Risk Assessment and Risk Management Plan for

DIR 134

Commercial import and distribution of genetically modified carnation cut-flowers with altered flower colour

Applicant: International Flower Developments Pty Ltd

October 2015
Summary of the Risk Assessment and Risk Management Plan for Licence Application DIR 134

**Decision**
The Gene Technology Regulator (the Regulator) has decided to issue a licence for the Australia-wide commercial scale import and distribution of genetically modified (GM) carnation cut-flowers. The licence authorises import, transport and disposal of GM carnation cut-flowers, and the possession or supply of the GMOs during any of these activities.

A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that this commercial release poses negligible risks to human health and safety and the environment and so no specific risk treatment measures are proposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the licence.

**The application**

<table>
<thead>
<tr>
<th>Application number</th>
<th>DIR 134</th>
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<tr>
<td>Applicant</td>
<td>International Flower Developments Pty Ltd (IFD)</td>
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<tr>
<td>Project title</td>
<td>Commercial import and distribution of GM carnations with altered flower colour¹</td>
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<tr>
<td>Parent organism</td>
<td><em>Dianthus caryophyllus</em> (carnation)</td>
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| Introduced genes and modified traits | - *F3’5’H* (flavonoid 3’5’-hydroxylase) gene from pansy or petunia – altered flower colour  
- *DFR* (dihydroflavonol reductase) gene from petunia – altered flower colour  
- *Cytb5* (cytochrome b5) from petunia – altered flower colour  
- Partial gene sequence from carnation *DFR* gene – altered flower colour  
- *SuRB* (acetolactate synthase) gene from tobacco – herbicide tolerance selectable marker |
| Proposed locations | Australia-wide |
| Primary purpose    | Commercial import and distribution of GM carnation cut-flowers with altered flower colour |

¹ The title of the licence application submitted by IFD is “Commercial import of three colour-modified genetically modified carnation varieties; Moonaqua, Moonberry and Moonvelvet”.
The licence authorises IFD to import and distribute three colour altered genetically modified carnation varieties: Florigene® Moonaqua™, Florigene® Moonberry™ and Florigene® Moonvelvet™. These carnations have been genetically modified to produce mauve, purple or violet coloured flowers. The licence does not permit growing the GM carnations in Australia, or their use in food or feed.

These GMOs have not been imported into Australia before but have been commercialised and are grown in Ecuador and Colombia, and the flowers are authorised for import into North America, Canada, Japan, Malaysia, Singapore and the EU. Similar GM carnation lines with altered flower colour have been grown in Australia since 1995, and are authorised through inclusion on the GMO Register (Register 001/2004).

**Risk assessment**

The risk assessment concludes that there are negligible risks to the health and safety of people, or the environment, from the proposed release.

The risk assessment process considers how the genetic modification and activities conducted with the GMO might lead to harm to people or the environment. Risks were characterised in relation to both the seriousness and likelihood of harm, taking into account current scientific/technical knowledge, information in the application, relevant previous approvals and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short and long term were considered.

Credible pathways to potential harm that were considered included exposure of people or other organisms through contact with or ingestion of GM carnation flowers and spread and persistence of GM plants or hybrid offspring leading to increased toxicity or allergenicity in people or increased toxicity in other desirable organisms, reduced establishment of desirable plants and reduced biodiversity.

The principal reasons for the conclusion of negligible risks are:

- the widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity or evidence of harm from them
- there have been no adverse reports from similar previous releases of GM carnations
- the inability of the GM carnation cut flowers to spread and persist.

**Risk management plan**

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions under post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.
Chapter 3 Risk Management Plan

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Section 3 General risk management

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3.2 Testing methodology

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

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3.5 Monitoring for compliance

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4.1 Adverse effects reporting system

4.2 Requirement to monitor specific indicators of harm

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Section 5 Conclusions of the RARMP

References

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Appendix B Summary of advice from prescribed experts, agencies and authorities on the consultation RARMP

