Risk Assessment
and
Risk Management Plan

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

DIR 030/2002

Title: **Commercial release of colour modified carnations (replacement of deemed licence GR-2)**

Applicant: Florigene Limited

June 2003

Office of the
Gene Technology Regulator

Abbreviations

AFFA	Agriculture, Fisheries, and Forestry Australia
ALS	Acetolactate Synthase
ANZFA	Australia New Zealand Food Authority (now FSANZ)
AQIS	Australian Quarantine Inspection Service
CaMV	Cauliflower mosaic virus
CHS	Chalcone synthase
DFR	Dihydroflavonol 4-reductase
DIR	dealing involving intentional release
DNA	Deoxyribonucleic acid
F3'5'H	Flavonoid 3', 5' hydroxylase
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
g	Gram
GM	genetically modified
GMAC	Genetic Manipulation Advisory Committee
GMO	genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IOGTR	Interim Office of the Gene Technology Regulator
MAC	Cauliflower mosaic virus/Mas chimeric promoter
mg/g	milligrams per gram
mRNA	messenger ribonucleic acid
ng/g	nanograms per gram
NRA	National Registration Authority for Agricultural and Veterinary Chemicals
OGTR	Office of the Gene Technology Regulator
ppm	parts per million
SuRB	Sulfonylurea resistance gene B
TGA	Therapeutic Goods Administrations
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
WHO	World Health Organisation
w/v	weight per volume
µg/g	Micrograms per gram

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EXECUTIVE SUMMARY

INTRODUCTION

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

Section 51 of the Act requires the Regulator to prepare a risk assessment and a risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and stakeholders, that addresses any risks to human health and safety and the environment posed by the dealings and considers how they can be managed.

THE APPLICATION

Florigene has applied for a licence for the continued commercial release of four lines of genetically modified carnation (*Dianthus caryophyllus*) that have been modified for flower colour. The current application (application number DIR030/2002) seeks to continue the dealings authorised by a general release approval (GR-2) issued on 25 September 1995 under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC). Section 190 of the Act includes arrangements for such dealings to be licenced for the duration of the transition period, which is stipulated as two years from the commencement of the Act on 21 June 2001. The Act requires that any dealings covered by 'deemed' licences that are proposed to continue beyond the two-year transition period, i.e. 21 June 2003, must be assessed and licensed under the provisions of the new regulatory system.

The present application is for a licence to deal with four GM lines (transformation events 123.2.38, 123.2.2, 11363, and 123.8.8) that have been produced after transformation with either of two binary vectors, pCGP1470 or pCGP1991. The release covers the propagation, growth, and distribution of both GM plants and cut flowers Australia-wide.

The GM carnation lines in this application contain two introduced genes in the anthocyanin biosynthetic pathway, DFR (dihydroflavonol 4-reductase) and F3'5'H (flavonoid 3', 5' hydroxylase), which are responsible for the production of purple, mauve, or blue flower colour. Each line also contains the selectable marker, SuRB (sulfonylurea resistance gene B), that confers tolerance to sulfonylurea herbicides and a range of other acetolactate synthase (ALS) inhibiting herbicides. It is intended that the GM carnation be used solely as ornamental plants.

Since 1992 there have been a number of field trials of colour modified carnation that were conducted under the former voluntary system, as well as the continued commercial release authorised in 1995. Florigene also conducted field trials of carnations modified for increased vase life between 1992 and 1995 and a commercial release was authorised in 1995 under the former voluntary system. The deemed licence for the latter release will lapse on 21 June 2003. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

THE EVALUATION PROCESS

A risk assessment and risk management plan was prepared in response to the application from Florigene in accordance with the Act and the Regulations, using a Risk Analysis Framework (available at www.ogtr.gov.au/pdf/public/raffinal.pdf). This framework was developed by the Regulator in consultation with the public, key State, Territory and Commonwealth government stakeholders, and the Gene Technology Technical Advisory Committee. Details of the process that the Regulator must follow and of the matters that the Regulator must consider in preparing a risk assessment and a risk management plan are set out in Appendix 7 of the RARMP. The complete RARMP can be obtained from the OGTR (freecall 1800 181 030) or from the OGTR web site at www.ogtr.gov.au.

Through the risk assessment process, a number of potential hazards that may be posed by the release of genetically modified carnation were evaluated on the basis of the likelihood of each hazard occurring and the likely impact of the hazard were it to be realised.

The potential hazards to human health and safety and the environment that were considered relate to:

- **Toxicity and allergenicity for humans:** GM carnation might be harmful to humans because it may be more toxic or allergenic than non-GM carnation as a result of the novel gene products or because of unforseen or unintended effects.
- **Toxicity and for other organisms:** GM carnation might be harmful to other organisms because it may be more toxic than non-GM carnation as a result of the novel gene products or because of unforseen or unintended effects of the modification.
- **Weediness:** GM carnation might be harmful to the environment because of an increased potential for weediness compared to conventional carnation.
- **Transfer of introduced genes to other organisms:** the new genes introduced into carnation might transfer to non-GM carnation, naturalised *Dianthus*, or to other plants or organisms, and this may have adverse consequences for the environment.

CONCLUSIONS OF THE RISK ASSESSMENT

In summary, the Regulator considers that the hazards posed by this commercial release of carnation modified to produce purple, blue or mauve flowers are unlikely to present any risks to the health and safety of people or the Australian environment that are different to conventional carnation. The assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans

GM carnation is unlikely to prove more toxic or allergenic to humans or other organisms than conventional carnation because:

- there have been no reports of adverse effects to human health and safety as a result of the current commercial release of carnation, which was approved in 1995;
- carnations are used for ornamental purposes only;
- concentrations of delphinidin in GM carnation are similar to a range of delphinidin producing plants including those commonly eaten by humans without adverse consequences, and toxicity studies of delphinidins and other anthocyanins using mammalian models indicate very low levels of toxicity;
- no differences were found in the biochemical profiles of GM and conventional carnation as revealed by chromatography studies;
- proteins related to the introduced proteins are common in edible plants;
- pollen is produced in very low quantities and is not aeroallergenic; and
- no homology of the novel proteins with sequences from known toxins or allergens was found.

Toxicity to other organisms

GM carnation is unlikely to prove more toxic to other organisms than conventional carnation because:

- concentrations of delphinidin in GM carnation are similar to a range of delphinidin producing plants, and toxicity studies of delphinidins and anthocyanins using mammalian models indicate very low levels of toxicity;
- no differences were found in the biochemical profiles of GM and conventional carnation as revealed by chromatography studies of phenolic acids and volatile gases;
- proteins related to the introduced proteins are common in edible plants;
- no reports of adverse toxicity have been found;
- no toxic effects of GM carnation were found on the germination and growth of a number of plants; and
- no differences were found in the quantities of bacteria and fungal spores in soil taken from around GM and conventional carnation.

Weediness

The risk of GM carnation establishing as a weed is negligible, and not likely to be greater than that of conventional carnation because:

- GM carnation does not share any life history characters with weedy species and the introduced proteins will not change these characters;
- the presence of the SuRB gene will only confer a selective advantage in those environments where weeds are controlled by ALS inhibiting herbicides. These herbicides are not used in the carnation industry and carnations exist exclusively as a managed cultigen;

- GM carnation has an extremely low potential for dispersal by natural means as it does not set seed;
- GM carnation does not spread by asexual reproduction without human intervention; and
- carnation has never been found as a weed in any of the countries that it is cultivated in, including Australia.

Transfer of introduced genes to other organisms

The likelihood of gene transfer from GM carnation to cultivated carnation is negligible because:

- GM carnation like many non GM carnation cultivars are effectively sterile;
- *Dianthus caryophyllus* is not sexually compatible with naturalized carnation species or with other species of the same family, and is geographically isolated from many of the populations of naturalized *Dianthus* species;
- there are no records of gene transfer from non-GM carnation to other plant species;
- natural events of horizontal gene flow from plants to distantly related organisms is extremely rare; and
- the probability of non-homologous recombination of intact plant DNA with the DNA of other organisms is extremely low.

Were this hazard to be realised, it would not pose any risks additional to those posed by the GM carnation itself.

THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)

Following a thorough and detailed assessment of the risks identified in the above section, it is considered unnecessary to impose any specific management conditions in relation to potential toxicity or allergenicity of GM carnation to humans or to other organisms, weediness, or gene transfer. In making a decision to issue a licence in respect of application number DIR 030/2002, the Regulator considers the licence need only contain minimal conditions to oversight the release on an ongoing basis.

General conditions

Any licence issued by the Regulator contains a number of general conditions, which may also be relevant to risk management. These include, for example, identification of the persons or classes of person covered by the licence and informing the Regulator if the applicant becomes aware of any additional information about risks to human health or safety or to the environment, or of any unintended effects.

Specific conditions

It is required that the licence holder provides the OGTR with a testing methodology that can reliably detect the presence of each of the four GM carnation lines and any transferred genetically modified material. The licence holder is required to provide an annual report on the commercial release. This includes information on any adverse impacts on human health and safety or the environment caused as a result of the GMO or viable material from the GMO. The licence holder must also maintain a written record of production, the site co-ordinates, and contact details of propagators and growers to whom Florigene gives or sells

the GMO, as well as the wholesale distributors of the GMO from whom Florigene receives royalties. These records must be included in the annual report and be made available to the Regulator on request.

Monitoring and enforcement of compliance by the OGTR

It should be noted that as well as imposing licence conditions, the Regulator has additional options for risk management. The Regulator has the legislative capacity to direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment.

CHAPTER 1 BACKGROUND

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

SECTION 1 THE APPLICATION

2. The application from Florigene is for a licence for the ongoing commercial release of genetically modified carnations (*Dianthus caryophyllus*) that have been modified for flower colour. Key information on the application is given below.

Project Title:	**Commercial release of colour modified carnations (replacement of deemed licence GR-2)**
Applicant:	Florigene
Common name of the parent organism:	Carnation
Scientific name of the parent organism:	*Dianthus caryophyllus*
Modified trait(s):	Modified flower colour Herbicide tolerance
Identity of the gene(s) responsible for the modified trait(s):	Modified flower colour • A gene coding for dihydroflavonol 4-reductase (DFR) • A gene coding for flavonoid 3', 5' hydroxylase (F3'5'H) Herbicide tolerance • A selectable marker gene (SuRB) whose protein confers resistance to acetolactate synthase (ALS) inhibiting herbicides
Location	• Research and stock plants: held at Florigene • Release of cuttings: a single propagator in Victoria • Release of rooted cuttings: between 3 and 6 growers in Australia (Victoria, South Australia, Queensland, Western Australia) • Release of cut flowers: Australia wide • Release of flowering plants: Australia wide
Release Size:	The release covers the propagation, growth and distribution of both GM plants and cut flowers Australia-wide
Time of Release:	June 2003

3. The current application is a replacement of the deemed licence to continue a general release (GR-2), which was authorised on 25 September 1995 under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC).

4. The Act includes transitional arrangements for dealings previously authorised under the voluntary system that was overseen by GMAC. Section 190 of the Act provides for those dealings for which an advice to proceed had been issued by GMAC prior to the commencement of the Act on 21 June 2001 to be 'deemed' to be licensed for the purposes of the Act. The transitional period stipulated by the Act is two years. The Act therefore requires that dealings covered by 'deemed' licences that are proposed to continue beyond the two year transition period, ie 21 June 2003, require assessment and licensing. The applicant sought to continue the dealings authorised by GMAC under GR-2 beyond 21 June 2003.

Section 1.1 The dealings

5. Florigene sought approval for the continued propagation, growth, and distribution of GM plants and cut flowers Australia-wide. Florigene holds research and stock plants (approx 700) and releases cuttings to propagators to multiply plants (approx. 250 000 per annum). Propagators release rooted cuttings to growers (approx. 25 000 annually), and the growers produce GM cut flowers (up to 2 million cut flowers annually) and plants for the Australian retail market (approx. 100 000 annually).

6. The present application was originally for a licence to deal with any transgenic carnation line produced after transformation with either of two binary vectors, pCGP1470 or pCGP1991. However, the licence has been granted for four transformation events (123.2.38, 123.2.2, 11363, and 123.8.8). Future events would require separate assessments of risks to human health and safety and the environment.

7. Florigene has indicated that, in the medium term, its intention is to seek approval for the products described under the dealings to be placed on the GMO Register. The purpose of the GMO Register is to enable certain dealings with GMOs to be undertaken without the requirement for a licence to be held by a named individual or organisation. No dealings with GMOs have yet been placed on the GMO Register. The Regulator would only approve the placement of dealings with a GMO on the Register if they have been previously licensed and the Regulator is satisfied that they are sufficiently safe that they can be undertaken by anyone without the need for the dealings to be licensed.

8. There have been no reports of adverse effects on human health or the environment resulting from previous releases of GM carnations. Note that GM carnations have been approved for commercial release in Australia since 1995.

Section 1.2 Parent organism

9. The parent organism is carnation, *Dianthus caryophyllus*. *D. caryophyllus* belongs to the Caryophyllaceae family, a temperate northern hemisphere family containing around 2100 species in 89 genera. The *Dianthus* genus contains approximately 300 species and is native to Europe, Asia, North Africa, and the Arctic region where one species is found. Carnation is exotic to Australia but has been grown commercially as a flower crop since 1954. At present the industry produces approximately 140 million cut flowers per annum across a total of 100 ha in Victoria, South Australia, Western Australia, and New South Wales. Victoria is the largest production centre.

Section 1.3 Genetic modification and its effect

10. Carnations have been genetically modified to produce violet, mauve, or purple coloured flowers. Colour in flowers is attributed to the presence of two pigment types - carotenoids and flavonoids. Carotenoids are responsible for yellow through orange colours however most plants do not contain carotenoid pigments. Many flavonoids are flower pigments such as the anthocyanins (water soluble plant pigments). There are three groups of anthocyanins, the delphinidins that generally produce blue flower colour, cyanidins that produce red or pink flower colour, and pelargonidins that produce orange or brick red flower colour. Non-genetically modified carnations lack the part of the anthocyanin biosynthetic pathway that is responsible for the production of delphinidins. This includes the enzyme flavonoid 3', 5' hydroxylase (F3'5'H) that converts either dihydrokaempferol (DHK) or dihydroquercetin (DHQ) to dihydromyricetin (DHM), and the dihydroflavonol 4-reductase (DFR) enzyme that converts DHM to leucodelphinidin which is subsequently modified to delphinidin-3-glycoside through the activity of endogenous enzymes.

11. The GM carnations in this application contain the genes coding for the enzymes F3'5'H and DFR, a selectable marker gene (SuRB) conferring resistance to ALS inhibiting herbicides (such as sulfonylureas), and regulatory sequences designed to enhance expression of the inserted genes.

12. Some of the regulatory sequences are derived from plant pathogens (Cauliflower Mosaic Virus – CaMV, and Crown Gall – *Agrobacterium tumefaciens*). However, they represent only a very small proportion of the pathogen genome and the sequences are not, in themselves, infectious or pathogenic.

Section 1.4 Method of gene transfer

13. Two binary vectors were constructed to contain the DFR, F3'5'H, and SuRB genes as well as associated regulatory sequences (see Tables 1 and 2 below). Each GM carnation line was produced by *Agrobacterium tumefaciens*-mediated transformation using the disarmed strain AGL0 to introduce the genes of interest from one of the two binary vectors. The *Agrobacterium*-mediated DNA transformation system is well understood and used extensively in genetic transformation of plants. The entire DNA sequence of the transgenes and the vectors used to transform the plants are known.

