), teratogenicity (rats, mice or rabbits), or toxicity (rats, dogs).

168. There is no direct evidence to indicate whether or not the introduced enzymes and their end-products are toxic to invertebrates. However, given that the introduced enzymes and their end-products are naturally present in many fruits, vegetables and plants, herbivorous vertebrates and invertebrates are already exposed to these enzymes and their end-products without any evidence of toxic effects.

169. Contact with introduced enzymes and their end-products will be limited because the proposed trial will be conducted in an enclosed insect-proof greenhouse for a limited duration of two years. Furthermore, the applicant proposes to spray the GM plants to keep them free of insect pests. There may be some contact between composted GM plant materials and vertebrates or invertebrates. However, the introduced enzymes and their end-products are not known to be toxic to these organisms. Therefore, **no risk is identified** and the potential for toxicity for organisms other than people as a result of direct or indirect ingestion of the GM plant materials containing the introduced enzymes and their end-products will not be assessed further.

2.3.2 Direct or indirect ingestion of the GM plant materials containing the introduced NPTII protein by vertebrates or invertebrates as a result of the release

170. As mentioned in Section 2.1.2, NPTII is a phosphorylating enzyme, which does not possess any properties that distinguish it toxicologically from other phosphorylating enzymes present in microorganisms, plants and animals (FDA 1994).

171. The NPTII protein is widespread in the environment since it is naturally produced by the common gut bacterium *Escherichia coli*. *E. coli* is widespread in human and animal digestive systems as well as in the environment. Humans (and, by implication, other animals) continually ingest kanamycin-resistant microorganisms, some containing the NPTII enzyme. Kanamycin-resistant bacteria have been isolated from soil, river water and sewage (Smalla et al. 1993).

172. Oral toxicity studies in mammals have not shown any adverse effects (Calgene 1990), (Berberich et al. 1993), (Fuchs et al. 1993b). There is no direct evidence to indicate whether or not the NPTII protein is toxic to invertebrates. However, given that the protein is widespread in the environment, invertebrates are expected to be exposed to NPTII, without evidence of toxic effects.

173. Any additional exposure resulting from the proposed release of the GM roses (via GM roses in the greenhouse or GM material in the compost) is not expected to result in significant toxic effects. Therefore **no risk is identified** and the potential for toxicity for vertebrates and invertebrates as a result of direct or indirect ingestion of the GM plant materials containing the NPTII protein will not be assessed further.

2.3.3 *Contact between microorganisms and the GM plant materials containing the introduced enzymes (including NPTII) and their end-products, as a result of the release*

174. As mentioned in Section 3.3 (Chapter 1), the applicant has measured the production of delphinidins in the GM rose flowers, leaves and roots. Delphinidin was detected in the flowers and leaves, but not in the roots. Consequently, soil microorganisms may be exposed to the introduced enzymes and their products through GM plant materials coming into limited contact with the greenhouse soil floor (eg leaves, petals, dead or diseased tissue dropping from the potted GM plants) for a short period of time before their removal, or through composting of GM plant waste material. This contact may lead to toxic effects for microorganisms caused by the introduced enzymes and their end-products.

175. Microorganisms are already exposed to the introduced enzymes and their end-products via contact with a wide variety of foods that contain them and plants that naturally produce them (refer to Section 2.1.1 and Section 2.1.2 for details).

176. The potential effects of delphinidins and other anthocyanins on microorganisms have been examined in GM blue carnations. Similar enzymes (F3'5'H from petunia and pansy, DFR from petunia) were introduced into GM carnations. Like roses, non-GM carnations lack the ability to produce delphinidins/blue coloured flowers. Experiments on the potential toxicity of GM blue carnations were carried out using seed germination and plant growth tests as direct bioassays and supplemented with direct counts of microorganisms. Further details are available in the GM blue carnation RARMP (DIR 030/2002), which is available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office.

177. To determine whether GM blue carnation plants had any effect on the soil microflora, bacteria and fungal counts were made from soil surrounding GM and control plants (unmodified plants of the same variety), using standard microbiological media. Similar quantities of bacteria and fungal spores were found in the soil from the control and GM plants.

178. The toxicity of the NPTII protein has been examined in detail in a number of other RARMPs (most recently DIR 055/2004 and DIR 056/2004), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. There is no direct evidence to indicate whether or not the NPTII protein or other similar proteins may be toxic to some organisms. However, these proteins are produced by common soil bacteria to protect them against exposure to the antibiotics neomycin and kanamycin (EFB 2001; EFSA 2004; Goldstein et al. 2005). Microorganisms are therefore expected to be exposed to these proteins in the natural and agricultural environment. Any additional exposure resulting from the production of the NPTII protein in the GM rose plants is not expected to result in significant toxic effects.

179. Microorganisms will be exposed to GM plant materials (stems, leaves, flowers) during composting. However, during the process of composting, the introduced genes, enzymes and their end-products will be degraded like any other plant enzymes—potentially by the action of microorganisms or by heat generated during composting. There will be no differences between composting the GM roses and other plants that naturally contain delphinidins.

180. The introduced enzymes and their end-products are not known to be toxic to microorganisms. As a result, contact between soil biota and the GM materials on the greenhouse floor or in the compost is not expected to lead to toxic effects for microorganisms. Therefore, **no risk is identified** and the potential for toxicity for microorganisms as a result of contact with the introduced enzymes and their end-products will not be assessed further.

2.4 Spread and persistence of the GM rose lines in the environment

2.4.1 Expression of *F3'5'H*, *DFR*, *5AT* and *nptII* genes, including the rose *DFR* gene with an inverted repeat, in the GM rose plants increasing the spread and persistence of the GM roses

181. The rose lines have been modified to express enzymes (*F3'5'H*, *DFR* and *5AT*) in the anthocyanin biosynthetic pathway that are essential for the formation of blue pigment in the flowers, namely delphinidin. The expression of these introduced genes (including the rose *DFR* gene with an inverted repeat) may provide a selective advantage for the GM roses over non-GM roses, leading to spread and persistence or weediness. Spread and persistence of the GM rose plants in the environment could lead to increased exposure of people and other organisms to the expressed enzymes and their end-products, resulting in allergic reactions for people or toxicity for people and other organisms.

182. As mentioned in Section 2.2 (Chapter 1), roses are long-living plants and may be found in isolation in situations where deliberately planted roses have been left unattended. Two rose species, *R. canina* and *R. rubiginosa*, are fully naturalised and have become noxious weeds in temperate Australia. However, no cultivated complex hybrid rose types (*Rosa X hybrida*) have become naturalised, despite a long history of cultivation. This information on non-GM roses is included here to establish a baseline for comparison with the GM rose lines being considered in this assessment.

183. None of the *F3'5'H*, *DFR* or *5AT* genes, or their combined effects (ie a change in flower colour) are associated with any life history or fitness characters. These introduced genes express delphinidin and other anthocyanins, which are secondary metabolites. Secondary metabolites are compounds produced by plants that do not appear to be essential for normal growth and development (Winkel-Shirley 2002).

184. However, secondary metabolites are important in the interaction of the plant and its environment, ensuring the survival of the organism in its ecosystem (Verpoorte 2002). The presence or absence of anthocyanins affects flower colour, which in turn attracts pollinators. Roses are insect pollinated and are capable of cross- and self-pollination. Therefore, anthocyanins may be important for reproductive success and gene transfer. However, while bee-pollinated flowers tend to be blue, it is not known if light purple or violet coloured GM rose flowers will alter pollinator behaviour (refer to Section 2.5.1 for more details). It should be noted that the colour of the GM rose lines is only marginally different in shade from the flower colour of existing non-GM rose varieties, including 'Rhapsody in Blue', 'Delilah' and 'Lava' (refer to Section 3.2 of Chapter 1 for more details).

185. Gene transfer via pollen dispersal from the WKS/82-130-4-1 and WKS82/130-9-1 GM rose lines will not result in transfer of the introduced genes. Breeding and *in situ* hybridisation experiments conducted in Japan show that the two WKS/82 GM rose lines appear to be periclinal chimeras. The introduced genes are only expressed in the outer epidermal layer of the WKS/82 GM rose lines (which forms a continuous layer over all tissues of the leaf, stem, flower petals, etc.), and are not in the pollen or anthers of these GM rose lines. However, the WKS/82 GM rose lines can still be vegetatively propagated. Data from similar *in situ* hybridisation experiments using the LA/919-4-10 GM rose line were not available. However, the breeding experiments show that the LA/919 GM rose line does produce transgenic seed, so this GM rose line is unlikely to be a chimeric plant (refer to Section 3.2 of Chapter 1 for details).