Appendix C Summary of submissions from the public on the consultation RARMP
### Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ALS</td>
<td>Acetolactate synthase</td>
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<tr>
<td>APVMA</td>
<td>Australian Pesticides and Veterinary Medicines Authority</td>
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<td>CaMV</td>
<td>Cauliflower mosaic virus</td>
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<td>Cyt b5</td>
<td>Cytochrome b5</td>
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<td>DIR</td>
<td>Dealings involving Intentional Release</td>
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<td>DFR</td>
<td>Dihydroflavonol reductase</td>
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<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>F3’5’H</td>
<td>Flavonoid 3’,5’-hydroxylase</td>
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<td>FSANZ</td>
<td>Food Standards Australia New Zealand</td>
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<td>GM</td>
<td>Genetically modified</td>
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<td>GMO</td>
<td>Genetically modified organism</td>
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<td>GTTAC</td>
<td>Gene Technology Technical Advisory Committee</td>
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<td>IFD</td>
<td>International Flower Developments Pty Ltd</td>
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<td>NLRD</td>
<td>Notifiable low risk dealings</td>
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<td>OGTR</td>
<td>Office of the Gene Technology Regulator</td>
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<td>PC2</td>
<td>Physical Containment level 2</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>RARMP</td>
<td>Risk Assessment and Risk Management Plan</td>
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<td>Regulations</td>
<td>Gene Technology Regulations 2001</td>
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<td>Regulator</td>
<td>Gene Technology Regulator</td>
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<td>SuRB</td>
<td>Sulfonylurea resistance</td>
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<td>TGA</td>
<td>Therapeutic Goods Administration</td>
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<td>the Act</td>
<td>The <em>Gene Technology Act 2000</em></td>
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Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the Gene Technology Act 2000 (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, comprise Australia’s national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

<table>
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<th>RISK ASSESSMENT CONTEXT</th>
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<td>LEGISLATIVE REQUIREMENTS</td>
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<td>(including Gene Technology Act and Regulations)</td>
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<td>RISK ANALYSIS FRAMEWORK</td>
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<td>OGTR OPERATIONAL POLICIES AND GUIDELINES</td>
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<td>PROPOSED DEALINGS</td>
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<td>Proposed activities involving the GMO</td>
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<td>Proposed limits of the release</td>
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<td>GMO</td>
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<td>Introduced genes (genotype)</td>
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<td>PARENT ORGANISM</td>
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<td>Origin and taxonomy</td>
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<td>Cultivation and use</td>
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<td>Biological characterisation</td>
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<td>Ecology</td>
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<td>RECEIVING ENVIRONMENT</td>
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<td>Environmental conditions</td>
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<td>Agronomic practices</td>
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<tr>
<td>Presence of related species</td>
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<td>Presence of similar genes</td>
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Figure 1 Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted with, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. This means that, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State...
and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, all Australian local councils and the Minister for the Environment. A summary of issues contained in submissions received is given in Appendix A.

6. Section 52 of the Act requires the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public. Advice from the prescribed experts, agencies and authorities for the second round of consultation, and how it was taken into account, is summarised in Appendix B. Four public submissions were received and their consideration is summarised in Appendix C.

7. The Risk Analysis Framework (OGTR 2013) explains the Regulator’s approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

8. Any dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme and Department of Agriculture. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

**Section 3 The proposed dealings**

9. International Flower Developments Pty Ltd (IFD) proposes to import and distribute carnations that have been genetically modified for altered flower colour. The three lines of GM carnations proposed for import and distribution are designated as Florigene® Moonaqua™ (Moonaqua), OECD unique identifier FLO-40689-6; Florigene® Moonberry™ (Moonberry), OECD unique identifier IFD-25958-3 and Florigene® Moonvelvet™ (Moonvelvet), OECD unique identifier IFD-26407-2.

10. The applicant is seeking approval for the distribution of cut flowers to occur Australia-wide. Cut GM carnation flowers would be imported in the same way as other GM and non-GM carnations. Once imported, the cut flowers would enter the retail chain in the floristry industry and be sold for ornamental purposes. There is no intention to grow these GMOs in Australia.

11. The dealings involved in the proposed intentional release are:
   a) importing the GMO
   b) transporting the GMO
   c) disposing of the GMO

and the possession, supply or use of the GMO for the purposes of, or in the course of, any of the above.

**Section 4 The parent organism**

12. The parent organism is carnation, Dianthus caryophyllus. Over 300 species of Dianthus are described, and they are commonly known as carnations or pinks (Galbally & Galbally 1997; Jurgens et al. 2003). Carnation is exotic to Australia, but has been grown commercially as a flower crop since 1954. The genus Dianthus contains several species that have been cultivated for hundreds of years for ornamental purposes (Ingwerson 1949). More detailed baseline information on carnation can be found in the document, The Biology of Dianthus.
caryophyllus L. (Carnation) (OGTR 2015), which was produced to inform the risk assessment process for licence applications involving GM carnations. This document is available on the OGTR website.