Table 1 – gene construct of binary vector pCGP1470

Promoter	**origin**	**Gene**	**origin**	**Terminator**	**origin**
35S	CaMV (cauliflower mosaic virus)	SuRB	*N. tabacum* (tobacco)	SuRB	*N. tabacum* (tobacco)
CHS	*A. majus* (snap dragon)	F3'5'H	*Petunia*	D8	*Petunia*
MAC	*CaMV A. tumefaciens (crown gall)*	DFR	*Petunia*	*mas*	*A. tumefaciens* (crown gall)

Table 2 – gene construct of binary vector pCGP1991

Promoter	**origin**	**Gene**	**origin**	**Terminator**	**origin**
35S	CaMV	SuRB	*N. tabacum* (tobacco)	SuRB	*N. tabacum* (tobacco)
CHS	*A. majus* (snap dragon)	F3'5'H	*Viola* (pansy)	D8	*Petunia*
DFR 5'	*Petunia*	DFR	*Petunia*	DFR	*Petunia*

14. For detailed information about the parent organism and the GMO please see Appendix 1.

SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

Section 2.1 Australian releases of GM carnation

15. Under the former voluntary system overseen by GMAC, Florigene carried out nine releases of GM carnations. Seven of these were limited and controlled planned releases (PR-19, PR-19X, PR-28, PR-28X, PR-29, PR-29X, and PR-84), and two were commercial releases (GR-1 and GR-2).

16. Florigene is the only company in Australia to release genetically modified carnations. These previous releases, conducted in accordance with GMAC guidelines, were assessed by GMAC as not posing any significant risks. No adverse effects on human health and safety or the environment have been reported in connection with any of these releases.

17. The releases assessed by GMAC are:

- PR-19 (1992-1995): Calgene Pacific (now Florigene) - *Planned release of transgenic carnation for trialing under commercial glasshouse production conditions.*

  The purpose of this release was to trial a small number (300) of GM carnations designed to prolong vase life of the flowers. GM carnations contained a gene encoding an ethylene-forming enzyme in its antisense orientation, and a selectable marker gene (*nptII*) that expresses NPTII protein conferring resistance to the antibiotic kanamycin.

- PR-19X (1993–1995): Calgene Pacific – *Planned release of carnation with various constructs aimed at prolonging flower life.*

  This extension included four additional GMOs to make a total of five GMOs. All GMOs contained the *nptII* gene as per PR-19, and either a gene encoding an ethylene-forming enzyme in its antisense orientation, or a gene from *A. tumefaciens* that is known to inhibit ethylene production. Approximately 4400 plants were trialed under commercial glasshouse production conditions.

- PR-28 (1994–1995): Calgene Pacific – *Planned release proposal for trialing transgenic carnation with modified flower colour under non-contained glasshouse conditions.*

This was a glasshouse trial of approximately 3500 plants. A total of 8 genetic modifications were trialed. All 8 constructs contained the gene encoding the flavonoid 3', 5' hydroxylase enzyme. Two of these also contained a gene encoding the dihydroflavonol 4-reductase reductase enzyme. Each of the modifications contained a selectable marker gene. Three of the eight modifications used the *nptII* gene as the selectable marker; it encodes the NPTII protein that confers resistance to the antibiotic kanamycin. The remaining five constructs contained the selectable marker gene (SuRB) that confers resistance to ALS inhibiting herbicides.

- PR-29 (1994-1996): Calgene Pacific – *Proposal for planned release of transgenic carnation modified for enhanced cutflower vase life.*

  This was a glasshouse trial of between 2000 and 4000 plants. Carnations were genetically modified by inserting either, a gene coding for aminocyclopropane cyclase (ACC) synthase, or ACC oxidase in order to enhance cutflower vase life. In addition both gene constructs also contained a selectable marker gene (SuRB) that confers resistance to ALS inhibiting herbicides.

- PR-28X (1994-1997): Florigene – *Proposal for extension of PR-28 to an igloo trialing area.*

  The purpose of extending PR-28 to an igloo trialing area was to test the performance of GM carnations modified for flower colour under commercial growing conditions.

- PR-29X (1994-1997): Florigene – *Proposal for extension of PR-29 to an igloo trialing area.*

  The purpose of extending PR-29 to an igloo trialing area was to test the performance of GM carnations modified for enhanced cutflower vase life under commercial growing conditions.

- PR-84 (1997-1999): Florigene – *Planned release of carnation modified for resistance to fungal pathogens.*

  Under this trial carnations were modified by insertion of one of eight genes thought to be important for resistance to fungal pathogens. Each GM carnation also contained a selectable marker gene (SuRB) encoding resistance to ALS inhibiting herbicides.

- GR-1 (1995-2003): Florigene – *General release for the commercialisation of GM carnation for improved vase life.*

  GR-1 is the commercial release arising from PR-19. GM carnations contain the antisense gene of ACC oxidase and a selectable marker gene (*nptII*) conferring antibiotic resistance. This licence will lapse on 21 June 2003.

- GR-2 (1995-2003): Florigene – *General release for the commercialisation of violet carnation developed using genetic engineering*.

  GR-2 is the commercial release arising from PR-28, and the present application seeks to continue this. There are two types of GM carnations; both contain the DFR gene from *Petunia hybrida*, and the SuRB gene from *Nicotiana tabacum*. The third gene (F3'5'H) is from *Viola* in vector pCGP1991 and from *P. hybrida* in vector pCGP1470.

Section 2.2 International approvals

18. Countries that have approved the release of GM carnations include

- The Netherlands: The Ministry of Housing, Spatial Planning, and the Environment approved 11 field trials between 1994 and 1998 and 11 market releases. Of these, one field trial and 10 market releases were for carnations genetically modified for colour. The Dutch legislation complies with the European directives 90/219/EEC and 90/220/EEC that relate to the contained use of genetically modified microorganisms, and the deliberate release into the environment of genetically modified organisms respectively.
- Japan: The Ministry for Agriculture, Forestry, and Fisheries approved nine field trials between 1994 and 1997, eight of these were for carnations genetically modified for colour. There are 11 colour modified carnation lines currently approved for commercial use in Japan.
- USA: In 1997 the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS), deregulated any carnation variety transformed with vectors pCGP1470 or pCGP1991. De-regulation means that flowers transformed with these constructs may be imported into the USA under normal market conditions, i.e. without special packaging or labelling. Transgenic carnations modified for colour have been sold in the USA since 1998.
- Ecuador: In 1997 the government of Ecuador approved the release of carnations genetically modified with binary vectors pCGP1470 or pCGP1991 for growing and export of flowers.
- Colombia. The Ministry of Agriculture approved the commercial release of carnations genetically modified with pCGP1470 and pCGP1991 vectors in 2000. GM carnation flowers have been exported from Colombia since 2001.
- Canada: Colour modified carnation flowers transformed with vectors pCGP1470 and pCGP1991 are approved for importation into Canada.
- Israel: In 1999 the National Committee for Transgenic Plants approved an application for general release of carnations genetically modified for colour. No commercial production or exports has taken place in Israel, however commercial trials have been carried out since 2000.
- Mexico, Kenya, and Singapore: Permission for general release of carnations genetically modified for colour using the vectors pCGP1470 and pCGP1991 have been obtained for these three countries. To date, no commercial activity has occurred.

CHAPTER 2 SUMMARY OF THE RISK ASSESSMENT & RISK MANAGEMENT PLAN (RARMP)

19. The Act and the Regulations require that risks associated with dealings with GMOs are identified and assessed as to whether they can be managed to protect human health and safety and the environment (see Appendix 7).

SECTION 1 ISSUES RAISED IN CONSULTATION ON THE RARMP

20. Comments received in response to the consultation on the risk assessment and risk management plan undertaken with expert groups and key stakeholders, as required by Section 50, of the Act and with the public, as required by Section 52 of the Act (see Appendix 7), were very important in shaping this risk assessment and risk management plan, which formed the basis of the final decision on the application.

21. The following issues were raised in written submissions received by the Regulator in relation to DIR 030/2002 and are addressed in the risk assessment and the risk management plan:

- Potential for toxic or allergic effects in humans (Appendix 2 refers);
- Ecotoxicity to non-target organisms, and persistence and accumulation of the expressed proteins (Appendices 2 and 3 refer);
- Potential for novel traits (including the selectable marker) to increase resistance to herbivores and disease, and to increase the weediness of GM carnation (Appendix 4 refers);
- The effects on the environment should horizontal gene flow occur especially in relation to the selectable marker gene (Appendix 5 refers);
- Potential for altered pollen biology and seed characteristics (Appendix 4 refers);
- Risk of gene transfer to non-transgenic carnation, naturalised *Dianthus* species or any species belonging to the Caryophyllaceae family, and the ecological impacts should such transfer occur (Appendix 5 refers); and
- Potential for the genetic modifications to confer increased fitness (Appendix 4 refers).

22. The Regulator received one submission from the public on this application. The key issue raised was the potential of GM carnation to detrimentally affect soil micro-organisms (Appendix 3 refers).

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

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

24. The risk assessment process, detailed in Appendix 7, identified a number of hazards that may be posed by the dealings. The risks posed by these hazards were assessed by considering:

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

25. The categories used, according to the level of risk are 'negligible', 'very low', 'low', 'moderate', 'high', or 'very high'.

26. The following table, Table 2.1, lists each of the hazards that were considered during the risk assessment process in the *Hazard Identification* column, summarises the assessment of each hazard under the column headed *Risk Assessment*, and identifies whether management is required in the final column. A comprehensive risk assessment of each identified hazard is provided in Appendices 2 - 5, as cross-referenced in the column headed *Summary of Risk Assessment*.

SECTION 3 DECISION ON THE APPLICATION

27. Details of the matters that the Regulator must consider in making a decision are provided in Appendix 7. In assessing the application for the commercial release of GM carnation, the Regulator considers the need to impose conditions to manage any risks to human health and safety or the environment.

28. Given the widespread scale and ongoing nature of a commercial release, the Regulator considers that the release should only be approved if the risks to human health and safety or the environment are low to non-existent and therefore do not require a range of specific licence conditions for them to be managed.

29. It was concluded that the release of GM carnation poses no greater risks to human health and safety and the environment than the minimal risks posed by conventional cultivated carnation. Therefore, only minimal licence conditions to oversight the ongoing commercial release have been imposed. These are detailed in Appendix 6

30. In accordance with matters required to be considered under section 58 of the Act, the Regulator has determined that Florigene is suitable to hold a licence for a dealing involving the continued commercial release of carnation genetically modified for flower colour into the environment. Further information on the process of assessment for the suitability of the applicant is contained in Appendix 7.

31. For the above reasons the Regulator has decided to issue a licence, number DIR 030/2002, in respect of this application.

Table 2.1. Summary of the risk assessment and risk management plan (including licence conditions)

Hazard Identification	Risk Assessment (RA; combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does risk require management?
TOXICITY FOR HUMANS	Negligible	**See Appendix 2** Available data indicate that the GM carnation will not be more toxic than conventional carnation. Carnations are not generally used as a food source, but even if ingested the likelihood of GM carnation being toxic is extremely low. The proteins for modified flower colour expressed in GM carnation are similar to those found in purple-coloured fruits and vegetables that are commonly consumed, and in ornamental flowers. ALS enzyme is not a known toxin or allergen and ALS enzymes are present in a wide variety of edible plants. No homology was found between the inserted genes and known toxins. Toxicity studies of delphinidins and anthocyanins indicate very low levels of toxicity. Humans are commonly exposed to and ingest delphinidins in fruits and vegetables at similar or greater concentrations than are found in GM carnation without adverse consequences.	No
ALLERGENICITY FOR HUMANS	Negligible	**See Appendix 2** Reports of allergenicity to carnations are rare and there are no reports of allergenicity to GM carnations. Pollen is produced in very low amounts, is not wind-dispersed and so has limited potential to act as an aeroallergen, and contains no delphinidins. No homology was found between the inserted genes and known allergens.	No
TOXICITY FOR OTHER ORGANISMS - invertebrates and soil biota	Negligible	**See Appendix 3** The evidence indicates that GM carnations will not be more toxic than conventional carnations. The introduced proteins occur widely in other edible plants and ornamental flowers and these are not known to be toxic for other organisms. Organisms are commonly exposed to and ingest delphinidins at similar or greater concentrations than are found in GM carnation. Germination of plant seed and plant growth, and bacterial and fungal population abundance does not differ in soil from GM and non-GM carnation	No
WEEDINESS - persistence in the environment	Negligible	**See Appendix 4** Non-GM carnation does not occur outside of the horticultural or garden environment. The inserted genes do not alter life history characters and naturalised GM or non-GM carnations have not been found in Australia. The presence of the SuRB herbicide tolerance gene could confer a selective advantage to carnation where ALS inhibitors are used to control weeds. However, ALS inhibitors are not used to control weeds in the carnation industry and carnations exist solely as managed cultigens.	No

WEEDINESS - spread in the environment	Negligible	**See Appendix 4** Non-GM carnation has poor dispersal abilities and the inserted genes in GM carnation are unlikely to alter this characteristic. No seed has ever been produced from GM carnation. Little pollen is produced and where it is present has low viability. Pollinators of carnation are limited to lepidopteran insects with long probosci but successful pollination does not occur frequently, if at all, in carnation crops.	No
GENE TRANSFER - Plants: other carnation and other *Dianthus* species	Negligible	**See Appendix 5** Pollen abundance and viability low. *D. caryophyllus* is not sexually compatible with *D. plumarius* or *D. barbatus*. Populations have limited geographical overlap	No
GENE TRANSFER - Plants: other genera	Negligible	**See Appendix 5** It is highly unlikely that inter-generic pollination would occur. *D. caryophyllus* pollen has low abundance and viability. No known hybrids between carnation and other Caryophyllaceae or any other plants	No
GENE TRANSFER - Microorganisms (bacteria)	Negligible	**See Appendix 5** The risk of the introduced genes transferring from GM carnation to humans and other organisms is negligible due to the limited probability of occurrence of uptake and integration of the DNA, and persistence of any novel organism. Natural events of horizontal gene flow from plants to distantly related organisms are extremely rare. Any organism that acquires the novel genes is unlikely to pose any additional risks to human health and safety, or the environment, compared to the GM carnations.	No

APPENDIX 1 INFORMATION ABOUT THE PARENT ORGANISM AND THE GMO

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

33. This part of the document addresses these matters and provides detailed information about the parent organism, the GMO for release, the genetic modification process, the genes that have been introduced, and the new gene products that are expressed in the genetically modified carnation.

SECTION 1 THE PARENT ORGANISM

34. The parent organism is carnation, *Dianthus caryophyllus*. *D. caryophyllus* belongs to the Caryophyllaceae family, a temperate northern hemisphere family containing around 2100 species in 89 genera. The *Dianthus* genus contains 300 species and is native to Europe, Asia, North Africa, and the Arctic region where one species is found. *D. caryophyllus* is widely cultivated as an ornament plant, but in recent Floras (databases describing the plants of a region or regions) it is recorded as not known in the wild, except perhaps in the Mediterranean countries of Greece, Italy, Sicily, and Sardinia (Tutin et al. 1993). Older Floras list it as growing wild in the Mediterranean areas of Italy, Sicily, Sardinia, Greece, France, Algeria, and Morocco.