186. In addition to pigment production, anthocyanin-related pigments are important in plant disease defence, pollen viability, microbial interactions and UV protection (Clegg & Durbin

2000). However, although non-GM roses do not naturally produce delphinidin, they do produce other anthocyanins (Ogata et al. 2005). Therefore, the GM roses will not have a selective advantage over non-GM roses through the production of these compounds.

187. The *nptII* gene or the introduced regulatory sequences in the GM rose plants are not expected to have any impact on the spread and persistence of the GM rose plants. Kanamycin and neomycin are not applied to plants outside the laboratory, nor does production by microorganisms result in high levels in the environment. Furthermore, the introduced regulatory sequences behave no differently to endogenous regulatory sequences in roses.

188. As discussed in Section 2.1.1, the introduced enzymes and their end-products (ie delphinidin and other anthocyanidins) are widespread in the environment and have a long history of safe use. Consequently, people, mammals, birds, invertebrates and microorganisms are already exposed to the introduced enzymes and their end-products via contact with many fruits, vegetables and plants. Literature searches have not found any reports of allergic reactions caused by the introduced enzymes and their end-products. Studies testing the toxicity of delphinidins and other anthocyanins for mammals have been performed (see Section 2.1.1 for details). No adverse effects of anthocyanins have been detected in studies testing for mutagenicity (*Salmonella*), teratogenicity (rats, mice or rabbits), or toxicity (rats, dogs). There is no direct evidence to indicate whether or not the introduced enzymes and their end-products are toxic to invertebrates. Results from experiments on the effect of delphinidins and other anthocyanins (produced in GM blue carnations) discussed in Section 2.3.3 suggest that the introduced enzymes and their end-products are not toxic to microorganisms.

189. Similarly, the NPTII protein is widespread in the environment and in food chains, without any suggested toxicity for humans or mammals (see Section 2.1.2 for details). Oral toxicity studies in mammals have not shown any adverse effects (Calgene 1990, Berberich et al. 1993, Fuchs et al. 1993b). Protein sequence comparisons using sequences from four separate protein databases (EMBL, Genbank, PIR29 and Swiss-Prot) indicated that NPTII does not have significant sequence identity to any known protein food allergens (Fuchs & Astwood 1996). The FDA has evaluated data submitted for deliberate releases of GMOs expressing the NPTII protein and concluded that NPTII does not have any of the characteristics associated with allergenic proteins (US FDA 1998) (see Section 2.2.2 for details).

190. There is limited scope for the spread and persistence of the GM rose lines beyond the release sites because the applicant proposes to grow the GM rose plants hydroponically in pots within an enclosed, insect-proof greenhouse. The applicant also proposes to harvest all flowers from bud (petals with colour visible) to partly open (petals beginning to expand from the flower). Furthermore, GM plant materials will be destroyed by shredding and composting.

191. Therefore, **no risk is identified** and the potential for spread and persistence or weediness as a result of expression of the introduced genes, or toxicity or allergic reactions in people as a result of the spread and persistence of the GM rose plants in the environment, will not be assessed further.

2.4.2 Dispersal of GM plant materials, pollen or seed by various means

192. The dispersal of GM rose plant materials, pollen or seed could lead to spread and persistence, which in turn could lead to weediness. Dispersal of the GM plant materials, pollen or seed could occur by a number of various means including dispersal by:

- (a) mammals, birds and invertebrates;
- (b) germination of seeds from compost or asexual reproduction;

- (c) spillage during transport;
- (d) flooding or movement of compost containing any viable GM plant materials from the release sites.

193. As mentioned in Section 2.2 (Chapter 1), roses are long-living plants and may be found in isolation in situations where deliberately planted roses have been left unattended. Two rose species, *R. canina* and *R. rubiginosa*, are fully naturalised and have become noxious weeds in temperate Australia. However, no cultivated complex hybrid rose types (*Rosa X hybrida*) have become naturalised, despite a long history of cultivation. This information on non-GM roses is included here to establish a baseline for comparison with the GM rose lines being considered in this assessment.

(a) Dispersal by mammals, birds and invertebrates

194. Rose seeds are dispersed by mammals and birds. Rose hips are brightly coloured and appear to attract birds and other animals which then excrete the seed in viable condition (Parsons & Cuthbertson 2001). Roses are insect pollinated and are capable of cross- and self-pollination. Rose pollen tends to be large, sticky and heavy, and is likely to be carried by insects rather than wind. An overview of the seed dispersal and pollination biology of *Rosa X hybrida* is provided in the document *Biology and Ecology of Roses (Rosa X hybrida)* (OGTR 2005b), which was produced to inform the risk assessment process for licence applications involving GM rose plants. This document is available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. It is unlikely that seed dispersal and pollination characteristics will change as a result of genetic modification.

195. Breeding and *in situ* hybridisation experiments conducted in Japan show that the WKS/82-130-4-1 and WKS82/130-9-1 GM rose lines appear to be periclinal chimeras. Therefore, pollen or seed dispersal from these two WKS/82 GM rose lines will not result in the spread and persistence of these GM rose lines. In contrast, breeding experiments show that the LA/919 GM rose line does produce transgenic seed (refer to Section 3.2 of Chapter 1 for details).

196. Furthermore, there is limited scope for the spread and persistence of the GM rose lines beyond the release sites because the trial will be conducted in an enclosed insect-proof greenhouse, with limited access, for a short duration of two years. Invertebrate contact with the GM roses will be further limited because the applicant proposes to spray the roses to keep them free of insect pests. In addition, the applicant proposes to harvest the GM rose flowers, thereby eliminating the possibility of rose hip formation and seed set. Pollen dispersal will be limited because the flowers will be harvested from bud (petals with colour visible) to partly open (petals beginning to expand from the flower).

197. Therefore, **no risk is identified** and the potential for weediness as a result of the dispersal of the GM plant materials, pollen and seed by mammals, birds or invertebrates will not be assessed further.

(b) Dispersal by germination of seeds from compost or asexual reproduction

198. As mentioned in Section 2.1 (Chapter 1), the applicant proposes to maintain the GM rose plants during the trial by pruning and removing dead or diseased tissue, and destroy GM plant material by composting. Details of the composting methods proposed by the applicant are described in Section 4.3 (Chapter 1). Germination of seeds from the compost may lead to spread and persistence or weediness. Asexual reproduction (ie re-growth or vegetative propagation) of GM rose cuttings from the release sites could also lead to spread and persistence or weediness.

199. Rose seeds generally require a period of stratification (ie alternate periods of freezing and thawing) before they will germinate. *Rosa multiflora* requires about 6 weeks of moist chilling at 3°C for optimum germination, but other species such as *R. rugosa* and *R. hygonis* require four to six months, and *R. blanda* requires ten months of stratification prior to germination. *Rosa canina* germinates best if the seeds are kept moist at room temperature for two months followed by an additional two months at 0°C (Hartmann & Kester 1975). However, in Australia seeds of *R. canina* and *R. rubiginosa* (both considered weedy species in Australia) may germinate at anytime of the year provided moisture is available. The seeds of *R. rubiginosa* may remain viable in the soil for 3 or 4 years (Parsons & Cuthbertson 2001).

200. The parent rose lines of the GM roses are cultivated hybrid roses (refer to Section 2.2, Chapter 1 for details). Hybrid rose seeds germinate best after 2 to 3 months at 1 to 4°C. However, a few seeds may germinate with no cold treatment at all. If seeds are produced from the GM roses, it is likely that seeds they will have similar requirements for germination (Hartmann & Kester 1975).

201. Roses are capable of asexual (ie vegetative) propagation. However, vegetative propagation of complex hybrid roses requires controlled horticultural conditions including shading, adequate moisture and good soil contact (Hartmann & Kester 1975). The applicant has provided preliminary data on adventitious root formation in the LA/919-4-10 GM line compared to its parent rose line. The GM rose line showed a lower percentage of rooting and fewer roots per rooted cutting when compared to the parent rose line (see Section 3.2 in Chapter 1 for more details). This suggests that root formation in plant material from the GM roses will be limited.

202. Wild (or species) roses are extremely hardy in their native environment (Ross 1991). Hybrid roses are less hardy than wild rose species. As a result, propagation of hybrid roses by grafting onto rootstock is commonly used. The rootstock can be chosen to suit particular growing conditions (eg alkaline or acidic soil, hot or cold climate). The rootstock tends to be more winter-hardy and disease-resistant than the hybrid rose (Hartmann & Kester 1975). As described in Section 4.3 (Chapter 1), the GMO proposed for release is a GM graft onto non-GM rootstock. Therefore, even if cuttings from the GM rose plants develop roots, it is unlikely that they will survive to develop into fully grown rose plants.