35. Very little documentation of the history of carnation exists, although it has been cultivated for over 2000 years (Vainstein et al. 1991). Carnation is exotic to Australia but has been grown commercially as a flower crop since 1954. At present the industry produces approximately 140 million cut flowers per annum across a total of 100 ha in Victoria, South Australia, Western Australia, and New South Wales. Victoria is the largest centre of flower production. Florigene states that relative to some overseas countries, carnation production is relatively low in Australia with China and Colombia producing carnations over 5 000 ha and 2 000 ha respectively, and Italy and Spain each having 1 000 ha under production. Other major producers are Mexico, Malaysia, India, Japan, Kenya, Turkey and Israel.

36. Although *Dianthus caryophyllus* is widely cultivated as an ornamental plant, there are few records of it being found as a naturalised plant even in Mediterranean countries, and there are no records of naturalised *D. caryophyllus* in Australia. Only two species of *Dianthus* are naturalised in Australia; *D. armeria* and *D. plumarius*. Both are restricted to south-eastern Australia and Tasmania (Figure A1.1).

37. The genus *Dianthus* contains several species that have been cultivated for hundreds of years for their ornamental value (Ingwerson 1949). Species of the genus *Dianthus* that are grown as cultivated garden plants are referred to as ‘pinks’. There are four types of ‘pinks’: cottage, rockery, annual and cluster-headed. The ‘Pinks’ usually have single open flowers with five petals. Species commonly grown as ‘pinks’ include *D. plumarius, D. alpinus, D. sylvestris, D. chinensis, D. deltoides, D. gratianopolitanus, D. carthusianorum, D. superbus, and D. armeria* although pinks have mainly been bred from *D. plumarius*. Sweet Williams are also classified as pinks, but are easily distinguishable by their sword-shaped leaves and ‘bunch’ or cluster of flowers. Sweet Williams have been developed from *D. barbatus*.

38. Carnations are double flowered cultivars of one 'pink' species, *Dianthus caryophyllus.* In its single flower form, *D. caryophyllus* is called the clove pink or Grenadine (Britannica 1999). Clove pink was grown in the Middle Ages for its clove-like perfume. However, modern cut flower varieties of carnation have been selected for flower size, petal number, stem length and disease resistance.

39. Cut flower varieties of *D. caryophyllus* grow to heights between 60 and 120 cm, and produce flowers with diameters of up to 60mm. 'Pinks', on the other hand, are generally between 30 and 40 cm tall with flowers up to 25mm in diameter. Petal number in cut flower varieties has increased from 5 to between 30 and 100 petals per flower, depending on variety. As a result, the reproductive tissues of the flower have become enclosed by petals, making insect access difficult especially for those without a long proboscis. In contrast, 'pinks' have open flowers, with the stigma and style protruding out of the flower.

40. There are many flower varieties of carnation. These are divided into groups based on plant form, flower size, and flower type: standards (sims), sprays (minis or miniatures), and midis (chinensii). Standards or sims flowers have a single large flower per stem, whereas sprays have a larger number of smaller flowers. The flowers of midis are smaller and the stem is shorter than the standard type, and there are twice as many flowers (per plant per annum as standards). Midis can produce either a single flower per stem, or have multiple side branches with flowers.

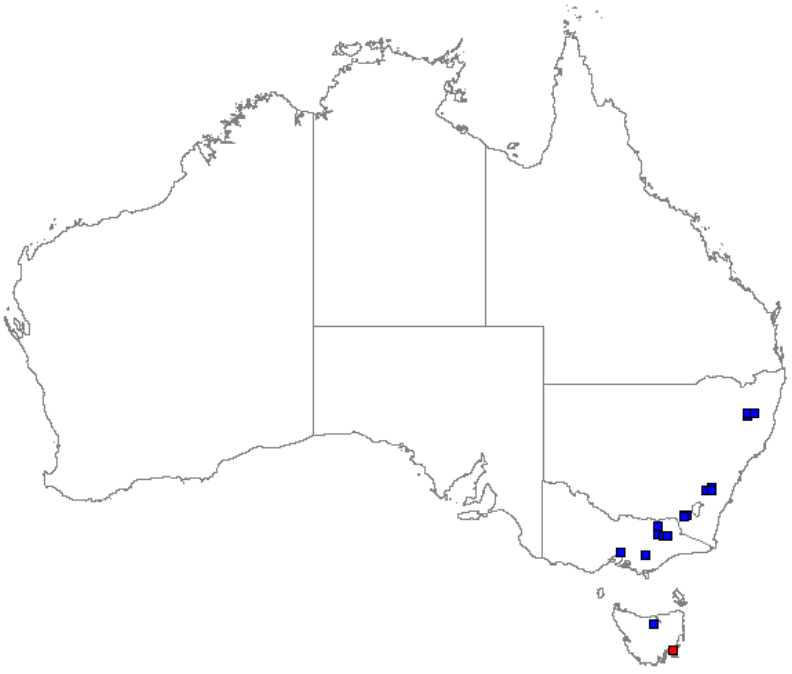

Figure A1.1 Map of Australia showing herbarium records for *D. armeria* (24 records) and *D. plumarius* (1 record in SE Tasmania).

41. Most commercially important carnation varieties are vegetatively propagated, and are not F1 hybrids. Carnation is detrimentally affected by inbreeding (Galbally and Galbally 1997). Inbred parental lines are necessary to breed F1 varieties, however inbreeding depression appears in the third selfed generation so that it is almost impossible to produce S4 seeds (Sato et al. 2000). Efficient, direct plant regeneration via adventitious shoot initiation has been obtained from petals (Kakehi 1979; Leshem, 1986; Nugent et al. 1991), receptacles, stems, and hypocotyl callus tissues (Petru and Landa 1974), calyxes, nodes, internodes, and leaves (Frey and Jannick 1991) of carnation. Regeneration from stems is apparently the preferred system, as plants grow faster, look healthier, and do not flower prematurely.

SECTION 2 THE ANTHOCYANIN PATHWAY

42. In general, colour in flowers is attributed to the presence of two pigment types – carotenoids and flavonoids. The carotenoids are responsible for yellow through orange colours. However, most plants do not contain carotenoid pigments. Many flavonoids are flower pigments such as the anthocyanins (water soluble plant pigments that accumulate in the vacuoles). There are three major types of anthocyanins that contribute to flower colour: delphinidins that produce blue or purple flower colour, cyanidins that produce red or magenta flower colour, and pelargonidins that produce orange, pink or brick red flower colour (Zuker et al 2002). Carnations do not naturally have blue or mauve flowers because they lack that part of the anthocyanin biosynthetic pathway that produces delphinidins or blue pigments.

43. Synthesis of all anthocyanins follows a similar pathway until the colourless naringenin is converted to dihydrokaempferol (DHK) (see Figure A1.2). In cultivated carnations DHK is either converted to the colourless leucopelargonidin by the enzyme dihydroflavonol 4-reductase (DFR) 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. Delphinidin synthesis requires the conversion of DHK or DHQ to dihyromyricetin (DHM) by flavonoid 3', 5' hydroxylase (F3'5'H). DFR, in conjunction with enzymes that oxidise, dehydrate and glycosylate, converts the colourless leucodelphinidin to coloured delphinidin. It is this part of the anthocyanin biosynthetic pathway that Florigene has modified so that delphinidins are expressed in carnations.

44. The GM carnations in this application contain the genes coding for the enzymes F3'5'H and DFR, as well as a selectable marker conferring resistance to acetolactate synthase (ALS) inhibiting herbicides, and regulatory sequences designed to enhance expression of the inserted genes.

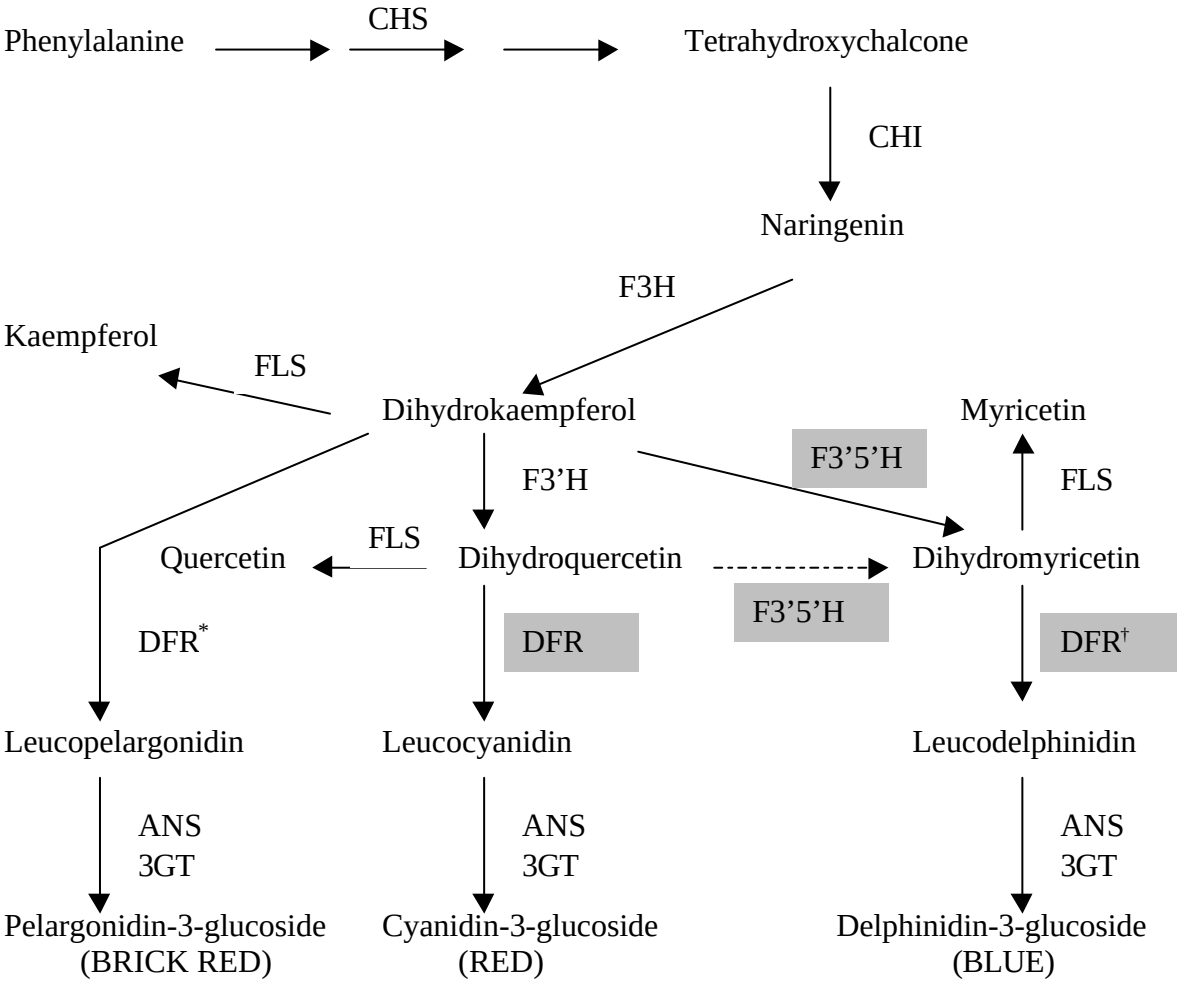

Figure A1.2 Anthocyanin biosynthetic pathway (adapted from Holton and Cornish 1995)
DFR*: *Petunia* DFR does not act on DHK, DFR†: *Petunia* DFR has highest activity on DHM.

Key to enzymes in figure:

ANS: anthocyanidin synthase	DFR: dihydroflavonol 4-reductase	F3'5'H: flavonoid 3',5' hydroxylase
CHI: chalcone isomerase	F3H: flavanone 3-hydroxylase	FLS: flavonol synthase
CHS: chalcone synthase	F3'H: flavonoid 3' hydroxylase	3GT: Flavonoid 3-glucosyltransferase

SECTION 3 INFORMATION ABOUT THE GMO

45. The organism to be released is GM carnation (*Dianthus caryophyllus*). There are a total of two genetic modifications using either of the binary vectors pCGP1470 and pCGP1991 that have resulted in the commercial release of 4 lines in Australia (see Table A1.1). These lines represent 4 transgenic events.

Table A1.1 showing details of GM carnation currently sold commercially in Australia.

Trade Name	Binary Vector	Flower type	Identifier
Florigene Moonlite	PCGP1470	Standard	40644 or 123.2.38
Florigene Moonshade	PCGP1470	Standard	40619 or 123.2.2
Florigene Moonshadow	PCGP 1991	Midi or standard	11363
Florigene Moonvista	PCGP1991	Standard	40685 or 123.8.8

46. Two binary vectors have been constructed to contain the DFR, F3'5'H, and SuRB genes as well as associated regulatory sequences (see Tables A1.2 and A1.3 below). Transgenic carnations have been produced by *Agrobacterium tumefaciens*-mediated transformation using the disarmed strain AGL0. The *Agrobacterium*-mediated DNA transformation system is well understood and used extensively in genetic transformation of plants. The entire DNA sequence of the transgenes and the vectors used to transform the plants are known.

Table A1.2 – gene construct of binary vector pCGP1470

Promoter	origin	Gene	origin	Terminator	origin
35S	CaMV (cauliflower mosaic virus)	SuRB	*N. tabacum* (tobacco)	SuRB	*N. tabacum* (tobacco)
CHS	*A. majus* (snap dragon)	F3'5'H	*Petunia*	D8	*Petunia*
MAC	CaMV and *A. tumefaciens* (crown gall)	DFR	*Petunia*	*mas*	*A. tumefaciens* (crown gall)

Table A1.3 – gene construct of binary vector pCGP1991

Promoter	origin	Gene	origin	Terminator	origin
35S	CaMV	SuRB	*N. tabacum* (tobacco)	SuRB	*N. tabacum* (tobacco)
CHS	*A. majus* (snap dragon)	F3'5'H	*Viola* (pansy)	D8	*Petunia*
DFR 5'	*Petunia*	DFR	*Petunia*	DFR	*Petunia*

SECTION 4 THE INTRODUCED GENES

Section 4.1 The genes for anthocyanin pathway enzymes

F3'5'H gene

47. All lines contain the F3'5'H gene, coding for flavonoid 3', 5' hydroxylase. Flavonoid 3', 5' hydroxylase, a member of the cytochrome P450 family, is a key enzyme in the synthesis of 3', 5' hydroxylated anthocyanins, which are generally required for purple or blue flowers (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 5, 7, 3, '4', 5' pentahydroxyflavonone.

48. The gene encoding the enzyme flavonoid 3', 5' hydroxylase in plasmid pCGP1470, is from the *hf1* gene in petunia (*Petunia hybrida*). Its expression is regulated by the chalcone synthase promoter from snapdragon (*Antirrhinum majus*) and by the 3' region of a phospholipid transfer protein gene derived from petunia (*Petunia hybrida*). The F3'5'H gene in plasmid pCGP1991 is derived from pansy (*Viola* sp.) and its expression is regulated by the chalcone synthase promoter from snapdragon (*Antirrhinum majus*) and the same 3' region as in pCGP1470.

49. 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).

DFR gene

50. Florigene has inserted the gene encoding the enzyme dihydroflavonol 4-reductase (DFR) reductase from *Petunia hybrida* into all the GM carnation lines. The DFR enzyme generally acts on dihydroflavonols such as dihydrokaempferol (DHK), dihydroquercetin (DHQ), and dihydromyricetin (DHM) to produce leucoanthocyanidins (see Fig A1.2). The leucoanthocyanidins are precursors to anthocyanin pigments. 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* has the highest activity with DHM as a substrate but does not reduce DHK (Meldgaard 1992). This specificity of *P. hybrida* ensures that most or all of the anthocyanidin produced in the transgenic carnation flowers is delphinidin.

51. The DFR gene family of *P. hybrida* consists of three genes, DFR*A*, DFR*B*, and DFR*C*, located on chromosomes IV, II, and VI respectively. Three DFR cDNA clones isolated from a petal limb cDNA library all originated from DFR*A* indicating that in petal limbs this is the most active DFR gene (Beld et al. 1989). The DFR*A* gene is also expressed in flower limbs, in the filament beneath the anther, the style just below the stigma, in the stem just above the soil in older plants, and in the seeds and seed pod tissue (Huits et al. 1994).

Section 4.2 Selectable marker gene

SuRB gene

52. The enzyme acetolactate synthase (ALS) catalyses the first common step in the biosynthesis of the amino acids isoleucine, leucine, and valine in bacteria, yeast, and higher plants (Keeler et al. 1993). ALS activity in plants can be inhibited by several classes of herbicides, including sulfonylureas, imidazolinones, and triazolopyrimidines (Mazure and Falco 1989). The herbicide works by blocking cell division in the active growing regions of stem and root tips (meristematic tissue) (Extoxnet 2003).

53. Florigene has inserted the selectable marker gene SuRB into GM carnation. This gene codes for an alternative form of the enzyme ALS that confers resistance to ALS inhibiting herbicides like sulfonylureas, imidazolinones, and triazolopyrimidines (Mazure and Falco 1989).

54. Mutant lines resistant to sulfonylureas have been isolated in *N. tabacum* by selection in tissue culture. Genetic analyses of these mutant lines identified two unlinked loci that were responsible for conferring the herbicide resistant trait, SuRA and SuRB (Chaleff and Ray 1984). Resistance is conferred due to the production of an altered form of the ALS enzyme which is less sensitive to ALS inhibiting herbicides, rather than due to the overproduction of a normal enzyme. This confers resistance to sulfonylureas and cross-resistance to many imidazolinone and triazolopyrimidine herbicides. Resistant phenotypes are the consequence of mutations in the coding regions of the ALS gene, and are not due to duplications or amplifications of the genes or to changes in the regulatory regions surrounding the genes (Lee et al. 1988). Florigene has used the SuRB gene from *Nicotiana tabacum* as the selectable marker gene in both pCGP1470 and pCGP1991 vectors.

Section 4.3 The regulatory sequences

CHS – chalcone synthase

55. Florigene has used the promoter region of the chalcone synthase gene to regulate expression of the gene coding for F3'5'H in both pCGP1470 and pCGP1991 vectors.

56. Chalcone synthase is a key enzyme in the anthocyanin biosynthetic pathway in plants. It catalyzes the condensation of three molecules of malonyl CoA with one molecule of *p*-coumaroyl CoA to produce naringenine chalcone (Niesbach-Klosgen et al. 1987). This product is important for the syntheis of a variety of compounds like anthocyanines, flavones, flavonols, flavonoids, and isoflavonoids (Reif et al. 1985).

57. The synthesis of CHS is highly regulated and the activity of the *chs* gene appears to be developmentally regulated. In most plants expression of the *chs* gene is also tissue specific (Reif et al. 1985). In *Antirrhinum majus* chalcone synthase has been shown to be encoded by the *nivea* locus (Spribille and Forkmann 1982). The complete coding sequence of the *chs* gene of *A. majus* is known and the gene codes for a protein of 390 amino acids (Sommer and Saedler 1986).

CaMV – Cauliflower mosaic virus promoter

58. In both of the pCGP1470 and pCGP1991 gene constructs, the promoter from the 35S region of Cauliflower Mosaic Virus (CaMV) has been inserted to drive expression of the SuRB gene in GM carnation. Note that only the promoter region of the 35S RNA gene was inserted, and no complete genes.

59. For the past 15 years or more, the CaMV has been used extensively in plant transformations. Although the natural host range of CaMV is limited to species in the Cruciferae (e.g. cabbages, cauliflowers, canola, mustard, and other brassicas), the promoter is active in the genome of a large variety of monocotyledonous and dicotyledonous plants (Blaich 1992). This promoter is considered to be constitutive, however some tissue specificity and cell cycle stage-dependent expression has been found (Benfey et al. 1989), and has been shown to significantly increase expression levels of the genes which it regulates.

***Mas* – Mannopine synthase terminater**

60. Florigene has used the terminator sequence from the mannopine synthase gene from *Agrobacterium tumefaciens* to regulate expression of the DFR gene in the binary vector pCGP1470.

61. Regulatory sequences from the mannopine synthase gene of *A. tumefaciens* are used to increase expression levels of transgenes.

MAC – Cauliflower mosaic virus/Mas chimeric promoter

62. Florigene has used a chimeric promoter to regulate expression of the DFR gene in plasmid pCGP1470. The promoter contains elements of the 35S promoter region of CaMV and of the mannopine synthase gene of the TR-DNA of *Agrobacterium tumefaciens* octopine Ti plasmid. Both promoters are commonly used to drive expression of novel genes in transgenic plants.

63. The chimeric promoter contains the 35S 5' region of CaMV but with the region containing the TATA box deleted, and in its place, a fragment containing the TATA box and about 300bp of the 5' upstream region of the *mas* promoter (Comai et al. 1990). The MAC promoter has been shown to have higher expression levels (as measured by GUS activity in leaves of transformed tobacco and tomato plants) than other promoters, and promoter expression increases with plant age (Comai et al. 1990).

D8 – Phospholipid transfer protein

64. Florigene has used the 3' region of a phospholipid transfer protein homologue gene to regulate the F3'5'H gene in both pCGP1470 and pCGP1991 vectors. The 3' region of D8 is from *Petunia hybrida*.

65. Phospholipid transfer proteins facilitate (*in vitro*) transfer of phospholipids between membranes (Kader 1985).

Section 4.4 The plasmid genes

66. Florigene reported extra-border integration of a small portion of the vector pCGP1470 backbone into lines 123.2.38 (Moonlite) and 123.2.2 (Moonshade). Because the application is for a commercial release, a full risk assessment of all the genes on the plasmid backbone of pCGP1470 has been done. Note that the entire plasmid sequences for both vectors (pCGP1470 and pCGP1991) are known. The two vectors are identical outside the T-DNA borders.

67. The plasmids are well characterised and contain the following on the vector backbone:

- Origin of replication for replication in *E. coli*
- Origin of replication for replication in *Agrobacterium tumefaciens*
- Tetracycline resistance gene for selection in the bacterial host

Tetracycline resistance gene

68. Tetracyclines are broad-spectrum antibiotics that inhibit bacterial growth by stopping protein synthesis. They are prepared from the cultures of Streptomycetes. (Streptomycetes belong to the bacterial group called Actinomycetes. They are superficially similar to fungi in

having filaments and spores and they occur in similar habitats). Tetracyclines have been widely used for the past 40 years in human therapy and veterinary medicine, but also as a growth promoter in animal husbandry. The emergence of bacterial resistance to tetracycline antibiotics has limited their use and cross-resistance is significant.

69. Several tetracycline resistance genes are currently used in molecular biology. The most common are the *tet*A genes of classes A (RP1, RP4, or TN*1721* derivatives), B (Tn*10* derivatives and C (pSC101 or pBR322 derivatives) that encode a tetracycline efflux system of tetracycline resistance. These genes are regulated by a repressor protein (*tet*R). This system and the ribosome protection system of tetracycline resistance are the most widespread mechanisms of tetracycline resistance. Most of the tetracycline resistance genes are acquired by bacteria via transferable plasmids and/or transposons. These two mechanisms have been observed in both aerobic and anerobic Gram-negative and Gram-positive bacteria demonstrating their wide distribution among the bacterial kingdom (http://biosafety.ihe.be/AR/Tetracycline/Menu_Tet).

70. Florigene has used a tetracycline resistance gene complex from *E. coli* to select for bacteria carrying the transformation vector. This complex contains *tet*A and *tet*R genes.

Origins of replication for replication in *E. coli* and in a broad bacterial host range

71. The plasmid vector also contains two origins of replication. One is for replication of the plasmid in *E. coli* so that large numbers of *E.coli* colony cells can be grown that contain the T-DNA. The second is for replication in *Agrobacterium tumefaciens* since the plasmids that have replicated in *E. coli* are unable to replicate autonomously in *A. tumefaciens*.

SECTION 5 METHOD OF GENETIC MODIFICATION

72. GM carnation lines were produced by inserting the F3'5'H, DFR, and SuRB genes into carnation genomic DNA. The genes were inserted by *Agrobacterium*-mediated DNA transformation (della-Cioppa et al. 1987) using the disarmed strain AGL0. Four transgenic lines were produced using either one of two binary vectors, pCGP1470 or pCGP1991.

73. The *Agrobacterium*-mediated DNA transformation system is well understood (Zambryski, 1992). The plasmic vectors, pCGP1470 and pCGP1991, contain well characterised DNA segments required for selection and replication of the plasmid in bacteria as well as *Agrobacterium* sequences essential for DNA transfer from *Agrobacterium* and integration in the plant genome (Bevan 1984, Wang et al. 1984, Klee and Rogers 1989).

74. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. It is the only prokaryotic organism known to be capable of transferring DNA to eukaryotic cells (Bundock and Hooykaas 1998).

75. The ability of *A. tumefaciens* to transfer genes evolved from bacterial conjugational transfer systems, which mobilise plasmids for transfer between bacterial cells. Usually, when using *Agrobacterium* vectors, only the T-DNA is transferred and integrated into the plant genome (de la Riva et al. 1998), although plasmid vector sequences can also be transferred. The transfer of the T-DNA (located between specific border sequences on a plasmid) from *A. tumefaciens* occurs through the mediation of the genes from the *vir* (virulence) 7 region of the Ti (tumour inducing) plasmids. It is generally accepted that T-DNA transfer into plant cells

by *Agrobacterium* is irreversible and cannot be re-mobilised to transfer elsewhere in the genome or to other organisms (Huttner et al. 1992).

76. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains do not contain the genes (*iaaM, iaaH* and *ipt*) responsible for the overproduction of auxin and cytokinin, which are required for tumour induction and rapid callus growth (Klee and Rogers 1989). A useful feature of the Ti plasmid is the flexibility of the *vir* region to act in either *cis* or *trans* configurations to the T-DNA. This has allowed the development of two types of transformation systems:

- co-integration vectors that join the T-DNA that is to be inserted into the plant and the vir region in a single plasmid (Stachel and Nester, 1986); and
- binary vectors that have the T-DNA and vir regions segregated on two plasmids (Bevan, 1984).

77. Both provide functionally equivalent transformation systems. *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants without causing biosafety problems.

78. In this instance and using standard *Agrobacterium* transformation protocols, disarmed binary vectors (pCGP1470 and pCGP1991) have been used to introduce the genes encoding the proteins F3'5'H and DFR, and acetolactate synthase as a selectable marker. Samples taken from all GM carnation lines that are in excess of 45 vegetative generations have been tested for the presence of *Agrobacterium tumefaciens* using PCR techniques. No presence has been detected.

SECTION 6 MOLECULAR CHARACTERISATION AND STABILITY OF THE GENETIC MODIFICATION

Section 6.1 Characterisation by Southern Blots

79. Southern blot analyses were used to demonstrate the approximate copy number of the inserted genes for each of the GM carnation lines (See Table A1.4), and to determine if any of the plasmid sequences were present in the carnation genome.

Table A1.4. Approximate copy numbers of each of the inserted genes of interest, the selectable marker, and the left and right borders of the T-DNA.

			Approximate Copy Number				
Event	**Line**	**Vector**	**RB**	**LB**	**DFR**	**F3'5'H**	**SuRB**
123.2.38	Moonlite	pCGP1470	1	1	1	nd	1
123.2.2	Moonshade	pCGP1470	nd	nd	nd	nd	nd
11363	Moonshadow	PCGP1991	5	4	4	4	4
123.8.8	Moonvista	PCGP1991	1	1	2	3	2

Key

RB: Right Border

LB: Left Border

nd = no data

F3'5'H: a 600bp fragment from the appropriate F3'5'H cDNA clone

SuRB: a 765bp fragment from the SuRb gene coding region

DFR: a 1400bp fragment from the petunia DFR cDNA clone

80. Southern blot analyses of the plasmid backbone (outside the left and right borders) were used to test whether there was any extra border integration. The following probes were used:

- A: *Eco*R V site of the tetracycline resistance gene (2720 bp). Outside left border.
- B: *Eco* R V site of the tetracycline resistance gene – *Sph* I site (2010 bp)
- C: *Sph* I- *Hinc* II fragment (2775 bp)
- D: *Hinc* II fragment (2315 bp)
- E1: *Hinc* II – *Eco*R I fragment (539 bp)
- E2: *Hinc* II fragment (1402 bp)
- F: Right Border Outside – *Eco*R I site (1608 bp)

81. It was found that two of the lines (123.2.38 and 123.2.2) contained a small portion of the plasmid vector sequence. However, in erring on the side of caution, the risk assessment also includes an assessment of the risks associated with the presence of the entire plasmid vector backbone in the GM carnation.

Section 6.2 Stability of the trait

82. Stability of the flower colour trait for the four lines is represented by analysis of the stability of each of the four phenotypes across numerous vegetative propagation cycles since GM carnation has never produced any seed and therefore analyses of trait inheritance cannot be done.

83. Approximately 10.4 million, 1.5 million, 1.9 million, and 1.0 million flowers have been produced from lines 11363 (Moonshadow), 123.2.38 (Moonlite), 123.8.8 (Moonvista), and 123.2.2 (Moonshade) respectively since commercial production began for each line. This translates to approximately 85 vegetative cycles for line 11363 and between 45 and 50 cycles for the remaining lines.

84. Anecdotal data from growers and site visits by Florigene indicate that the rate of unintended colour changes to the flowers (offtypes or sports) is very low in lines 11363 (Moonshadow), 123.2.38 (Moonlite), and 123.2.2 (Moonshade). For example after production of over 260,000 flowers had been produced of line 11363, only one sport was observed. Line 123.8.8 (Moonvista) shows higher numbers of sport types compared to the other three lines, but even these numbers are low. In a recent survey of approximately 7000 Moonvista flowers, approximately 0.3% of the flowers showed sport types.

85. Offtypes or sports can arise spontaneously in vegetatively propagated plants through somatic mutation that results in vegetative offspring that are phenotypically different to the mother plant. This phenomenon has been capitalised by plant breeders for new sources of plant varieties. For example the Australian Pelargonium varieties 'Flush striped', 'Jackie', and 'Pearl Colwell' are all sports of the varieties 'Princess Victoria', 'Sybil Holmes', and 'Jester' respectively.

86. Florigene have also tested the stability of the genotype of line 123.8.8 using Southern hybridisation. Southern hybridization analyses were performed on genomic DNA isolated from GM carnation in 1999 and again in 2002 following digestion with the restriction endonuclease *Eco*RI using probes for F3'5'H and SuRB. These showed that both copy number (3 and 2 respectively) and size of the inserted genes were the same in both years indicating stability of the genotype over a three year period.