203. Germination of seeds from the WKS/82-130-4-1 and WKS82/130-9-1 GM rose lines will not result in the spread and persistence of these two GM rose lines. As mentioned previously, breeding and *in situ* hybridisation experiments conducted in Japan show that the WKS/82-130-4-1 and WKS82/130-9-1 GM rose lines appear to be periclinal chimeras. However, the WKS/82 GM rose lines can still be vegetatively propagated. In contrast, breeding experiments show that the LA/919 GM rose line does produce transgenic seed (refer to Section 3.2 of Chapter 1 for details).

204. There is limited scope for the dispersal of the GM rose lines beyond the release sites through germination of seeds from the compost, or through asexual reproduction. The proposed release will be conducted in an enclosed greenhouse, with limited access. The GM rose flowers will be harvested, thereby eliminating the possibility of rose hip formation and seed set. Furthermore, GM plant materials, along with the non-GM rootstock, will be destroyed by shredding and composting, which will reduce the ability of GM plants to propagate through seed germination (in the unlikely event that seeds are produced), or through cuttings.

205. Therefore, **no risk is identified** and the potential for spread and persistence or weediness as a result of germination of seeds from the compost and asexual reproduction will not be assessed further.

(c) Dispersal by spillage during transport

206. The applicant proposes to transport the GM rose plants from the Florigene PC2 facility to the release site (ie the greenhouse). From the onset of first flowering, GM plant materials will be transported from the greenhouse to the Florigene laboratories. The applicant has stated that plant material transported to the Florigene laboratories will be primarily flowers. GM plant waste material from the greenhouse will be transported to the composting site for shredding. Any accidental spillage of plant material during transport could allow the GM rose plants to spread and persist in the environment.

207. The applicant proposes to transport GM plant materials according to OGTR transportation guidelines (*Guidelines for the transport of GMOs, June 2001; Policy on transport and supply of GMOs, July 2005*). Therefore, any spillage of GM plant materials during transport to and from the release sites would be rare.

208. In addition, the opportunity for any adverse outcome from any such rare occurrence is further diminished by the need for appropriate horticultural conditions for germination, survival and persistence of escaped GM plant material (refer to the above information on 'Dispersal by germination of seeds from compost or asexual reproduction').

209. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of spillage of GM plant material during transport or storage will not be assessed further.

(d) Dispersal by flooding or movement of compost containing any viable GM plant materials from the release sites

210. The release sites include the greenhouse, where the GM roses will be grown, and the compost, where GM plant waste material will be destroyed. GM plant materials, pollen or seed may be dispersed by flooding of the greenhouse or compost, or by movement of the compost to areas where the GM seed could germinate and GM rose plants could establish. This could result in spread and persistence or weediness of the GM rose plants.

211. The GM rose plants will be grown in an enclosed greenhouse, with limited access. The GM roses will be grown in pots, above the soil, and will remain in the same pots in the same coco peat soil medium for the duration of the trial. Therefore, there is limited scope for the dispersal of viable GM materials due to flooding from this release site.

212. There is also limited scope for the dispersal of the GM roses due to flooding of the compost. The applicant proposes to harvest the GM rose flowers, thereby eliminating the possibility of rose hip formation and seed set. The GM plant waste material (including the soil used to grow the GM plants in pots) will be destroyed by shredding and composting. Shredding will limit the possibility of asexual reproduction or seed germination (in the unlikely event that seeds are produced from the GM rose plants). Furthermore, asexual reproduction and seed germination from the composted GM plant materials is unlikely outside of controlled horticultural conditions. As mentioned above in 'Dispersal by germination of seeds from compost or asexual reproduction', appropriate horticultural conditions are required for germination, survival and persistence of complex hybrid roses. Furthermore, germination of seeds from the WKS/82-130-4-1 and WKS82/130-9-1 GM rose lines will not result in the spread and persistence of these two GM rose lines. Breeding and *in situ* hybridisation experiments conducted in Japan show that the WKS/82-130-4-1 and WKS82/130-9-1 GM rose lines appear to be periclinal chimeras. Consequently, these two GM rose lines do not produce transgenic seed (refer to Section 3.2 of Chapter 1 for details).

213. Therefore, **no risk is identified** and the potential for spread and persistence or weediness as a result of the dispersal of the GM plant material by flooding or movement of compost from the release sites will not be assessed further.

Uncertainty

214. There is limited data on whether agronomic characteristics which are indicative of weediness will be acquired as a result of the genetic modifications. This information would be useful for determining the opportunity for the spread and persistence of the GM roses in possible future applications involving release of the GM rose lines into the open environment (ie outside of a greenhouse). However, this information is not required for assessing the risk of this proposed release of GM rose lines because the trial is limited in size, duration and location.

2.5 Gene flow by vertical gene transfer

215. Transfer of genetic material to offspring by reproduction (vertical gene transfer) could result in the transfer of the introduced genes or their associated regulatory elements to other plants.

216. As mentioned in Section 2.2 and 4.4 (Chapter 1), interspecific and intraspecific gene transfer is possible among many of the *Rosa* species. There are a number of sexually compatible species present in Australia that could receive genes from the GM rose lines, including naturalised rose species and other cultivated rose species. The applicant proposes to grow sexually compatible non-GM rose plants alongside GM rose plants, including the parent rose lines. Therefore, transfer of genetic material to offspring by reproduction, either asexual or sexual (vertical gene transfer) could result in the transfer of the introduced genes or their associated regulatory elements to other rose plants.

217. Vertical transfer of the introduced genes to other rose species or cultivars may lead to a chain reaction of potential adverse outcomes. Firstly, expression of the introduced genes in other rose species or cultivars, as a result of vertical gene transfer, may lead to spread and persistence or weediness. Secondly, spread and persistence or weediness may lead to the increased exposure of people and other organisms to the introduced enzymes and their end-products, which may lead to toxicity or allergic reactions in people, or toxicity for mammals, birds, invertebrates or microorganisms.

2.5.1 Production of the introduced enzymes and their end-products, in other rose species or cultivars as a result of gene transfer

218. Expression of the introduced genes in other rose species or cultivars, as a result of vertical gene transfer, may lead to spread and persistence or weediness as a result of selective advantage. The spread and persistence of the GM rose plants in the environment could lead to increased exposure of people and other organisms to the expressed enzymes and their end-products, resulting in allergic reactions for people or toxicity for people and other organisms.

219. As mentioned in Section 2.2 (Chapter 1), interspecific and intraspecific hybridisation is common in the *Rosa* genus. Roses are insect pollinated and are capable of cross- and self-pollination. Rose pollen tends to be large, sticky and heavy, and is likely to be carried by insects rather than wind (OGTR 2005b). Pollinating insects can include bumble bees, honey bees, wasps, and flies. An overview of the pollination biology of *Rosa X hybrida* is provided in the document *Biology and Ecology of Roses (*Rosa X hybrida*)* (OGTR 2005b), which was produced to inform the risk assessment process for licence applications involving GM rose plants. This document is available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office.

220. Vertical gene transfer between the WKS/82-130-4-1 and WKS82/130-9-1 GM rose lines and other rose species and cultivars will not result in transfer of the introduced genes. Breeding and *in situ* hybridisation experiments conducted in Japan show that the two WKS GM rose lines appear to be periclinal chimeras. However, the breeding experiments show that the pollen from the LA/919 GM rose line is viable and crosses using this pollen produce transgenic seed (refer to Section 3.2 of Chapter 1 for details).

221. Flower colour is one of several chemical signals that attract insects and animals for pollination and seed dispersal, and could have an impact on the number of pollinator visitations (Schemske & Bradshaw 1999). Different pollinators see flower colour in different ways. Flowers attractive to bees are usually yellow or blue, and some are white (Seigler 1998). Red flowers are colourless to bees, but are seen by birds.

222. Research into two monkeyflower species (*Mimulus cardinalis* and *M. lewisii*) found that allele substitutions at a flower colour locus resulted in a change in pollinator preference for flowers (Bradshaw & Schemske 2003). The wildtype *M. lewisii* is pink flowered and pollinated by bumblebees, while the wildtype *M. cardinalis* is white-flowered and pollinated by hummingbirds. In this experiment, the gene responsible for flower colour in these plants was substituted between plants, such that *M. cardinalis* produced pink flowers and *M. lewisii* produced white flowers. The pink flowered *M. cardinalis* received 74-fold more bee visits compared to its white flowered wildtype. Similarly, the white flowered *M. lewisii* received 68-fold more hummingbird visits than the pink flowered wildtype.

223. While bee-pollinated flowers tend to be blue (Harborne & Grayer 1993), it is not known if light purple or violet coloured GM rose flowers will alter pollinator behaviour. It should be noted that the colour of the GM rose lines are only shades different from the flower colour of existing non-GM rose varieties, including 'Rhapsody in Blue', 'Delilah' and 'Lava' (refer to Section 3.2 of Chapter 1 for more details).