SECTION 7 EXPRESSION OF THE INTRODUCED PROTEINS

87. The expression and activity of the introduced proteins F3'5'H and DFR is demonstrated by the change in carnation flower colour to blue/purple in the GM carnation. The blue/purple phenotype of GM carnation in the result of the production of delphinidins, which can only occur when the introduced proteins are expressed.

88. The concentration of delphinidin and other anthocyanidins in flower samples was determined by HPLC (see Table A1.5 below). These data represent a single assay of bulked petal samples, and are expressed as mg/g fresh weight (of petal).

Table A1.5. Concentration of anthocyanins in carnation lines.

Event	**Line**	**Concentration (mg/g FW)**			
		Delphinidin	**Cyanidin**	**Petunidin**	**% Delphinidin**
-	Non GM Parent (1)	0	0	0	0
-	Non GM Parent (2)	0	0	0	0
123.2.38	Moonlite	0.093	0.031	0	75
123.2.2	Moonshade	0.479	0.018	0.013	94
11363	Moonshadow	0.348	0.024	0	94
123.8.8	Moonvista	2.542	0.007	0	99

89. Delphinidin concentration in GM carnation is within the range expressed by common and widely cultivated ornamental plants containing delphinidin (see Table A1.6).

Table A1.6. Concentration of delphinidins in common ornamental plants

Species	**Delphinidin (mg/g FW)**	**Delphinidin as % anthocyanins**
Agapanthus	0.12	82
Brachycome	0.75	83
Cineraria	0.96	71
Delphinium	0.52	98
Dampiera	1.64	100
Hibiscus species	1 – 10	<50
Iris	1.26	100
Lisianthus	2.8	90
Pansy	3.9	84
Rhododendrum	0.14	50
Wisteria	0.39	89

90. The introduced ALS protein encoded by the SuRB gene is expressed and active in GM carnation since it is used as a selectable marker to identify successfully transformed carnation plants.

APPENDIX 2 HUMAN HEALTH AND SAFETY

91. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. In this part of the document, risks posed by the dealing to human health and safety are considered in relation to toxicity and allergenicity.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENCITY HAZARD

92. GM carnation differs from cultivated carnation in the expression of three additional proteins: flavonoid 3', 5' hydroxylase, dihydroflavonol 4-reductase, and acetolactate synthase. Carnations normally carry genes encoding DFR and ALS activity. The potential for carnation expressing these proteins to be toxic or allergenic to humans has been considered in detail in this Appendix. This could occur if genetically modified carnation were toxic or allergenic because of the novel gene products expressed in the plants or because of unforeseen, unintended effects of the genetic modification.

Section 1.1 Exposure of people to GM carnation

93. GM carnation is not intended for use as food. Adverse effects to human health and safety potentially could occur if GM carnation became toxic or allergenic for people. Humans are in contact with carnation flowers and plants through:

- Working with carnation (e.g. growers, propagators, florists, flower sellers); or
- Living in or near areas where carnation is commercially grown; or
- Recreational growing of carnation plants or having cut flowers in a residential environment.

94. It should be noted that GM carnation has been approved for commercial release since 1995. There have been no reports of any adverse toxic or allergic effects on human health and safety through exposure to GM carnation.

SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENCITY HAZARD OCCURRING

95. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of GM carnation on human health and safety, a number of factors were considered including;

- the toxicity and allergenicity of cultivated carnation ;
- the toxicity and allergenicity of the protein products;
- the toxicity and allergenicity of GM carnation (the GMO);

Section 2.1 Toxicity and allergenicity of cultivated carnation

96. Despite a long history of floriculture, there are few reports of occupational respiratory allergy within the floral industry and no reports of toxicity. Allergic disorders induced by ornamental flower exposure are usually manifested by dermatologic symptoms (eczema,

urticaria, and contact dermatitis) associated or not with respiratory manifestations, but sometimes exclusively respiratory symptoms are seen (Sanchez-Guerrero 1999).

97. There have been only two reports of occupational allergy to *Dianthus caryophyllus.* In the first, a commercial flower seller/distributor developed severe rhinoconjunctivitis, and contact urticaria and dermatitis from respiratory and skin exposure to *D. caryophyllus* (as well as *Gypsophila paniculata* and *Lilium longiflorum*). These symptoms arose only after eight years of working with these flowers and showing no allergic symptoms (Vidal and Polo 1998). In the second, 16 patients employed in the carnation industry with occupational respiratory symptoms (and 15 allergic asthmatic patients who were not exposed to carnations) were subjected to skin prick, nasal provocation, RAST, and immunoblotting tests using carnation extract. Skin prick tests with carnation extract produced positive results in 15 of the 16 experimental patients and negative results in the control group. Nasal provocation test results with carnation extract were positive with 13 of the 16 patients and negative in all control subjects. There was also a significant correlation between serum-specific IgE levels to carnation and nasal challenge test results indicating that exposure to carnations can produce an allergic response (Sanchez-Guerrero et al. 1999), although it is uncommon.

98. It should be noted that neither of these studies was conducted to establish whether GM carnations are allergenic as they concern conventional carnation. They are included here to establish a baseline of allergenicity of non-GM carnations for comparison with the GM carnations. It should also be noted that cultivated carnations produce very little pollen (refer Appendix 4) which is sticky and therefore limited in its ability to act as an aeroallergen.

Section 2.2 Toxicity and allergenicity of the introduced proteins

99. None of the introduced proteins are known toxins or allergens. The proteins responsible for modified flower colour (F3’5’H and DFR) are similar to those found in purple-coloured fruits and vegetables which are commonly consumed and have a long history of safe use. The SuRB gene codes for an alternative form of the acetolactate synthase enzyme. This enzyme is not a known toxin or allergen and related enzymes are expressed in a variety of edible plants (e.g. soybean and rice).

100. BLAST (Basic Local Alignment Search Tool) searches of the open reading frames contained in the T-DNAs of the binary transformation vectors pCGP1470 and pCGP1991 have been done against the GenBank and SwissProt databases. The sequences analysed contained the selectable marker enzyme (SuRB) from tobacco, the dihydroflavonol 4-reductase (DFR) coding region from *Petunia*, the genes encoding flavonoid 3’5’hydroxylase (F3’5’H) from *Petunia* and *Viola.* None of the deduced amino acid sequences appear to be homologous to any known allergens or toxic proteins.

Section 2.3 Toxicity and allergenicity assessment of the introduced protein products

101. Delphinidin is produced as a result of the combined expression of the introduced genes DFR and F3’5’H together with endogenous genes in the anthocyanin biosynthetic pathway. The maximum concentration of delphinidin found in GM carnation is 2.542 mg/g FW (fresh weight) for line 123.8.8 (Moonvista). This is approximately the level in bilberry (*Vaccinium myrtillus*) which may have up to 3.70 mg/g FW anthocyanin content of which approximately 43% is delphinidin (Kalt et al. 1999). Anthocyanins are antioxidants (Wang et al. 1997) and

dietary antioxidants are believed to play a role in reducing the risks of various human degenerative diseases (Priot and Cao 1998). Anthocyanins from the European bilberry have been well studied with regard to human health (Morazzoni and Bombardelli 1996), and bilberry anthocyanin extract is currently marketed in a variety of pharmaceutical and food supplement products (Kalt and Dufour 1997).

102. Delphinidin and other anthocyanidins are found in many raw foods (see Table A2.1). Delphinidin is not known to be a toxic compound when consumed or handled. There are no toxicity data in the Merk Index for the aglycone, the mono-glucoside or the 3'5' di-glucoside of delphinidin.

Table A2.1. Anthocyanin content of some common fruits and vegetables (http://www.does.org/images/TabF1_2_3.jpg)

Source	Anthocyanin content (mg/g FW)
Apples (Scugog)	0.1
Bilberries	3 – 3.2
Blackberries	0.8 – 3.2
Black currants	1.3 – 4.0
Blueberries	0.25 – 4.95
Red cabbage	0.25
Black chokeberries	5.6
Cherries	0.04 – 4.5
Cranberries	0.6 – 2
Elderberry	4.5
Grapes	0.06 – 6
Kiwi	1
Red Onions	0.07 – 0.21
Plum	0.02 – 0.25
Red radishes	0.11 – 0.6
Black raspberries	3 – 4
Red raspberries	0.2 – 0.6
Strawberries	0.15 – 0.35

103. Humans are naturally exposed to anthocyanins through the ingestion of fruit and vegetables. The available information indicates that anthocyanins are poorly absorbed from the gastrointestinal tract.

104. 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% of 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).

105. Delphinidin has effects on enzymes and other biochemical parameters. It has been shown to inhibit aldoreductase in the lens of rats (Varma and Kinoshita 1976 cited in IPCS 2003). Delphinidin-3-glycoside extracted from grapes has been found to increase the activity of alpha glucan phosphorylase and glutamic acid dicarboxylase but inhibit glycerol dehydrogenase, malate dehydrogenase and hexokinase (Carpenter et al. 1967 cited in IPCS 2003).

106. In a special study on pharmacology short-term improvements in visual acuity and darkness adaptation have been reported in humans after receiving oral doses of up to 700mg of the anthocyanins (Pourrat et al. 1967 cited in IPCS 2003).

107. In special studies on mutagenicity, delphinidin was inactive in the Ames assay system using 5 different strains of *Salmonella typhimurium* with and without activation (Brown and 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 will 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.

108. In special studies on teratogenicity, anthocyanin glycosides extracted from currants, blueberries and elderberries were reported not to be 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). LD_{50} values for oral administration of this extract was reported to be 25 and 20 g/kg of body weight for mice and rats respectively (IPCS 2003). (Teratogens are agents that raise the incidence of congenital malformations).

Section 2.4 Toxicity and Allergenicity of GM Carnation (the GMO)

109. GM carnations have been commercially available since 1995. There have been no reports of any adverse effects of GM carnation since their commercial release. This is in the context of the production of approximately 15 million flowers since commercial production began.

SECTION 3 CONCLUSIONS REGARDING TOXICITY AND ALLERGENICITY

110. The risk of GM carnation being toxic or allergenic to humans is considered to be very low because:

- Humans are commonly exposed to and ingest delphinidins at similar or greater concentrations than are found in GM carnation without adverse consequences and toxicity studies of delphinidins and anthocyanins using mammalian models indicate very low levels of toxicity;
- No differences were found in the biochemical profiles of GM and conventional carnation as revealed by chromatography studies;
- Proteins related to the introduced proteins are common in edible plants and no sequence homologues for the inserted genes (F3’5’H, DFR, and SuRB) were found with known toxins or allergens;

- Like non-GM carnation, GM carnation pollen is produced in very low quantities contains no delphinidins, and is not wind-dispersed so has limited potential to be aeroallergenic; and
- There have been no reports of adverse effects on human health and safety as a result of commercial release of GM carnation.

111. The licence holder will be required to report any adverse effects on human health and safety (for example allergic reactions as a result of occupational exposure to the carnation) or to the environment.

APPENDIX 3 TOXICITY/PATHOGENICITY TO OTHER ORGANISMS

112. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. In this part of the document, risks posed by the dealing to the environment were considered in relation to the potential toxicity of the GMO to organisms other than humans.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR PATHOGENICITY HAZARD

113. The possibility was considered that GM carnation may be harmful to other organisms. GM carnation differs from cultivated carnation in the expression of two additional proteins in the anthocyanin biosynthetic pathway: flavonoid 3', 5' hydroxylase and dihydroflavonol 4-reductase; and a protein for herbicide tolerance: acetolactate synthase.

114. Hazards to the environment could occur if novel gene products expressed in the genetically modified carnation were toxic. If GM carnation is toxic for other organisms, the potential hazards would most likely have adverse impacts on:

- Invertebrates, including insects;
- Microbial organisms, particularly soil microorganisms, with direct impact on the growth of flower crops on farms,

115. It should be noted that during cultivation of non-GM carnation, growers typically eliminate as many pests of the flowers as possible using chemical sprays and physical containment. Exposure of mammals, birds, and insects to conventional carnation is limited because it is a horticultural crop that is grown intensively. The growing environment is highly managed as unblemished flowers fetch the highest price. Exposure of the above organisms to GM carnation would be similarly limited.

SECTION 2 LIKELIHOOD OF THE TOXICITY OR PATHOGENICITY HAZARD OCCURRING

116. In assessing the likelihood of adverse impacts due to toxicity of GM carnation, a number of factors were considered including:

- information about the likely routes of exposure to GM carnation and the introduced protein products, for example through direct contact with the flowers or through contact with soil in which the flowers are grown;
- the toxicity of non-GM carnation (refer to Appendix 2);
- the toxicity of the new protein products expressed in GM carnation (refer to Appendix 2); and
- other information relating to the toxicity of GM carnation for particular species, including mammals (refer to Appendix 2), other plants, invertebrates, and soil microorganisms.

117. It should be noted that since its commercial release in 1994/95, there have been no reported adverse toxic effects on other organisms through exposure to GM carnation. No

adverse effects of anthocyanins have been detected in studies testing for mutagenicity (*Salmonella*), teratogenicity (rats, mice, or rabbits), or toxicity (rats) (see Appendix 2 for details). Furthermore, proteins related to the ones that have been introduced to carnation are common in edible plants that are often eaten by organisms other than humans including insects.

118. Experiments on the potential toxicity of GM carnation modified with vectors pCGP1470 or pCGP1991 were carried out using seed germination and plant growth tests as direct bioassays, supplemented with direct counts of micro-organisms and biochemical analysis of secondary compounds.

119. Soil from around the root zone of several GM carnation lines and from controls was used to germinate and grow Chinese cabbage seed. There was no significant difference in the % germination, root length, shoot length, and shoot fresh weight between soil taken from the root zone of the GM lines and the controls.

120. Leaves from controls and GM carnation were frozen in liquid nitrogen and then mixed with soil before germinating Chinese cabbage seed in the soil. There was no significant difference in the % germination, root length, and shoot fresh weight between the GM lines and the controls.

121. Petal homogenates from each of the GM carnation lines and from non-GM carnation were applied to two plant bioassay systems to determine if the homogenates had herbicidal activity. Surface sterilised lettuce seeds were placed onto filter paper and a diluted (1:20) petal homogenate applied. The control had only water applied to the seeds. There were no inhibitory effects of petal homogenates on % germination of lettuce seed. The control, conventional carnation and transgenic carnation lines had between 97% and 100% germination. There was no difference found in shoot length at day six between any of the lines. Seeds treated with homogenates of Moonvista petal had slightly longer radicles at day 6 than any of the other lines but as the experiment finished it is difficult to put any meaningful interpretation on this result.

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

123. Finally, experiments have been carried out to determine whether there are any differences in the biochemical profile between GM carnation and their parental variety. Phenolic acids were extracted from leaves and roots, and volatile gases produced from the flowers were analysed by chromatography. GM carnation and the controls produced very similar chemical profiles indicating their biochemical similarity.