224. As discussed in Section 2.4.1, the introduced anthocyanin biosynthesis enzymes and their end-products do not provide a selective advantage over non-GM roses. Delphinidins and other anthocyanins are secondary metabolites, and are therefore not involved in plant growth, development or reproduction (Winkel-Shirley 2002).

225. There is limited scope for gene transfer to other rose species or cultivars. Insect pollinators will not gain access to the GM rose plants because the release will be conducted in an insect-proof greenhouse. Also, the applicant proposes to harvest all flowers produced by the three GM rose lines and the non-GM parent rose lines, thereby limiting the possibility of hip formation and seed set. Pollen flow will also be limited because the applicant intends to harvest flowers from bud to partly open.

226. As discussed in Section 2.1.1 and Section 2.1.2, the introduced anthocyanin biosynthesis enzymes and their end-products (ie delphinidin and other anthocyanidins) and the NPTII enzyme are widespread in the environment and have a long history of safe use. Consequently, people, mammals, birds, invertebrates and microorganisms are already exposed to the introduced enzymes and their end-products via contact with many fruits, vegetables and plants. Literature searches have not found any reports of allergic reactions caused by the introduced enzymes and their end-products. Studies testing the toxicity of delphinidins and other anthocyanins for mammals have been performed (see Section 2.1.1 for details). No adverse effects of anthocyanins have been detected in studies testing for mutagenicity (*Salmonella*), teratogenicity (rats, mice or rabbits), or toxicity (rats, dogs). There is no direct evidence to indicate whether or not the introduced enzymes and their end-products are toxic to invertebrates. Results from experiments on the effect of delphinidins and other

anthocyanins (produced in GM blue carnations) discussed in Section 2.3.3 suggest that the introduced enzymes and their end-products are not toxic to microorganisms.

227. Since there is limited scope for gene transfer to other rose species or cultivars, there is limited chance that the introduced genes will be expressed in other rose plants. Therefore, **no risk is identified** and the potential for spread and persistence or weediness as a result of expression of the introduced genes, or toxicity or allergic reactions in people as a result of the spread and persistence of the GM rose plants in the environment, will not be assessed further.

Uncertainty

228. There is limited data on the changes in pollinator behaviour as a result of GM rose lines with light purple or violet coloured flowers. This information would be useful for determining the opportunity for, and changes in the frequency of, gene transfer between the LA/919-4-10 GM rose line and non-GM rose lines in possible future applications involving release of the GM rose lines into the open environment (ie outside of a greenhouse). However, this information is not required for assessing the risk of this proposed release of GM rose lines because the trial is limited in size, duration and location.

2.5.2 Presence of the introduced regulatory sequences in other rose species or cultivars as a result of gene transfer

229. There is limited scope for gene transfer to other rose species or cultivars (refer to Section 2.5.1). The proposed trial will take place in an enclosed insect-proof greenhouse, and all flowers will be harvested from bud to partly open.

230. Furthermore, all of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to rose plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. The impacts from the introduced regulatory elements are equivalent and no greater than the endogenous regulatory elements.

231. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of vertical gene transfer of introduced regulatory sequences will not be assessed further.

2.6 Gene flow by horizontal gene transfer

2.6.1 Presence of the introduced genes or regulatory sequences to other plants, animals or microorganisms

232. Transfer of the introduced genes, or the introduced regulatory sequences, from the GM plants to sexually incompatible plants, animals or microorganisms (horizontal gene transfer) can occur only rarely without human intervention.

233. Transfer of the *F3'5'H*, *DFR* and *5AT* genes to other organisms could cause the spread of these genes in the environment. However, these enzymes and their end-products (ie delphinidin and other anthocyanins) already occur naturally in many fruits, vegetables and plants. No adverse effects of anthocyanins have been detected in studies testing for mutagenicity (*Salmonella*), teratogenicity (rats, mice or rabbits), or toxicity (rats, dogs). There is some evidence that anthocyanins may have beneficial health properties, including anti-oxidant, anti-inflammatory and anti-mutagenic activity (Harborne & Williams 2000; Nakayama et al. 2003) (see Section 2.1.1 for more details).

234. The *nptII* gene is already naturally present in bacterial species, any transfer of this gene is much more likely to occur between bacteria, or bacteria and other organisms, than between plant and other organisms. In the extremely unlikely event that transfer of *nptII* to other organisms were to occur, it could result in antibiotic medicines containing kanamycin or

neomycin becoming less effective due to spread of resistance to these antibiotics in the environment. Currently kanamycin is not in human clinical use, while neomycin has limited and very specific clinical uses (TGA, MIMS Online database). Neomycin is registered with the APVMA for limited veterinary uses (APVMA, Pubcris database), however resistance to neomycin and kanamycin are widespread and other antibiotics are preferred (Flavell et al. 1992).

235. The *nptII* was originally isolated from mobile genetic elements (transposons) found in the plasmids and chromosomes of *E. coli*. *E. coli* is widespread in human and animal digestive systems as well as in the environment. Transposons are readily transferable between bacterial species in nature. The *nptII* gene is associated with transposon Tn5 (Beck et al. 1982) and is observed in gram negative bacteria and *Pseudomonas sp.* While it is widely dispersed in the environment, other genes that also confer resistance to neomycin and kanamycin are more common, and also readily transferable among bacterial species (Smalla et al. 1994; Belgian Biosafety Server 1999).

236. Transfer of the regulatory sequences to other organisms could alter the expression of endogenous genes in unpredictable ways. However, all of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to rose plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. As there is no difference between the two events, this does not represent a novel adverse outcome as a result of the genetic modification.

237. Horizontal gene transfer has been examined in detail in a number of other RARMPs (most recently DIR 057/2004), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. These assessments have concluded that horizontal gene transfer from plants to other sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. An extensive search of the relevant literature did not reveal evidence of gene transfer between roses and unrelated plant species. Reports of horizontal gene transfer from plants to bacteria occurring during laboratory experiments have relied on the use of highly similar sequences to allow homologous recombination to occur, and conditions designed to enhance the selective advantage of gene transfer events (Nielsen et al. 1998; Mercer et al. 1999; Nielsen et al. 2000; Gebhard & Smalla 1998; De Vries et al. 2001). Horizontal gene transfer is not expected to produce any adverse outcomes during this proposed release. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of horizontal gene transfer will not be assessed further.

2.7 Unintended changes in biochemistry or physiology

238. Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such effects may include:

- altered expression of an unrelated gene at the site of insertion
- altered expression of an unrelated gene distant to the site of insertion for example, due to changes in chromatin structure, methylation patterns, transcriptional read-through
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions of an introduced gene product with endogenous non-target molecules

- secondary effects arising from altered substrate or product levels in the biochemical pathway of the introduced gene product.

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

240. Unintended changes in gene expression could alter either the biochemistry or the physiology of the GM rose plants. Biochemical or physiological changes to the GM rose lines proposed for release could occur either as a result of the expression of the introduced genes or of the transformation process itself. However, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

2.7.1 Altered biochemistry or physiology of the GM roses resulting from the introduction or expression of the introduced genes, including the rose *DFR* gene with an inverted repeat

241. The introduced enzymes (F3'5'H, 5AT and iris *DFR*) and their end-products function in the same way as the native enzymes and end-products (see Section 2.4 and 2.5 in Chapter 1 for more details).

242. Expression of the rose *DFR* gene with an inverted repeat results in the expression of a dsRNA product. The dsRNA product is degraded into small nucleotide dsRNA fragments (~21 nucleotides), which then degrade mRNAs that are complementary to the dsRNA fragments (Waterhouse and Helliwell 2002). The end result is the suppression of the endogenous *DFR* gene. Mismatches between the rose *DFR* dsRNA fragments and other genes may occur, leading to the suppression of other related genes. For example, *DFR* dsRNA fragments may act to suppress the endogenous rose *DFR* gene, as well as the introduced iris *DFR* gene and other related genes. This may lead to altered biochemistry or physiology of the GM rose lines. However, information supplied by the applicant indicates that the rose flowers from the LA/919-4-10 GM rose line have mauve coloured flowers, suggesting that the rose *DFR* dsRNA fragments do not suppress expression of the iris *DFR* gene.

243. As discussed in Section 2.4.1, the introduced enzymes and their end-products do not provide a selective advantage over non-GM roses. Delphinidins and other anthocyanins are secondary metabolites, and are therefore not involved in plant growth and development (Winkel-Shirley 2002).

244. The applicant has provided preliminary data on changes in phenotype between the parent rose lines and the GM rose plants (see Section 3.2 in Chapter 1 for more details). Preliminary results from these trials indicate that there are no apparent morphological differences between the WKS82/130-4-1 and the WKS82/130-9-1 GM lines and their parent rose line (hybrid tea rose). Some morphological differences were detected between the LA/919-4-10 GM line and its parent rose line (floribunda rose) (Table 1.7 in Chapter 1). Plant height and petal number are lower in the GM line compared to the parent line, while petal height, petal width and flower width are greater. The only significant difference was detected for the lower petal number in the GM line. The applicant states that these results are indicative of suppressed vigour in the LA/919-4-10 GM line compared to its parent line.

245. The applicant has also provided preliminary data on adventitious root formation in the LA/919-4-10 GM line compared to its parent rose line. The GM rose line showed a lower

percentage of rooting and fewer roots per rooted cutting when compared to the parent rose line (see Section 3.2 in Chapter 1 for more details).

246. None of the morphological differences between the parent rose line and the LA/919-4-10 GM line are likely to pose any harm to people or other organisms, or provide a selective advantage for the GM rose line from the proposed release. Therefore, **no risk is identified** and the potential for toxicity or weediness as a result of unintended changes in biochemistry or physiology will not be assessed further.

2.8 Unintended presence of *Agrobacterium* during release

247. *Agrobacterium* has been shown to be persistent in *in vitro* plant tissues and shoots or plants arising from *Agrobacterium*-mediated genetic modification experiments. Broad spectrum antibacterial compounds tend to have a bacteriostatic effect, suppressing, but not eliminating bacterial growth and when removed the bacteria may resume growth. In particular, Gram-negative bacteria (such as *Agrobacterium*) are difficult to eradicate completely from *in vitro* cultures (Leifert & Cassells 2001).

248. The transfer of GM rose plants/cuttings potentially carrying *Agrobacterium* vectors into the environment may result in the transfer of genes to non-target plants or other microorganisms (Leifert 2000).

2.8.1 Carry over of *Agrobacterium* with gene constructs from genetic modification experiments on GM rose plants (ie cuttings)

249. The three GM rose lines were each generated by *Agrobacterium*-mediated genetic modification (refer to Section 2.3 in Chapter 1 for more details). The applicant proposes to harvest flowers from the GM roses, as well as maintain the plants through pruning and the removal of dead or diseased tissue. Waste GM plant material will be destroyed by shredding and composting. The unintended transfer of *Agrobacterium* vectors from these GM rose plant materials to the environment may lead to potential impacts on biodiversity or increased pathogenicity (if the *Agrobacterium* conjugates with a virulent wildtype strain).

250. *Agrobacterium tumefaciens* is a soil-borne, Gram-negative bacterium that causes crown gall on plants. *Agrobacterium* contains a large tumour inducing (Ti) plasmid. The Ti plasmid contains a section of DNA called T-DNA, which harbours genes encoding for the production of amino acid derivatives such as octopine or nopaline, and plant hormones such as auxin and cytokinin. A separate region of the Ti-plasmid contains the genetic machinery for the transfer of the T-DNA region. *Agrobacterium* infects plant tissue (generally at a wound site) and transfers the T-DNA region from the Ti plasmid into the plant genome. The infected plant tissue produces octopine or nopaline, which is required for proliferation of the *Agrobacterium*, as well as the plant hormones, which causes proliferation of undifferentiated plant cells resulting in the characteristic ‘gall’ formation seen on infected plants.

251. *Agrobacterium* used for genetic modification of plants is disarmed, meaning the T-DNA region of the Ti-plasmid has been deleted. In general, only the left and right T-DNA borders remain. However, in some strains of *Agrobacteria*, only one border of the ‘disarmed’ Ti-plasmid may remain. Disarmed *Agrobacteria* are capable of infecting plants, but as there are no plant hormone genes to transfer, there is no gall formation. The T-DNA region can be replaced by inserting other genes of interest between the T-DNA borders. Alternatively, the T-DNA borders, flanking genes of interest, can be incorporated into a separate plasmid (called a binary plasmid) and this plasmid can co-exist with the disarmed Ti plasmid in *Agrobacterium*. Under appropriate laboratory conditions, these genes can be transferred from the disarmed Ti-plasmid or the binary plasmid in *Agrobacterium* into plants or plant tissues, from which GM plants can be generated by tissue culture.

252. Overall there is evidence to suggest persistence of *Agrobacterium* in *in vitro* plant tissue cultures but not in the regenerated GM plants. (Barrett et al. 1997) used *Agrobacterium* to generically modify *Brassica oleraceae*. Their study showed that after 24 weeks of culture on antibiotic media, 24% of the regenerated *Brassica oleraceae* shoots were still contaminated with *Agrobacterium*, suggesting persistence of *Agrobacterium* in the *in vitro* plant tissues. However, the *B. oleraceae* shoots were not regenerated on selective media and no evidence was provided to suggest the shoots were indeed genetically modified. Thus, it is not possible to conclude that *Agrobacterium*-mediated GM plants were contaminated with *Agrobacterium*, nor is there any evidence to suggest persistence of *Agrobacterium* in the *B. oleraceae* shoots once they were removed from *in vitro* culture.

253. Hammerschlag et al. (2000) also supported observations on the persistence and difficulty in eliminating *Agrobacterium* from *in vitro* apple (*Malus x domestica*) cultures. They examined a number of different treatment regimes and were able to regenerate 26 *Agrobacterium*-mediated GM apple plants that were *Agrobacterium*-free.

254. Cooke et al. (1992) indicate that persistence of *Agrobacterium* in *in vitro* and *in vivo* plant tissues was variable depending upon plant species. After inoculation with *Agrobacterium*, *Aster* cultures remained contaminated during 16 weeks of *in vitro* culture and *in vivo* after transfer to soil. The *Agrobacterium* contamination was not always visible, but a sterility test revealed the persistence of the *Agrobacterium* in the *Aster* plants. In contrast, after *Agrobacterium* inoculation, *in vitro* and *in vivo* cultures and plants of *Iris* and *Rosa* remained free of *Agrobacterium* contamination for up to 18 months after transfer to soil. Cooke et al. (1992) noted that the pH of the *Rosa*-culture media was 3.9 and that *Agrobacterium* would not grow at such a low pH.

255. The GM *Agrobacterium* (containing the gene construct) would be able to conjugate with other *Agrobacterium* and many other bacteria. Generally the possibility of gene flow from GM plants to bacteria is rare (refer to Section 2.6.1), but the spread from bacteria to bacteria would be very likely. It is also possible that the GM *Agrobacterium* could genetically modify cells of other plants. Although the conditions for *Agrobacterium* infection and gene transfer would exist in nature, the creation of a GM plant would be unlikely because: (1) it would be unlikely that the *Agrobacterium* would genetically modify a cell or cells that would give rise to a new organism, (2) it is unlikely that conditions in nature would exist that would select for the survival of the infected GM plant cells, and (3) not all the GM plant cells would have expression of the introduced genes (eg due to position effect, genetic re-arrangements, silencing due to multiple copies). Should a new GM plant arise it is unlikely that it would have a selective advantage and would not likely persist under natural conditions.

256. The GM roses proposed for release have gone through a number of generations, so it is unlikely that the *Agrobacterium*-based transformation vectors persist on the GM roses. The genetic modification of the three GM rose lines was carried out in Japan, in September 2000. GM plantlets were transferred to a glasshouse in May 2002. The GM rose lines were then imported from Japan as tissue cultures, under AQIS permit in October 2003. One of the conditions of their entry into Australia was that the tissue culture growth media must not contain antibiotics or other microbial suppressants. The GM tissue cultures must also be free of bacterial contamination. Furthermore, leaves from similar GM and non-GM parent rose lines held in Japan have been tested for the presence of the *Agrobacterium* strain carrying the transformation vector. No bacterium was detected (information supplied by the applicant).

257. In Australia, the GM rose plants were transferred from tissue culture as rooted plantlets. The LA/919 GM rose plants were transferred to the Florigene PC2 greenhouse in June 2004, then WKS/82-9-1 GM rose plants were transferred in January 2005 and the WKS/82-4-1 GM

plants were transferred in March 2005. A small number of cuttings have been made from the imported GM plants.

258. The potential for transfer of GM rose plants/cuttings carrying *Agrobacterium* vectors into the environment is limited because the proposed trial will be carried out in an enclosed, insect-proof greenhouse. In addition, the applicant proposes to grow the GM roses hydroponically in pots above the soil, and keep them free of insect pests and disease by spraying. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of unintended presence of *Agrobacterium* will not be assessed further.

2.9 Unauthorised activities

2.9.1 Use of GMOs outside the proposed licence conditions (ie non-compliance)

259. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM rose lines outside of the proposed release area. The adverse outcomes that this event could cause are discussed in Section 2.4. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of unauthorised activities will not be assessed further.

Section 3 Conclusions of risk assessment

260. The hazard identification process considered the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials used in food (eg honey, rose water, rose hips), GM plant by-products (eg soil used in the greenhouse, compost, cut flowers), the introduced genes, or products of the introduced genes.