SECTION 3 CONCLUSIONS REGARDING TOXICITY AND PATHOGENICITY

124. It is considered that the risk of GM carnation being toxic to non-target organisms is very low because:

- Levels of delphinidin in GM carnation are similar to a range of delphinidin-producing plants, and toxicity studies of delphinidins and anthocyanins using mammalian models indicate very low levels of toxicity;

- No differences were found in the biochemical profiles of GM and conventional carnation as revealed by chromatography studies.
- Proteins related to the introduced proteins are common in edible plants and no sequence homologues for the inserted genes (F3'5'H, DFR, and SuRB) were found with known toxins;
- No reports of adverse toxicity have been found;
- No toxic effects were seen when Chinese cabbage plants were germinated and grown in soil from the root zone of GM and conventional carnation;
- No toxic effects of GM and conventional carnation petal homogenates were found as measured by lettuce seed germination and shoot length; and
- No differences were found in the quantities of bacteria and fungal spores in soil taken from around GM and conventional carnation.

APPENDIX 4 WEEDINESS

125. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. In this part of the document, risks posed by the dealing to the environment were considered in relation to the potential for the GMO to become a weed.

126. There are numerous definitions of weeds including 'a plant growing where it should not be'. Weeds become a problem to the community when their presence or abundance interferes with the intended use of the land they occupy. Weeds are thought to share a number of life history characters that enable them to rapidly colonise and persist in ecosystems, particularly those that are regularly disturbed. These characteristics include:

- The ability to germinate, survive, and reproduce under a wide range of environmental conditions;
- Long-lived seed with extended dormancy periods;
- Rapid seedling growth;
- Rapid growth to reproductive stage;
- Long continuous seed production;
- The ability to self pollinate but are not exclusively autogamous;
- Use of unspecialized pollinators or wind when outcrossing;
- High seed output under favourable conditions;
- Special adaptations for long distance and short distance dispersal; and
- Being good competitors (Baker 1965, 1974).

127. From analysis of global data sets it has been found that agricultural weeds tend to be herbaceous, rapidly reproducing, abiotically dispersed species, whereas plants that are most likely to become invaders of native ecosystems tend to be primarily aquatic or semi-aquatic, grasses, nitrogen-fixers, climbers, and clonal trees (Daehler 1998).

SECTION 1 NATURE OF THE WEEDINESS HAZARD

128. The possibility was considered that GM carnation might have the potential to be harmful to the environment, because of an increased potential for weediness either as a direct result of genetic modification or as a result of pleiotropic effects (i.e. a single gene responsible for a number of distinct and seemingly unrelated phenotypic effects). This could result from changes in life history characters such as increased fitness due to increased fecundity of GM carnation relative to cultivated non-GM carnation.

SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

129. In assessing the likelihood of adverse impacts due to weediness of GM carnation on human health and safety and the environment, the following factors were considered including;

- the inherent weediness of cultivated carnation;

- the weediness and selective advantage of GM carnation;
- the distribution of GM carnation and other *Dianthus*; and
- weeds of the family Caryophyllaceae.

Section 2.1 Inherent weediness of cultivated carnation

130. According to Tutin et al. (1993) *D. caryophyllus* is 'apparently not known wild except perhaps in some Mediterranean countries'. From recent Floras (databases describing the plants of a region or regions), carnation's natural distribution appears to be restricted to the Mediterranean regions of Greece, Italy, Sicily, and Sardina.

131. Carnation is not known outside its native habitat except as a cultivated plant. Carnation is grown in many countries including in Europe, Israel, Japan, South America and Australia, and sold more widely including the USA and Canada. It is not a weed or an invasive species in any of these countries, nor are there any records of it being a pest species.

132. Cultivated carnation shares few life history strategies with plants that are classed as weeds or invasive species. It does not reproduce rapidly, is not abiotically dispersed, and is not a nitrogen-fixer, climber, or clonal. Also, as a result of its long history of cultivation, carnation generally does not produce much pollen and consequently seed set is low or absent (Galbally and Galbally 1997). Although cultivation of carnation is via vegetative reproduction, carnation does not naturally reproduce asexually.

Section 2.2 Weediness and selective advantage of GM carnation

EFFECT OF THE DFR AND F3'5'H GENES ON WEEDINESS POTENTIAL

133. Carnation has been modified to express proteins in the anthocyanin biosynthetic pathway that are essential for the formation of blue pigment in the flowers, namely delphinidin. Neither the DFR gene or the F3'5'H gene or their combined effects (i.e. a change in flower colour) is associated with any life history or fitness characters. On this basis it is highly unlikely that there would be any selective advantage of GM carnation over non-GM carnation.

134. Florigene has conducted trials to examine aspects of fitness of GM carnation compared to non-GM carnation. These include assessments of pollen viability as measured by staining in acetocarmine and percentage pollen germination for GM and non-GM carnation lines.

135. Although 90% of Moonshadow (11363) flowers had anthers, none of the pollen germinated when placed on a pollen germination medium. The morphology of anthers in Moonshadow is different to the standard varieties in that they are petaloid in appearance.

136. In trials using Moonlite (123.2.38), only one anther was found in 20 flowers and none of the pollen germinated. Pollen viability as assessed by acetocarmine staining was 40% compared to 66% in control lines. For Moonvista (123.8.8) slightly higher numbers of flowers contained anthers compared to the control, but there was no difference in the either the number of anthers per anther-bearing plant or in the percentage pollen viability (approx 70%) as measured by acetocarmine staining, or the percentage pollen germination (approx 15%). Moonshade (line 123.2.2) showed a similar pattern to Moonvista. There were slightly more flowers with anthers and with viable anthers in the GM carnation compared to the

control, but no significant difference between the two groups in their percentage pollen viability (approx 70%) and percentage pollen germination (approx 7%). It should be noted that the numbers of anthers is variable and some trials have shown fewer anthers in the GM lines compared to a control.

137. Florigene has deliberately tried to cross-pollinate GM carnation with the objective of establishing whether viable seed could be produced. Both transgenic and non-GM carnations (6 lines in total) were used in a reciprocal pollination experiment, including two of the transgenic lines (123.1.36 and 123.2.38) for commercial release. In each experiment (12 in total) irrespective of whether the transgenic line was the recipient or the donor, no seed was set and in many cases a shrunken receptacle was observed.

138. Florigene has measured and compared the size of the reproductive organs of GM carnation (line 123.2.2) and non-GM carnation. Summary results are given in Table A4.1 below. The findings show that there is no change in the size of the reproductive organs as a result of genetic modification (for line 123.2.2).

Table A4.1. Measurements of reproductive organs for non-GM carnation and transgenic Moonshade (123.2.2).

Reproductive Organ		**Non-GM**	**123.2.2**
No. of stamens	Flowers sampled Range **Mean**	20 0-8 **4.8**	20 1-7 **3.65**
Stamen length (cm)	Sample number Range **Mean**	20 1.6-3.1 **2.21**	20 1.5-3 **2.21**
Number of styles	Flowers sampled Range **Mean**	20 4 **4**	20 3-4 **3.65**
Style length (cm)	Sample number Range **Mean**	20 1.7-3.1 **2.25**	20 1.4-3 **2.23**
Style width (mm)	Sample number Range **Mean**	20 0.7-1.2 **1.37**	20 0.7-2 **1.01**
Anther length (mm)	Sample number Range **Mean**	13 3.0-4.1 **3.6**	20 2.2-4.4 **3.7**
Anther width (mm)	Sample number Range **Mean**	13 1-3 **1.4**	20 0.8-1.6 **1.2**

EFFECT OF THE SELECTABLE MARKER GENE ON WEEDINESS POTENTIAL

139. The acetolactate synthase (ALS) protein could only confer a selective advantage to GM carnation plants if these plants were established in areas where ALS inhibiting herbicides were in use. These herbicides are not used in the carnation industry but are used to control annual and perennial grasses and broad-leaved weeds mainly in cereal, legume and cotton crops. The expression of the SuRB gene is highly unlikely to confer any selective advantage to GM carnation in relation to weediness. As discussed previously carnations are not found in the environment in Australia, existing exclusively as managed cultigens.

Section 2.3 Distribution of GM carnation and other *Dianthus* species

140. The dealings include propagation of GM carnation at a single location (at Monbulk) in Victoria, growth of GM plants to flowering stage at between 3 and 6 locations in Victoria, South Australia, Queensland, and Western Australia, and distribution of cut flowers and whole plant retail throughout Australia.

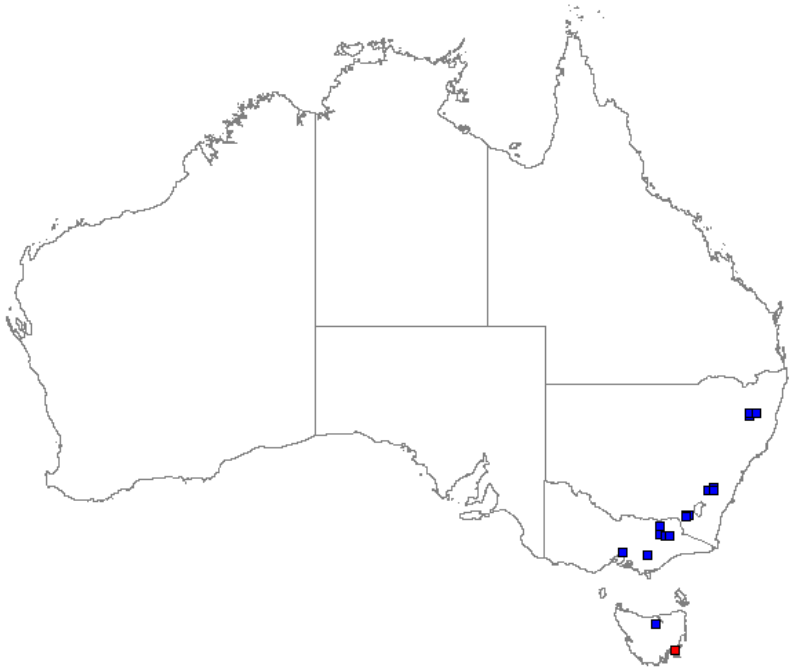

Figure A4.1 Map of Australia showing herbarium records for *D. armeria* (24 records) and *D. plumarius* (1 record in SE Tasmania).

141. Species of the genus *Dianthus* are not classed as noxious or invasive weeds. There are only three listed species in this genus that are weeds in Australia: *Dianthus*: *D. armeria*, *D. barbatus* (Lazarides et al. 1997), and *D. plumarius* (Groves 1998). Figure A4.1 shows the distributions of *D. armeria* and *D. plumarius* in Australia.

142. Based on collections deposited at herbaria in each of the States and Territories, *Dianthus armeria* (Deptford Pink) is a garden escapee and occurs in Victoria, NSW, and Tasmania. There is only one record of *D. plumarius* in SE Tasmania, although the first

record of its occurrence was at Dunalley Beach in NE Tasmania (Groves 1998). *D. barbatus* is listed as a weed in NSW (Lazarides et al. 1997) (Figure A4.2).

Figure A4.2. Map of New South Wales showing the distribution of *Dianthus barbatus* (Royal Botanic Gardens Sydney (03/03/03). PlantNET - The Plant Information Network System of Royal Botanic Gardens, Sydney (version 1.4). http://plantnet.rbgsyd.gov.au)

Section 2.4 Weeds of the Caryophyllaceae family in Australia

143. The parent organism, *Dianthus caryophyllus*, belongs to the Caryophyllaceae family. The Australian Plant Name Index lists 92 species in this family. Of these there are approximately 62 species in 22 genera that are considered to be weeds in Australia (see Table A4.2). A search of both the Noxious Weeds Database and the list of Weeds of National Significance revealed that only one of these 62 weed species of the Caryophyllaceae is considered noxious. *Silene vulgaris* or Bladder Campion is classed as a noxious weed in South Australia and a prohibited plant in Western Australia.

Table A4.2. Caryophyllaceae weed species in Australia, their habitat in Australia, and their distributions overseas. Light shaded grey indicate weeds of the genus *Dianthus;* darker shaded grey indicates declared noxious weed.

Name	Common Name	Location in Australia	Overseas Distribution
Agrostemma githago	Corn Cockle	Weed of cereal crops and waste ground, and garden escape	Mediterranean
Arenaria leptoclados	Lesser Thyme-leaved Sandwort	Disturbed ground	Europe, Western Asia
Arenaria serpyllifolia	Thyme-leaved Sandwort	Disturbed ground	Europe, temperate Asia, North America, LHI
Cerastium balearicum (*C. semidecandrum*)	Balearic Mouse-eared Chickweed	Lawns, disturbed and waste ground	Europe, Middle East, west Asia

Name	Common Name	Location in Australia	Overseas Distribution
C. comatum	Levantine Mouse-eared Chickweed	Disturbed ground	Eastern Greece and Turkey
C. diffusum	Sea Mouse-eared Chickweed	Disturbed ground	Europe
C. fontanum (*C. vulgare*)	Mouse-eared Chickweed	Lawns, waste ground, and cultivated areas	Europe. LHI
C. glomeratum	Mouse-eared Chickweed	Gardens in winter-spring, disturbed ground and pastures	LHI, Europe, cosmopolitan
C. pumilum	Curtis' Mouse-eared Chickweed	Disturbed ground	Europe, north Africa, west Asia
Corrigiola litoralis	Strapwort	Damp sandy places	Western, central and southern Europe, Africa, west Asia, Mediterranean
Dianthus armeria	Deptford Pink	Garden escape	Europe, Asia
D. barbatus	Sweet William	Garden escape	Europe, Asia
Drymaria cordata	Tropical Chickweed	-	Pantropics
Gypsophila paniculata	Baby's breath	-	Europe
G. tubulosa (*G. australis*)	Chalkwort	Disturbed often sandy soils	Asia Minor, temperate Europe, New Zealand
Herniaria cinerea (*H. hirsuta*)	Hairy Rupturewort	Weed of often poor, sandy or clay soils	Europe, Mediterranean, south west Asia
Lychnis alba (*Silene pratensis*)	Campion		
Lychnis chalcedonica	Maltese-cross Campion	Garden escape	West Asia
Lychnis coronaria	Rose Campion	Garden escape	Mediterranean, Middle East
Minuartia mediterranea	Slender Sandwort	Weed of sandy, often coastal soils	Europe, Mediterranean
Moenchia erecta	Erect Chickweed	Disturbed soils	Europe
Paronychia argentea	Whitlowwort	Weed of dry places	Southern Europe

Name	Common Name	Location in Australia	Overseas Distribution
P. brasiliana	Brazilian whitlow	Waste and cultivated land	South America, South Africa, Norfolk Island
P. franciscana	Whitlowwort	Waste and disturbed ground	Chile
Petrorhagia nanteuilii	Proliferous Pink	Damp disturbed and waste ground, and sometimes native grassland	Mediterranean, western Europe
P. velutina	Velvet Pink	Higher rainfall areas	Mediterranean, Europe
Polycarpon tetraphyllum	Four-leaved allseed	Weed of lawns, gardens, and habitation	LHI, Europe, Mediterranean
Sagina apetala	Annual Pearlwort	Lawns, cultivation, waste ground, and stone and brick work	LHI, Europe, North Africa, west Asia, South America
S. maritima	Sea Pearlwort	Coastal southern Australia, often on sandy and rocky areas	Coastal Europe, North Africa
S. procumbens	Spreading Pearlwort	Damp places, lawns, cultivation and waste ground	Europe, Asia, North America, NZ
Saponaria calabrica	Adriatic Soapwort	Only one record in 1899 as naturalised	Mediterranean
S. officinalis	Soapwort	Waste ground and stream fringes	Europe, west Asia
Scleranthus annuis	Knawel	-	Europe, north Africa, Asia
S. apetala	Mallee Catchfly	-	South west Europe, Mediterranean
S. armeria	Sweet William Campion	-	Europe
S. atocioides (*S. schafta*)	Turkish Catchfly	-	Europe
S. conica	Striated Catchfly	-	Eurasia
S. dichotoma	Two-branched Catchfly	-	Europe, west Asia
S. dioica	Red Campion	Garden escape	Europe
S. gallica	French Catchfly	-	LHI, Europe, west Asia