261. Sixteen events were identified and assessed whereby the proposed release of three GM rose lines might give rise to harm to people or the environment.

262. These sixteen events included consideration of whether, or not, expression of the introduced genes could result in products that are toxic to people or other organisms, allergenic, produce unintended changes in biochemistry or physiology, or alter characteristics that may impact on spread and persistence of the GMOs. In addition, consideration was given to the opportunity for gene flow to other organisms, and unauthorised activities.

263. None of the sixteen events are considered to give rise to an identified risk that requires further assessment. The principle reasons include:

- small scale of the trial that is limited in both area and duration;
- containment and disposal measures proposed by the applicant to limit the spread of GM plant materials;
- none of the GM plant materials will be used in food;
- widespread presence of the same or similar introduced genes in the environment and lack of evidence of harm from these genes;
- the lack of toxicity or allergenicity of enzymes and end-products from the introduced genes;
- limited capacity of the GM rose to spread and persist except by intentional horticultural techniques;

- limited opportunity and ability of the GM rose lines to transfer the introduced genes to other organisms;
- limited morphological differences between the GM rose lines compared with the parent rose lines.

264. Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM rose lines into the environment is considered to be **negligible**.

Chapter 3 Risk management

265. Risk management includes evaluation of risks identified in Chapter 2 to determine whether or not specific treatments are required to mitigate harm to human health and safety, or the environment, from the proposed release. Other risk management considerations required under the Act are also addressed in this Chapter, including conditions that limit and control the proposed release. Together, these risk management measures are used to inform the decision-making process and determine licence conditions that may be imposed by the Regulator under section 62 of the Act.

Section 1 Background

266. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

267. Under section 62 of the Act, 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. Licence conditions can be imposed to limit and control the scope of the dealings and the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Other Australian regulators

268. Australia's gene technology regulatory system operates as part of an integrated legislative framework (OGTR 2005a). Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, TGA, NICNAS, NHMRC and AQIS. Dealings conducted under any licence issued by the Regulator may also be subject to regulation by one or more of these agencies.

269. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. The *Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purposes of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

270. AQIS is responsible for monitoring imports to prevent the introduction of exotic pests and diseases into the environment. An importer is required to notify AQIS if they are importing GMOs, or products known to be mixed with any amount of GM material. As the importation would also constitute a dealing under the Act, the importer requires an authorisation under this Act for the import to lawfully proceed. Florigene imported tissue cultures of the three GM rose lines from Japan under AQIS permits 200319814, 200403322, 200515938 in relation to NLRD 1011/2003.

271. FSANZ is responsible for human food safety assessment. FSANZ may approve products derived from GM food crops approved for release overseas but not in Australia for use in food. Approval has not been sought for the use of products derived from the GM rose lines. Florigene does not intend to use any products derived from this proposed release in human food.

Section 3 Risk treatment measures for identified risks

272. The risk assessment of events listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed release. These events were considered in the context of the proposed release on two sites over two years (March 2006 - April 2008)

and the receiving environment. The first site is an enclosed, insect-proof greenhouse where the GM rose lines will be grown hydroponically. The total size of the greenhouse is 100m². The second site of approximately 25m² is nearby and will be used for shredding and composting of GM and other plant materials cultivated during the trial. The proposed release sites are in the Shire of Yarra Ranges, Victoria.

273. The *Risk Analysis Framework* (OGTR 2005a), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. However, containment measures have been imposed to limit the release to the location, size and duration proposed by the applicant.

Section 4 General risk management

4.1 Licence conditions associated with managing limited and controlled releases

4.1.1 Measures to limit and control the proposed release

274. A number of licence conditions have been imposed to limit and control the release, including requirements to:

- grow the GM roses in a greenhouse (the first site);
- grow the GM plants hydroponically in pots above the soil;
- measures to minimise insects in the greenhouse;
- control weeds on the greenhouse floor and vegetation immediately adjacent to the outside of the greenhouse;
- harvest flowers inside the greenhouse at before anthers are visible to limit the possibility of pollen flow and seed set;
- destroy GM and other plant waste material (including soil medium) by incineration, or by shredding and composting at the second site; and
- measures to contain compost at the second site;
- cleaning of equipment used in the greenhouse and in shredding and composting GM plant waste material; and
- monitor compost for volunteers once every 60 days, until no volunteers have been found for a period of at least 6 months.

4.1.2 Measures to control other activities associated with the release

275. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, June 2001; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have been imposed regarding transportation and storage, and to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

4.2 Other risk management considerations

276. All DIR licences issued by the Regulator contain a number of general conditions that also relate to risk management. These include, for example:

- identification of the persons or classes of persons covered by the licence
- applicant suitability
- contingency and compliance plans

- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment
- a requirement that the applicant allows access to the release sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

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

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

278. Before making the decision to issue a licence for this application (DIR 060/2005), the Regulator determined that Florigene Pty Ltd (Florigene) is suitable to hold a licence.

279. Conditions in the licence include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.

280. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Compliance and contingency plans

281. The licence requires Florigene to submit a plan detailing how it intends to ensure compliance with the licence conditions and document that compliance. This plan is required before the planting of any of the GM rose lines occurs.

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

283. Florigene is also required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic material in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

4.2.3 Reporting structures

284. The licence obliges the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the release
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the release.

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

286. A number of written notices are also required under the licence that will assist the OGTR in designing and implementing its risk based monitoring program for all licensed dealings. The notices would include:

- expected and actual dates of planting
- expected and actual dates of commencement of flowering
- expected and actual dates final cleaning at the end of the trial
- dates and location of any incineration or composting of GM plant waste material.

Section 5 *Monitoring and Compliance*

287. A range of monitoring and compliance activities are undertaken on behalf of the Regulator (OGTR 2005a) to check compliance with licence conditions.

288. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

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

Section 6 *Issues to be addressed for future releases*

290. The risk assessment identified additional information that may be required if the applicant were to submit an application for a larger scale release of these GM rose lines or apply for changes in containment conditions, including:

- concentration levels of delphinidin in the three GM rose lines; and
- altered agronomic characteristics indicative of weediness as a result of genetic modifications.

And, if the release was proposed to occur outside enclosed greenhouse facilities:

- changes in pollinator behaviour as a result of altered flower colour in the GM rose lines, that may increase gene flow under outdoor field conditions; and
- level of gene flow between the GM rose lines and compatible rose cultivars and related species under Australian field conditions.

Section 7 *Conclusions of the RARMP*

291. The risk assessment concludes that this limited and controlled release of three GM rose lines into the Shire of Yarra Ranges, Victoria poses **negligible** risks to the health and safety of people and the environment.

292. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to contain the release to the proposed location, size and duration requested by the applicant.

Chapter 4 Licence Conditions

Section 1 Interpretations and Definitions

This licence does not authorise dealings with GMOs that are otherwise prohibited as a result of the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

In this licence:

- (a) unless defined otherwise in this licence, words and phrases used in this licence have the same meaning as they do in the *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001*;
- (b) words importing a gender include any other gender;
- (c) words in the singular include the plural and words in the plural include the singular;
- (d) words importing persons include a partnership and a body whether corporate or otherwise;
- (e) 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 and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;
- (f) where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning;
- (g) specific conditions prevail over standard conditions to the extent of any inconsistency.

In this licence:

‘Act’ means the *Gene Technology Act 2000* (Cth) and equivalent provisions in corresponding State law.

‘Annual Report’ means a written report provided to the Regulator within 90 days of each anniversary of the date of issue of this licence containing all the information required by this licence to be provided in the Annual Report.

‘Clean’ (or **‘Cleaned’**), as the case requires, means the removal and destruction of the GMOs and any Plant Material and in relation to Equipment, the removal of the GMOs and any Plant Material from the Equipment and their destruction.

‘Compost Heap’ includes the contents of the compost heap, and means a compost heap within the premises of the Australian Roses facility in Silvan, Victoria.

‘Deal With’ in relation to a GMO means the following,

- (a) conduct experiments with the GMO;
- (b) make, develop, produce or manufacture the GMO;
- (c) breed the GMO;
- (d) propagate the GMO;
- (e) use the GMO in the course of manufacture of a thing that is not a GMO;
- (f) grow, raise or culture the GMO;

and includes the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing mentioned in any of paragraphs (a) to (f).

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

‘GM’ means genetically modified.

‘GMOs’ means the genetically modified organisms listed in Attachment B and authorised for release by this licence.

‘Greenhouse’ means a Greenhouse within the premises of the Australian Roses facility in Silvan, Victoria.

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

‘Plant Material’ means viable parts of the GMOs or non-GM rose grown at the Greenhouse or at the Compost Heap including seed, pollen, flowers, leaves, cuttings, rootstock and root ball, whether from the plant itself or derived from or produced by the plant.

‘Regulator’ means the Gene Technology Regulator.

‘Rose’ means plants of the species *Rosa X hybrida*, *Rosa multiflora* and *Rosa canina*.

‘Sign-off’ means a notice in writing from the Regulator stating that the inspection conditions of this licence no longer apply.

‘Volunteer Plants’ means progeny of the GMOs or non-GM roses at the Greenhouse and Compost Heap or regrowth of previous GM or non-GM rose plants at the Greenhouse and Compost Heap.

Section 2 General Conditions

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 Florigene Pty Ltd.

Project Supervisor

3. The Project Supervisor in respect of this licence is identified at Attachment A.

4. The licence holder must immediately notify the Regulator in writing if any of the contact details of the Project Supervisor change.

No dealings with GMOs except as authorised by this licence

5. Persons covered by this licence must not deal with the GMOs except as expressly permitted by this licence.

Persons covered by this GMO licence

6. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with GMOs grown pursuant to this licence.

Informing people of their obligations

7. The licence holder must inform any person covered by this licence, to whom a particular condition of this licence applies, of the following:

- (a) the particular condition (including any variations of it);
- (b) the cancellation or suspension of the licence;
- (c) the surrender of the licence.

8. The licence holder must provide the Regulator, on the Regulator's written request, signed statements from persons covered by this licence that the licence holder has informed those people of the conditions of this licence that apply to them and that they have understood the conditions and agree to be bound by them.

Applicant to notify of circumstances that might affect suitability

9. 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 Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment;
- (c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of this licence to meet the conditions in it.

Licence holder must provide information on matters related to suitability

10. The licence holder must provide information related to the licence holder's ongoing suitability to hold a licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Additional information to be given to the Regulator

11. It is a condition of a licence that the licence holder inform the Regulator if the licence holder:

- (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.

12. The licence holder must provide the information required by paragraphs (a) (b) and (c) of the immediately preceding condition to the Regulator as soon as practically and reasonably possible and must also include the information in the Annual Report.

People dealing with GMOs must allow auditing and monitoring of the dealing

13. If a person is authorised by this licence to deal with GMOs 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

14. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and comply with its instrument of accreditation.

Notices

15. The licence holder must provide all notices to the Regulator required to be given by this licence and each notice must be provided in the manner required by Section 10 of this licence.

Section 3 Growing the GMOs**GMOs covered by this licence**

16. The GMOs covered by this licence are described at Attachment B.

Permitted dealings

17. Subject to any applicable conditions, this licence authorises all Dealings with the GMOs.

Locations, Growing Seasons and size of trial

18. The permitted Dealings with the GMOs may be undertaken for two years from the date of issue of the Licence.

19. All Dealings except experiments, transport, shredding and composting of the GMOs and Plant Material must be undertaken in a Greenhouse.

20. The maximum size of the Greenhouse is 100m² and no more than one Greenhouse must be used for growing the GMOs at any one time.

21. If the GMOs and/or Plant Material are shredded, shredding must take place either in the Greenhouse or within 5 metres of the Compost Heap.

22. The maximum total size of the Compost Heap is 25m².

23. The licence holder must be able to access and control the Greenhouse and Compost Heap to the extent necessary to comply with this licence, for the duration of the life of the licence.

Note: Details relating to controlling the Greenhouse and Compost Heap must be provided in the Compliance Management Plan (see Section 9 of this licence)

Measures to manage Gene Flow – Greenhouse

24. Non-GM roses may be planted and grown in the Greenhouse, but if planted, must be handled and controlled as if they were GMOs for purposes of this licence. All conditions which refer to and apply to a GMO are taken to refer to and apply equally to non-GM roses.
25. Each of the following measures to manage gene flow must be implemented:
- (a) the Greenhouse must be made of framed heavy duty plastic, with a soil floor;
 - (b) the GMOs must be grown in pots above the floor;
 - (c) the Greenhouse, including ventilation into the Greenhouse, must be insect-proofed;
 - (d) any insects observed in the Greenhouse must be killed immediately;
 - (e) the Greenhouse floor must be kept clean of Plant Material and weeds;
 - (f) all flowers must be harvested before anthers are visible;
 - (g) all grafting, propagation and maintenance of GMOs (i.e. pruning, spraying for pests, harvesting flowers) must occur within the Greenhouse;
 - (h) the Greenhouse must be secured against unauthorised entry when not occupied; and
 - (i) any weeds within 1 metre of the perimeter of the Greenhouse must be removed so as to allow an assessment of the insect-proofing of the Greenhouse.

Measures to manage Gene Flow – Compost Heap

26. Plant Material from the GMOs, not used for analysis or storage, must be destroyed by shredding and composting.
27. Shredded Plant Material must be deposited on the Compost Heap.
28. There must be at least ten metres between the Compost Heap and any non-GM plants including any non-GM plants in other greenhouses.
29. The Compost heap must be contained in a manner which does not allow the escape of its contents.
30. Containment measures must be implemented to ensure the Compost Heap does not at any time exceed a total of 25 square metres.
31. The Compost Heap must remain in the same location throughout the trial.
32. Compost, including soil and Plant Material from the Compost Heap must not be removed until after Sign-Off.

Section 4 Disposal of the GMO

Storage, Relocation or Destruction

33. Plant Material from the GMOs must be:
- (a) stored in a container that is labelled to indicate that it contains Plant Material, within a locked facility that is signed so as to indicate that Plant Material is stored within the facility; or
 - (b) relocated to a facility approved by the Regulator to at least physical containment level 2; or
 - (c) destroyed by shredding and composting.

Cleaning

34. Within 14 days of the storage, relocation or shredding and composting of the last GMO, from a Greenhouse, the Greenhouse must be Cleaned.

35. Equipment and any areas used to Clean Equipment used in connection with the GMOs at the Greenhouse or Compost Heap must be Cleaned immediately or as soon as practicable after use and before it is used for any other purpose.

Section 5 Use of Plant Material

Material from the GMOs may be collected

36. Plant Material may be collected from the Greenhouse for the purpose of conducting experiments on it.

37. Plant Material must not be used, sold or otherwise disposed of for any purpose which would involve or result in their use as food for animals or humans.

Transportation of the GMOs and Plant Material

38. Plant Material may only be transported to the extent necessary to store it, shred and compost it, Clean it from Equipment, relocate it to a facility approved by the Regulator or to physical containment level 2, or relocate it to the Greenhouse from a facility approved by the Regulator or from physical containment level 2.

39. If Plant Material is transported, it must be transported in the manner required by this licence. To the extent that any of the requirements of this licence are inconsistent with the OGTR Guidelines for the Transport of GMOs (2001) the requirements of this licence prevail.

40. Every container used to transport Plant Material must be labelled:

- (a) to indicate that it contains genetically modified rose; and
- (b) with telephone contact numbers for the licence holder and instructions to contact the licence holder in the event that the container is broken or misdirected.

41. The licence holder must have in place accounting procedures to verify whether the same quantity of GMOs or Plant Material sent is delivered and must document routes, methods and procedures used for transportation of GMOs and Plant Material.

Section 6 Use of Site

Inspection

42. The Compost Heap must be inspected for the existence of Volunteer Plants.

43. Inspection must be performed by a person who is able to recognise Volunteer Plants.

44. Volunteer plants found must be shredded and composted prior to the plant flowering.

45. Inspections of the Compost Heap must begin immediately after the first Plant Material is composted and are to occur at least once every 60 days, until the Regulator has issued a Sign-off.

Section 7 Sign Off

46. If the Greenhouse has been Cleaned the licence holder may make written application to the Regulator for a Sign Off in respect of the Greenhouse.

47. If inspections of the Compost Heap have been routinely completed for a period of at least 6 months beginning on the day that the last of the Plant Material was added to the Compost Heap, and if inspection records for the Compost Heap show that no Volunteer Plants have

been observed in the most recent 6 month inspection period, the licence holder may make written application to the Regulator for Sign off in respect of the Compost Heap.

Section 8 Contingency Plans

48. Within 30 days of the date of issue of this licence, a written Contingency Plan must be submitted to the Regulator detailing measures to be taken in the event of the unintended presence of the GMOs or Plant Material outside an area that must be inspected.

49. The Contingency Plan must include details of procedures to:

- (a) ensure the Regulator is notified immediately if the licence holder becomes aware of the event;
- (b) destroy any of the GMOs or Plant Material; and
- (c) inspect for and destroy any Volunteer Plants that may exist as a result of the event.

50. The Contingency Plan must be implemented in the event that the unintended presence of the GMOs and Plant Material is discovered outside an area that must be inspected.