Name	Common Name	Location in Australia	Overseas Distribution
S. latifolia	White Campion	-	Europe, west Asia
S. longicaulis	Portuguese Catchfly	-	Spain, Portugal
S. nocturna	Mediterranean Catchfly	-	Mediterranean
S. pratensis (*S. alba*)	White Campion	Disturbed ground	Europe, west Asia
S. pseudoatocion	North African Catchfly	-	North Africa
S. tridentata	Spanish Catchfly	-	Spain, north Africa
S. uniflora (*S. maritima*)	Sea Campion	Disturbed and salt-affected soils	West and north west European coasts, southern Europe
S. vulgaris	Bladder Campion	Pastures and crops	Europe, west Asia, Mediterranean, Africa, America, Indonesia, New Zealand
Spergula arvensis	Corn Spurrey	Disturbed ground	Europe, north Asia, Africa
S. pentandra	Five-anther Spurrey	Disturbed ground	Europe
Spergularia bocconii	Bocconii's Sandspurrey	-	Western Europe, Mediterranean, New Zealand
S. diandra	Lesser Sandspurrey	-	Mediterranean, southern Europe, SE Russia, Asia Minor, Middle East
S. levis	Sandspurrey	-	South America
S. marina (*S. salina*)	Salt Sandspurrey	-	Europe, northern temperate New Zealand
S. media	Coast Sandspurrey	-	Eurasia, New Zealand
S. rubra	Sandspurrey	Garden escape	Europe
Stellaria graminea	Starwort	-	Europe, Asia

Name	Common Name	Location in Australia	Overseas Distribution
S. media	Chickweed	Weed of gardens, habitation, cultivation and pasture	LHI, Europe, Northern Hemisphere
S. pallida	Lesser Chickweed	-	Europe
S. palustris	Swamp Starwort	-	Eurasia
S. pungens	Pungent Starwort	-	-
Vaccaria hispanica	Bladder soapwort	Wheat	Europe, west Asia

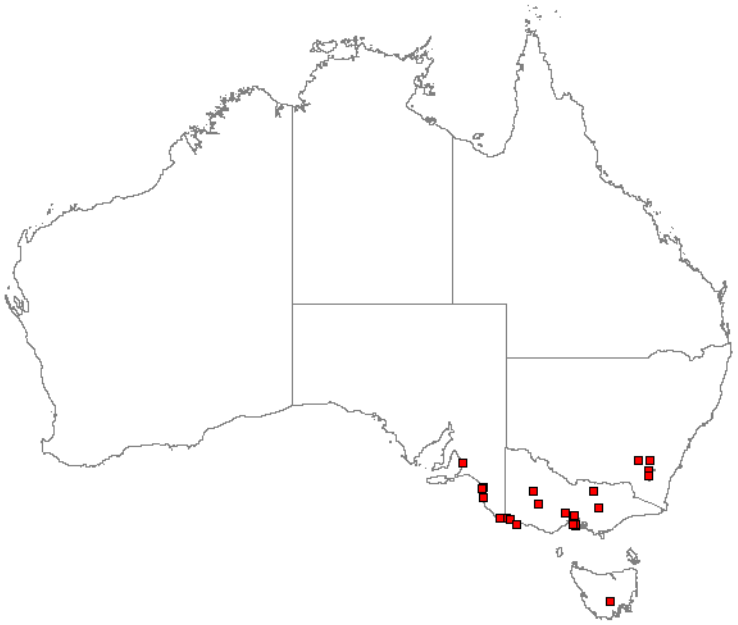

Figure A4.3 Distribution of the noxious weed *Silene vulgaris* in Australia.

144. Figure A4.3 shows the distribution of *Silene vulgaris* in Australia. There is some overlap in the distribution of *S. vulgaris* and where *D. caryophyllus* is grown. However, there is no evidence to indicate that these species are reproductively compatible, therefore there is no risk of any weediness characters being passed on to *D. caryophyllus*. Furthermore, although the distributions of *S. vulgaris* and *D. armeria* and *D. barbatus* overlap to some extent, there are no records of hybridisation between *S. vulgaris* and *Dianthus* species and no reports of invasiveness of either *D. armeria* or *D. barbatus*.

SECTION 3 CONCLUSIONS REGARDING WEEDINESS

145. It is concluded that there is negligible or effectively zero risk of GM carnation establishing as weed in Australia because:

- It does not share any life history characters with weedy species and the introduced proteins will not change these characters;
- The presence of the SuRB gene will only confer a selective advantage in those environments where weeds are controlled by ALS inhibiting herbicides and these herbicides are not used in the carnation industry and carnations exist exclusively as a managed cultigen;
- It has an extremely low potential for dispersal by natural means as pollen viability is low and seed set has never occurred;
- It does not spread by asexual reproduction without intervention; and
- Has never been found as a weed in any of the countries that it is cultivated in, including Australia;

APPENDIX 5 ENVIRONMENTAL SAFETY — TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

146. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. In this part of the document, risks posed by the dealing to the environment were considered in relation to the potential for the introduced genes to transfer from the GMO to other organisms.

147. In general terms, the types of hazards that might result from transfer of the genes introduced into GM carnation to other organisms differ depending on the recipient organism. Theoretically, transfer of the novel genes (and the vector sequences) to plants may result in herbicide tolerant or colour modified plants. Transfer of the novel genes (and vector sequences) to microorganisms may result in organisms that are able to express acetolactate synthase and/or tetracycline resistance. The consequences of either of these scenarios may include the potential to compete with naturalised or native flora thereby reducing biodiversity or disrupting ecosystems.

148. The potential hazards are addressed in the following sections, with respect to:

- other plants (Section 1 of this Appendix); and
- other organisms (Section 2 of this Appendix).

SECTION 1 TRANSFER OF INTRODUCED GENES TO OTHER PLANTS

Section 1.1 Nature of the gene transfer hazard

1.1.1 TRANSFER OF GENES TO CULTIVATED CARNATION

149. Transfer of the introduced genes or regulatory sequences to cultivated non-GM carnation plants would present the same hazards, and have the same potential impacts as the presence of the genes in GM carnation. These risks are considered in Appendices 2 - 4. If such a transfer occurred, it would increase the possibility that the novel genes could spread in the environment, with flow-on impacts depending on the nature of the gene and the species to which it transfers.

1.1.2 TRANSFER OF GENES TO OTHER DIANTHUS SPECIES AND OTHER PLANT SPECIES

150. Transfer of the introduced genes or regulatory sequences into other plant species, in particular to native flora, may have adverse effects on biodiversity if the recipient plants gained a selective advantage and were better competitors than other plants. Other potential hazards specific to the transferred gene sequences are as follows:

- F3’5’H and DFR (Anthocyanidin biosynthetic genes):

  If these genes were transferred modifications to flower colour of the recipient plant species may result. This may change the type of pollinators or predators of the plants, and perhaps result in altered fitness. If relative fitness was increased these plants containing the novel proteins may increase in number.

- SuRB gene:

  If this gene were transferred it may result in herbicide tolerance in recipient plant species. This may result in selective advantage for recipient plants if ALS inhibiting herbicides were used to control those plants.

- CaMV 35S promoter and other regulatory sequences:

  If gene transfer did occur, there may be unintended or unexpected effects if the introduced regulatory sequences altered the expression of endogenous plant genes. If such perturbation of normal plant gene expression did occur, the impact would depend on the phenotype.

  Some of these regulatory sequences are derived from plant pathogens (cauliflower mosaic virus, *Agrobacterium tumefaciens*). The possibility has been considered as to whether they might have pathogenic properties. Other regulatory sequences are derived from flowering plants. The possible effects of gene transfer of these sequences have also been considered.

151. Florigene found that a small portion of the plasmid vector was transferred into the carnation genome with the genes of interest and the selectable marker in two of the GM carnation lines (123.2.38 and 123.2.2). A risk assessment of the entire plasmid vector was undertaken so that all potential hazards were considered. It should be noted that two steps would need to occur before there was any potential hazard of the plasmid backbone being present in the carnation genome. Firstly the transfer into the carnation genome would have to occur and secondly the vector sequences would have to be transferred from the carnation genome into other plants or microorganisms.

152. The plasmid vector contains a tetracycline resistance gene complex and two origins of replication (see Appendix 1 for full details). The nature of the gene transfer to plants is considered below, while the risk assessment of the transfer to organisms other than plants is considered in Section 2 of this appendix.

- Tetracycline resistance gene from the plasmid vector (pCGP1470)

  If transfer of the tetracycline resistance gene complex from the plasmid vector pCGP1470 occurred there may be transfer of tetracycline resistance genes to the recipient organism(s). Even if transfer occurred proteins conferring resistance to tetracycline antibiotics are unlikely to be expressed in plants since the regulatory genes work best in prokaryotes and very rarely in eukaryotes. The impact of such a transfer has been considered.

- Origins of replication from the plasmid vector (pCGP1470)

  The two origins of replication (*ori)* on the plasmid vector allow for replication of the plasmid in bacteria. They enable replication in both *E. coli* (specific host) and *Agrobacterium*. The ability of the plasmid to replicate in *Agrobacterium* indicates its potential to replicate in a broad bacterial host range. The possible effects of gene transfer of the origins of replication have been considered.

Section 1.2 Likelihood of the gene transfer hazard occurring

1.2.1 TRANSFER OF GENES TO OTHER CULTIVATED CARNATION

153. Carnation has been cultivated for over 2000 years and new varieties have been developed mainly by the selection of desirable individuals from inter and intraspecific crosses. For example, hybrids were produced between *D. caryophyllus* and other *Dianthus* species as early as 1717, and a large number of *Dianthus* species and cultivars are sexually compatible. *Dianthus* species are obligate outcrossers because they are protandrous (i.e. the anthers and pollen mature before the pistils) thereby preventing self-pollination.

154. In the wild, cross-pollination of carnation relies on insect pollinators. There are no known reports of insect pollinators of *D. caryophyllus* either in Australia or elsewhere. However, pollination is likely to be effected by lepidopteran pollinators since they are the only insects with probosci long enough to reach the nectaries which are situated at the base of the flower in all *Dianthus* species.

155. Lepidopterans of the genera *Macroglossum, Plusia, Pieris, Hesperia, Aphantopus, Aporia, Cyaniris, Ochlodes, Mesoacidalia, Polyommatus, Thymelicus* are recorded as pollinators of other *Dianthus* species. Of these only *Macroglossum, Plusia* and *Pieris* occur in Australia, and according to the applicant *Macroglossum* is recorded as pollinating *D. barbatus*.

156. *Pierus rapae* (family Pierinae) is an introduced lepidopteran and occurs in the south-east and south-west of Australia including Tasmania. The larvae damage cruciferous plants (e.g.mustard, radish, turnip etc). *Plusia argentifera* and *P. chalcites,* in the family Plusinae, are pests of dicotyledonous plants. Moths of the genus *Macroglossum* pollinate a number of different *Dianthus* species. This genus belongs to the Philampelinae family and there are 14 species in this family, most of which occur in northern Australia in the tropics. Members of this family have a highly developed proboscis that is often long (Britton et al. 1979). It should be noted that none of the propagators or growers has noticed lepidopterans in their carnation crops.

157. In a horticultural setting, pollination between *Dianthus* species rarely occurs without human intervention. This is not only because the crosses can be controlled but also because with continual breeding of carnation many cultivars have become infertile. Carnation generally produces only small quantities of pollen and its quantity and quality varies according to cultivar and species (Kho and Baër 1973, Galbally and Galbally 1997). The pollen of carnation is heavy and sticky, is not wind-dispersed, and has low viability (percentage germination for some lines is less than 10%).

1.2.2 TRANSFER OF GENES TO OTHER DIANTHUS SPECIES AND OTHER PLANT SPECIES

158. There is an effectively zero probability of gene transfer to any other plant species even for the most closely related naturalised *Dianthus* species. This is due to the very low fertility of *D. caryophyllus*, the lack of sexual compatibility between *D. caryophyllus and D. plumarius* or *D. barbatus*, and the limited overlap of the geographic distributions of naturalised *Dianthus*.

1.2.3 CONSEQUENCES OF GENE TRANSFER TO PLANTS

159. The consequences of gene transfer from GM carnation to plants, including non-GM carnation, naturalised *Dianthus* species, and other plants including weeds of the Caryophyllaceae family, has been considered with respect to specific gene sequences, as follows:

- Anthocyanin biosynthetic genes (DFR and F3'5'H):

  It is possible that if these genes were transferred to non-GM carnation or other *Dianthus* species, the plants may have altered petal colour. However, petal colour is a complex phenotype that relies not only on the genes and resultant proteins, but also on optimal cellular and environmental conditions. There is no evidence to suggest that flower colour is linked to any life history traits and therefore even if transfer of these genes were to occur there is no evidence and no *a priori* reason to indicate that they would have a survival advantage. It is possible that altered petal colour may affect pollinators of the plants that use colour as a nectar guide. However, there is no evidence that a lack of pollinators is limiting for related plants in any area.

- Herbicide tolerance marker gene (SuRB):

  There would be no adverse impacts even if gene transfer occurred to non-GM carnation, since ALS inhibiting herbicides are not used for weed control in the carnation industry. It is unlikely that there would be adverse consequences if the SuRB gene were transferred to naturalized *Dianthus* or other weeds of the Caryophyllaceae since ALS inhibiting herbicides are not generally used for widescale control of weeds outside of agriculture.

- CaMV 35S promoter and other regulatory sequences:

  The likelihood of a hazard arising due to transfer of these sequences to other plants is remote. Plants are already exposed in nature to *Agrobacterium tumefaciens* and Cauliflower Mosaic Virus from which these sequences are derived. Although these regulatory sequences are derived from plant pathogens, they only represent a very small proportion of the pathogen genome. The sequences are not, in themselves, infectious or pathogenic. It should be noted that CaMV is already ubiquitous in the environment (Hodgson 2000).

- Tetracycline resistance gene complex

  The likelihood of a hazard arising due to the transfer of the tetracycline resistance gene complex is remote. Plants are already exposed in nature to tetracycline resistance genes because they originate from streptomycetes (Gram positive bacteria that resemble fungi) which are ubiquitous in the environment. Streptomycetes are common in soil, plant debris, dung, house dust, and many other habitats. Furthermore expression of the tetracycline resistant gene complex is driven by a bacterial promoter and so even if it was transferred to plants it would not be expressed.

- Origins of replication

  The plasmid contains two origins of replication (*ori*), one for replication in *E. coli* and one for replication in *Agrobacterium tumefaciens*. Plasmids with rolling circle modes of replication are unlikely to be able to either mulitply or initiate DNA synthesis once integrated into the plant genome. There is no additional risk of gene transfer of these origins of replication than is already present due to the presence of these bacteria in the environment.