Section 9 Compliance Management Plan

51. Prior to growing the GMOs, a written Compliance Management Plan must be provided to the Regulator. The Compliance Management Plan must describe in detail how the licence holder intends to ensure compliance with each of these conditions and document that compliance and must include:

- (a) a list of the names of all organisations or natural persons who will be persons covered by this licence. Where a name of a person is not known at the time of submitting the Compliance Management Plan the function or position of the person to be covered must be provided.

Note: Examples of functions or positions are 'Site manager', 'Farm labourer' etc.

- (b) an explanation of how the licence holder has informed, or proposes to inform, each person covered by the licence of the conditions of this licence.
- (c) a description of the responsibilities of the licence holder and of each person covered by the licence in relation to the requirements of this licence.
- (d) a description of how any contracts, agreements, or other enforceable arrangements between the licence holder and persons covered by the licence will allow the licence holder to access and control the Greenhouse and Compost Heap to the extent necessary to comply with this licence, for the duration of the life of the licence or if contracts, agreements, or other enforceable arrangements are not in place how the licence holder proposes to access and control the Greenhouse and Compost Heap to the extent necessary to comply with this licence, for the duration of the life of the licence.
- (e) a description of how the licence holder will comply with the conditions of this licence.

52. Where any of the details of the compliance management plan change, the Regulator must be notified of the changes within 14 days of the change occurring.

Section 10 Reporting and Documentation Requirements

53. At least seven days prior to the date on which planting of the GMOs is intended to commence, the licence holder must provide a notice in writing to the Regulator which contains:

- (a) the date on which the GMOs are moved to the Greenhouse; and
- (b) the period during which the licence holder considers the GMOs are likely to flower; and
- (c) the GPS coordinates and street address for the Greenhouse.

54. Within seven days of planting of the GMOs, notice of the actual date of planting must be provided to the Regulator.

55. The licence holder must provide the Regulator with a notice of the date on which the first shredding and composting of Plant Material commences. The notice must be given at least 7 days, and not more than 21 days, in advance of the intended date of the first shredding and composting. Any change of intention prior to the intended date must be notified to the Regulator as soon as is reasonably and practically possible.

56. Within seven days of the date on which the Plant Material is first shredded and composted the licence holder must provide a notice in writing to the Regulator which contains:

- (a) the date on which the first GM plant material is shredded and moved to the compost; and
- (b) the GPS coordinates, street address and area for the Compost Heap.

57. The licence holder must provide a notice in writing to the Regulator when the Greenhouse is Cleaned. The notice must be provided to the Regulator within 14 days of the date on which Cleaning concluded.

58. On the request of the Regulator, the Regulator must be provided with written documentation of the procedures in place to ensure continuing compliance with the Cleaning conditions in this licence.

59. The results of inspection activities must be recorded in a logbook or paper file. The findings of the inspections as recorded in the logbook or paper file must be forwarded to the Regulator within 14 days of inspection taking place and must also be included in the licence holder's Annual Report to the Regulator. The logbook or paper file must contain at least the following:

- (a) details of the areas inspected;
- (b) details of the date of inspection;
- (c) the names of the person or persons who undertook the monitoring and details of the experience, training or qualification that enabled them to recognise Volunteer Plants;
- (d) the means of inspection used;
- (e) the number of Volunteer Plants observed, if any;
- (f) details of the development stages reached by the Volunteer Plants, if any; and
- (g) details of methods used to destroy Volunteer Plants, if any.

60. The licence holder must keep records of the number and type of rose plants grown at the Greenhouse as part of the trial, and the number of rose plants from the Greenhouse stored, at the end of the trial.

61. The licence holder must have in place accounting procedures to ensure that the same quantity of GMOs or Plant Material sent is delivered and must document routes, methods and procedures used for transportation of GMOs and Plant Material.

Annual Report

62. The licence holder must provide an Annual Report to the Regulator.

Testing methodology

63. The licence holder must provide a written instrument to the Regulator describing an experimental method that is capable of reliably detecting the presence of the GMOs and the presence of the genetic modifications described in this licence (at Attachment B) in a recipient organism. The instrument must be provided within 30 days of the issuing of this licence.

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Appendix A Definitions of risk analysis terms

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

Consequence

outcome or impact of an adverse **event**

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

Event*

occurrence of a particular set of circumstances

Hazard*

source of potential harm

Hazard identification

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

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

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

Risk

the chance of something happening that will have an undesired impact

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

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

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

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

Risk analysis

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

Risk analysis framework

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

Risk assessment

the overall process of **hazard identification** and **risk estimation**

Risk communication

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

Risk Context

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

Risk estimate

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

Risk evaluation

the process of determining risks that require treatment

Risk management

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

Risk management plan

integrates **risk evaluation** and **risk treatment** with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

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

States

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

Uncertainty

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

Appendix B Summary of submissions received from prescribed experts, agencies and authorities³ on the application

All issues raised relating to risks to human health and safety and the environment were considered in the context of currently available scientific evidence that was used in the preparation of the consultation RARMP.

Issues relating to the Risk Assessment and where they have been considered:

- Risks relating from occupational exposure (see Chapter 2, Sections 2.1 & 2.2)
- Persistence of GM plant material (see Chapter 2, Section 2.4)
- Risk of toxicity to all organisms (including people) (see Chapter 2, Sections 2.1 & 2.3)
- Risk of adverse pleiotropic effects (see Chapter 2, Section 2.7.1)
- Risks resulting from persistence and/or accumulation of the expressed proteins and anthocyanins in the environment (see Chapter 2, Sections 2.1-2.4)
- Risk resulting from any gene flow (both vertical and horizontal) (see Chapter 2, Sections 2.5 & 2.6)
- Risk resulting from dissemination of the GM pollen and/or seed beyond the intended areas (see Chapter 2, Section 2.4)
- Risk of weediness (see Chapter 2, Section 2.4)
- Independent research to show that GMOs are harmless to health and environment (see Chapter 2, Section 1).

Issues relating to the Risk Management Plan:

- Adequate containment measure (see Chapter 3 & 4)
- Labelling and cleaning of equipment (see Chapters 3 & 4)
- Monitoring the location for volunteers (see Chapters 3 & 4)
- Disposal of GM plant material (see Chapters 3 & 4)
- Transport of GM plant material (see Chapter 3 & 4).

Issues that are outside the scope of assessments under the *Gene Technology Act 2000*:

- Market and trade concerns (Outside the scope of the assessment)
- Strong national laws regarding gene technology be implemented (refer *Gene Technology Act 2000*)
- Mandatory labelling of GMO products (FSANZ assesses this issue— refer Chapter 3).

³ Gene Technology Technical Advisory Committee, State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and local councils where the release may occur.

Appendix C Summary of public submissions received on the application

All issues raised relating to risks to human health and safety and the environment were considered in the context of currently available scientific evidence that was used in the preparation of the consultation RARMP.

One submission was received that raised the following issues relevant to the preparation of the RARMP:

- Buffer zones between areas planted with GM and non-GM plants (see Chapter 4)
- Concerns about the unknown effects of GMOs on the environment and food chains (see Chapter 2, Section 2.7.1).

The submission also raised a number of issues that could not be considered:

- Concerns about the potential for breeding poison-resistant weeds and pesticide-resistant bugs (GMOs proposed for release are not herbicide tolerant or insecticidal)
- Marketing concerns following contamination (Outside the scope of the assessment)
- Concerns about the ethical consequences of biotechnology (refer section 112 of the *Gene Technology Act 2000* or Gene Technology Ethics Committee).

Appendix D Summary of submissions received from prescribed experts, agencies and authorities on the consultation RARMP

None of the experts, agencies and authorities prescribed for consultation under the *Gene Technology Act 2000* (the Act), other than the local council where the release may take place, raised any issues on the RARMP relating to human health and safety and the environment that required further consideration.

The local council provided a submission that, while acknowledging marketing and labelling issues are outside the scope of assessments conducted under the Act, raised concerns regarding Licence conditions that have been addressed in Chapters 3 and 4:

- Licence condition that no other plants can be grown in greenhouse for the duration of the trial
- Licence conditions must include stringent reporting and monitoring processes

The Council also sought assurances that the OGTR would monitor the conduct of the trial and investigate issues of public concern in relation to activities at the site. The OGTR provides information on routine monitoring and the outcome of investigations in the Regulator's Quarterly Report (available on the website <<http://www.ogtr.gov.au>>, under 'Publications and Forms').

Appendix E Summary of public submissions received on the consultation RARMP

The Regulator received one submission from the public on the consultation RARMP.

All issues relating to risks to human health and safety and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Issues raised relating to Risk Assessment:

- Risks relating from exposure (see Chapter 2, Sections 2.1 & 2.2)
- Risks of toxicity and allergenicity to humans (see Chapter, Sections 2.1 & 2.2).