Section 1.3 Conclusions regarding gene transfer to other plants

160. It is considered that the likelihood of any gene transfer from GM carnation to non-GM cultivated carnation is low, and the overall risk posed by such gene transfer is negligible, because:

- GM carnation like many non GM carnation cultivars are effectively sterile. Little pollen is produced and no seeds or seed pods have been found on GM carnation plants; and
- Gene transfer would not pose any additional risks to the low risks posed by GM carnation itself.

161. It is considered that the risk of gene transfer from GM carnation to naturalised *Dianthus* populations is negligible, because:

- *Dianthus caryophyllus* is not compatible with *D. plumarius* or *D. barbatus*. *D. barbatus* is not compatible with *D. armeria*. Crosses between *D. barbatus* and *D. armeria* produce ovular swelling but the ovules degenerate and no embryo is produced; and
- Geographical isolation of many of the populations of naturalized *Dianthus* species significantly decreases the likelihood of gene transfer.

162. It is considered that the risk of gene transfer from GM carnation to weed species of the Caryophyllaceae family is negligible, because:

- Geographical isolation and/or genetic incompatibility prevent the production of fertile hybrids;

163. It is considered that the risk of gene transfer from GM carnation to other plant species is negligible, because:

- There are no records of gene transfer from non-GM carnation to other plant species, genetic incompatibility would prevent the production of fertile hybrids and therefore it is highly improbable that there would be gene transfer from GM carnation to other plant species.

SECTION 2 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS (MICROORGANISMS AND ANIMALS)

Section 2.1 Nature of the gene transfer hazard

164. Transfer of the introduced genes to other organisms (microorganisms and animals) could only happen as a result of horizontal gene transfer (non-sexual, non-parental-to-offspring gene transfer). Three different mechanisms of horizontal gene transfer (HGT) in bacteria have been described: transduction, conjugation, and transformation (see Nielsen et al. 1998 for a full explanation).

165. Transduction is a bacterial cell-virus interaction that can mediate gene transfer between bacteria in the environment (e.g. on plant leaf surfaces, in soil or water). Viruses that function in more than one species are known, but viruses that function in both plants and bacteria, and thereby facilitate HGT from plants to bacteria have not been identified (Nielsen et al. 1998).

166. Conjugation is a mechanism of cell-to-cell interaction that can mediate gene transfer between bacteria in the environment (e.g. in soil, on plant surfaces, in water etc). Conjugation is known to occur frequently between compatible bacteria with the transferable genes usually residing on plasmids. Transfer of chromosomal genes is much less frequent, except for some high frequency recombination strains. Conjugative gene transfer has been regarded as the most frequently occurring mechanism of HGT between bacteria (Sprague 1991, Amabile-Cuevas and Chicurel 1993, Dreiseikelmann 1994, Souza and Equiarte 1997). However, mechanisms that support conjugative gene transfer from higher plants to bacteria (e.g. transposons that function in both plants and prokaryotes) are not known (Nielsen et al. 1998).

167. Gene transfer by transformation results in the uptake of naked DNA by bacteria, and has been shown to occur in the environments such as in soil, on plants, and in water. Most studies describing natural transformation have been conducted *in vitro* (Lorenz and Wackernagel 1994, Streips 1991) but often are of little relevance to most natural terrestrial environments.

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

- DFR and F3’5’H (anthocyanin biosynthetic genes):

  Transfer of the genes to animals (including humans) or microorganisms including bacteria and viruses would not present a hazard, since the genes coding for the proteins flavonoid 3’, 5’ hydroxylase, and dihydroflavonol 4-reductase are only two of many proteins in the anthocyanin biosynthetic pathway. This pathway is not present in organisms other than plants. Horizontal transfer to bacteria is also extremely unlikely (see Section 2.2.1 of this Appendix). Even if it did occur they are not toxic, and would not increase the virulence or pathogenicity of the recipient organism. Furthermore, there would be no positive selection acting to retain these genes and they would be lost. It is therefore considered that the transfer of these genes, if it occurred, would pose negligible risks to the environment.

- SuRB (herbicide tolerance gene):

  Even in the extremely unlikely event of transfer of the SuRB gene, it would not present a risk to the environment. Many organisms across a wide range of taxa, including flowering plants, mammals, yeast and bacteria, have either an acetolactate synthase gene or an acetolactate synthase-like gene (in the case of mammals) that shows substantial homology with nucleotide sequences from the SuRB gene of *N. tabacum*. BLAST searches (Basic Local Alignment Search Tool - http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) showed homologies of over 89% with species of the same family of *N. tabacum* (Solanaceae) over the entire gene sequence (over 2400 nucleotides), and substantial homologies with a broad range of taxa including other dicotyledons, monocotyledons and chlorophytes.

- CaMV 35S promoter and other regulatory sequences:

  If gene transfer occurred, there could be unintended or unexpected effects if the introduced regulatory sequences alter the expression of endogenous genes. If such perturbation of normal gene expression occurred, the impact would depend on the resultant phenotype. Some of these sequences are derived from plant pathogens (cauliflower mosaic virus, *Agrobacterium tumefaciens*). The possibility should be considered that they might have pathogenic properties.

  While Ho *et al*. (2000) have postulated that there are risks posed through recombination of the CaMV 35S promoter with the genomes of other viruses infecting the plants to create new viruses, or of integration of the CaMV 35S promoter into other species causing mutations, cancer or reactivation of dormant viruses, these claims have been challenged in the scientific literature (e.g. Hull et al. 2000; Morel & Tepfer 2000; Hodgson 2000b; Hodgson 2000c; Tepfer 2002).

  Although some of the regulatory sequences transferred to the plants are derived from pathogens, the pathogens only infect plants. In any case, the regulatory sequences only represent a very small proportion of the pathogen genome and are not, in themselves, infectious or pathogenic. It should be noted that CaMV is already ubiquitous in the environment (Hodgson 2000a).

- Tetracycline resistance gene complex

  Tetracycline resistance genes are very common in a broad range of bacteria because of their natural occurrence in the environment and through the widespread and frequent use of these antibiotics in human therapy, veterinary medicine, and as livestock growth promoters. They are readily transferred in nature already, and therefore it is considered that there is no additional risk of transfer of tetracycline resistant genes that is not already present in the environment.

- Origins of replication (*ori*s)

  The types of *ori*s found in both *E. coli* and *A. tumefaciens* are common and ubiquitous in the environment. There is no additional risk associated with any possible transfer of these *ori*s over and above that already present due to the abundance of these bacteria in the environment.

Section 2.2 Likelihood of the gene transfer hazard occurring

169. The likelihood of genes transferring from carnation to other organisms has been considered below. In summary, the risk of transfer of the introduced genes from GM

carnation to humans or other animals, or microorganisms including bacteria and viruses is negligible.

170. Theoretically, horizontal gene transfer from GM carnation to other organisms, including humans, and microorganisms is possible, but it is extremely unlikely. This is because HGT does not appear to happen frequently as inferred from phylogenetic analyses, and because there are a number of possible barriers to horizontal gene transfer including temporal and spatial, transfer, establishment, expression and evolutionary barriers (Nielsen 1998). The main barriers to exchange of genes are probably transfer and establishment barriers. Also, barriers related to spatial and temporal localization of available DNA and competent bacterial cells are likely to constrain successful HGT.

171. The stability of released DNA in the terrestrial environment and competent bacteria are essential for transformation to occur successfully. Competence in bacteria is not usually constitutively expressed and bacterial cells that are transformable need to enter a physiologically regulated state of competence for the uptake of exogenous DNA (Lorenz and Wackernagel 1994).

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

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

2.2.1 GENE TRANSFER FROM PLANTS TO MICROORGANISMS

174. Horizontal gene transfer from plants to bacteria has not been experimentally demonstrated under natural conditions (Syvanen, 1999; Nielsen et al. 1997; Nielsen et al. 1998) and deliberate attempts to induce such transfers have so far failed (e.g. Schlüter et al. 1995; Coghlan, 2000). Transfer of plant DNA to bacteria has been demonstrated under highly artificial laboratory conditions (Mercer et al. 1999; Gebhard and Smalla, 1998; Nielsen et al., 1998), but even then only at a very low frequency. Phylogenetic comparison of the sequences of plant and bacterial genes suggests that horizontal gene transfer from plants to bacteria during evolutionary history has been extremely rare (Doolittle, 1999; Nielsen et al. 1998).

175. Horizontal gene transfer from plants to plant-associated fungi has been reported. Fungi are known to be transformable and uptake of DNA from the host plant has been claimed for

Plasmodiophora brassicae (Bryngelsson et al 1988, Buhariwalla and Mithen 1995). Also, the hygromycin gene from a genetically modified plant was reported to be taken up by *Aspergillus niger* (Hoffman et al. 1994). However, stable integration and inheritance of the plant DNA in the genome of these fungi has not been substantiated by experimental evidence (Nielsen et al. 1998).

176. There is a theoretical possibility of recombination between sequences that have been introduced into the GM carnation plant genome, and the genome of viruses that might infect carnation plants. This type of phenomenon has been observed once, and only between homologous sequences under conditions of selective pressure. In this example, regeneration of a defective virus (containing a deletion mutation in its coat protein) back to an infectious virus occurred by complementation of sequences transcribed from a viral coat gene introduced into a transgenic plant genome of (Greene and Allison, 1994, Teycheney and Tepfer, 1999).

Section 2.3 Conclusions regarding gene transfer to other organisms

177. The likelihood of gene transfer from plants to other organisms is considered negligible because:

- Limited probability of occurrence. The chance of interaction, uptake and integration of intact plant DNA by other organisms is extremely low, especially if it involves unrelated sequences (non-homologous recombination).
- Limited probability of persistence. The chance that any novel organism that does arise from gene transfer will survive, reproduce and have a selective advantage (competitiveness or fitness) is extremely low.
- Natural events of horizontal gene flow from plants to distantly related organisms are extremely rare.
- Experimental horizontal gene transfer has generally been achieved only with related gene sequences (homologous recombination) using high selective pressure and sensitive detection systems to identify very rare events.

178. Furthermore, any organism that acquires the novel genes is unlikely to pose any additional risks to human health and safety, or the environment, compared to the GM carnations.

APPENDIX 6 LICENCE CONDITIONS AND REASONS FOR SPECIFIC LICENEC CONDITIONS

PART 1 INTERPRETATION AND DEFINITIONS

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

Words importing a gender include any other gender.

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

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

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

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

In this licence:

'Carnation' means plants of the species *Dianthus caryophyllus.*

'GM' means genetically modified.

'GMO' means genetically modified organisms authorised for release by this licence.

'OGTR' means the Office of the Gene Technology Regulator.

PART 2 LICENCE CONDITIONS

The licence holder must comply with the conditions of this licence.

Section 1 General Conditions

Additional information to be given to the Regulator

1. 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.

(*Explanatory note: if the Licence holder observes or becomes aware of adverse effects these must be immediately reported to the Gene Technology Regulator, who will then vary the Licence conditions to protect the health and safety of people and the environment*).

Material Changes in circumstances

2. The licence holder must immediately, by notice in writing, inform the Regulator of:

   (a) any relevant conviction of the licence holder occurring after the commencement of this licence;
   (b) any revocation or suspension of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country, being a law relating to the health and safety of people or the environment;
   (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.

Remaining an Accredited organisation

3. The licence holder must, at all times, remain an accredited organisation and comply with any conditions of accreditation set out in the Guidelines for Accreditation of Organisations.

Changes to details

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

Section 2 Specific Conditions

Testing methodology

1. 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 GMO and any transferred genetically modified material that might be present in a recipient organism. The instrument must be provided within 30 days of this licence being issued.

Reporting

2.1 In addition to the requirements under General Condition 1, the licence holder must provide the Regulator with a written report within 90 days of each anniversary of this licence, in accordance with any Guidelines issued by the Regulator in relation to annual reporting. This report must include information on any adverse impacts on human health and safety or the environment, caused as a result of the GMO or viable material from the GMO.

2.2 The licence holder must keep written records of the names, site co-ordinates, addresses and contact telephone numbers of:

(a) all persons or entities who are given or sold the GMO by the licence holder for the purposes of propagating or growing the GMO; and

(b) all persons and entities who are wholesale distributors of the GMO and who Florigene receives royalties from.

These records must be included in the annual report and be made available to the Regulator on request.

2.3 The licence holder must keep written records of the number of plants propagated and grown each year. These records must be included in the annual report and be made available to the Regulator on request.

PART 3 REASONS FOR SPECIFIC LICENCE CONDITIONS

The reasons for inclusion of the specific licence conditions follow (with reference to the numbering of the conditions in the licence). As no significant risks to human health and safety and the environment were identified in relation to the ongoing release of the GM carnations in Australia, only minimal oversight licence conditions have been imposed.

Condition 1 This condition will enable the presence of the GMO and any transferred genetically modified material to be reliably detected.

Condition 2 The annual report is required for administrative and auditing purpose, but also includes information on any adverse impacts on human health and safety or the environment, caused as a result of the GMO or viable material from the GMO. This is in addition to requirements under the Act for all licence holders to inform the Regulator as soon as they become aware of any new information about risks to human health and safety and the environment, or of any unintended effects (general condition 1).

APPENDIX 7 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES

SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

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

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

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

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

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

184. Detailed information about the national regulatory system and the gene technology legislation is also available from the OGTR website (www.ogtr.gov.au)

SECTION 2 THE LICENCE APPLICATION

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

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

186. The application must also contain supporting information from the Institutional Biosafety Committee and additional information required for a GMO that is:

- a plant;
- a micro-organism (not living in or on animals and not a live vaccine);
- a micro-organism that lives in or on animals;
- a live vaccine for use in animals;
- a vertebrate animal;
- an aquatic organism;
- an invertebrate animal;
- to be used for biological control;
- to be used for bioremediation; and
- intended to be used as food for human or vertebrate animal consumption.

SECTION 3 THE INITIAL CONSULTATION PROCESSES

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

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

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

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

SECTION 4 THE EVALUATION PROCESSES

190. The risk assessment process was carried out in accordance with the *Act* and the *Regulations*, using the Risk Analysis Framework (the Framework) developed by the Regulator (available on the OGTR website). It also took into account the guidelines and risk assessment strategies used by related agencies both in Australia and overseas. The Framework was developed in consultation with the States and Territories, Commonwealth government agencies, GTTAC and the public. Its purpose is to provide general guidance to

applicants and evaluators and other stakeholders in identifying and assessing the risks posed by GMOs and in determining the measures necessary to manage any such risks.

191. In undertaking a risk assessment, the following were considered and analysed:

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

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

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

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

- any previous assessment, in Australia or overseas, in relation to allowing or approving dealings with the GMO;
- the potential of the GMO concerned to:
  - be harmful to other organisms;
  - adversely affect any ecosystems;
  - transfer genetic material to another organism;
  - spread, or persist, in the environment;
  - have, in comparison to related organisms, a selective advantage in the environment; and
  - be toxic, allergenic or pathogenic to other organisms.

- the short and long term when taking these factors into account.

SECTION 5 FURTHER CONSULTATION

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

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

196. Comments received in written submissions on this risk assessment and risk management plan were very important in shaping the final risk assessment and risk management plan and in informing the Regulator's final decision on an application.

197. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings can be managed so as to **protect human health and safety and the environment**.

SECTION 6 DECISION ON LICENCE

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

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

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

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

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

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

- limit the scope of the dealings;
- require documentation and record-keeping;

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

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

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

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