13. The main use of carnation is for decorative purposes, either as cut flowers or as an ornamental grown in gardens.

14. While not a food, carnation can be used as a garnish. The flower petals of older varieties have a strong smell of cloves and can be crystallised or used as a garnish in salads or for flavouring many foods including fruit, fruit salads, butter, lemonade, vinegars, conserves and syrups (Facciola 1990; Hughes 1993). Carnation petals are one of the ingredients that have been used to make the French liqueur, green chartreuse.

15. Carnation petals can be used in perfume (Pieroni et al. 2004). Modern floriculture carnations, however have little or no scent, and scent loss is often correlated with increased vase-life in cut flowers (Chandler & Brugliera 2011).

16. Carnation has been used in European traditional herbal medicine for coronary and nervous disorders (McGeorge & Hammett 2002) and previously used to treat fevers, although this practice is now obsolete (Bown 1995; Lim 2014). Carnation flowers are considered to be alexiteric (counteracting the effects of poison), antispasmodic (counteracting spasms of smooth muscle, usually in the gastrointestinal tract), cardiotonic (having a favourable effect on the heart), diaphoretic (promoting sweating) and nervine (acting therapeutically on the nerves) (Chopra et al. 1956). Compounds from carnation buds have exhibited in vitro antibacterial activity against several bacteria including Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus and Escherichia coli (Lim 2014). Furthermore, antiviral compounds have been isolated from the leaves and seeds of carnation (Lim 2014).

4.1 Weediness

17. Cultivated carnation is exotic to Australia.

18. Carnations have not been reported as a weed, invasive species or pest species in any of the countries where they are grown as floriculture crops, including Israel, Japan and parts of Europe and South America. Wild type Dianthus caryophyllus, i.e. forms that do not produce double flowers like the currently cultivated varieties, can be found in Greece, Italy, Sardinia and Sicily, where they may be native, and in France and Spain (Tutin & Walters 1993). In Australia, carnation itself has not been reported as a weed, nor is it closely related to any Weeds of National Significance (Department of Environment 2015).

4.1.1 Potential to cause harm

Adverse health effects on people or animals

19. Despite carnation having a long history of floriculture, there are few reports of occupational allergy within the floral industry and no reports of toxicity in humans. Dianthus species contain saponins in their leaves. These can cause minor, short-lived skin irritation upon dermal exposure and low toxicity when ingested (Stefanaki & Pitsios 2008; Vidal & Polo 1998). Contact dermatitis and respiratory allergy associated with carnation-handling generally develops following a latency period (Lamminpaa et al. 1996; Sanchez-Guerrero et al. 1999). Contact dermatitis has been observed in workers who had previously handled carnations for four (Stefanaki & Pitsios 2008) and eight years (Vidal & Polo 1998). Only one case of respiratory allergy without prior occupational exposure to carnation was found in the literature (Brinia et al. 2013).
20. Sensitivity to associated pests must be considered when an allergy to carnations is suspected. The two-spotted mite *Tetranychus urticae* is a parasite of the flowers and a reported allergen, and simultaneous allergies to both carnations and *T. urticae* have been observed (Cistero-Bahima et al. 2000; Sánchez-Fernández et al. 2004). A case of occupational asthma was shown to be caused by carnation in a patient sensitized previously to *T. urticae* (Cistero-Bahima et al. 2000).

21. Carnation petals and leaves are reportedly mildly toxic to dogs and cats, causing gastrointestinal upset if ingested (ASPCA 2015). Petals may also cause contact dermatitis in pets, but it is not known if it occurs spontaneously following exposure, or whether it develops following a latency period and repeated contact (ASPCA 2015). No toxicity or ill-effects were observed in mice fed extracts of carnation petals in an acute toxicity test (Chandler et al. 2013).

**Adverse environmental effects**

22. Carnation is a cultivated plant and only grown in highly managed areas: it is grown as a monoculture in horticulture and as a deliberately-planted ornamental in gardens. Carnation volunteers have not been observed outside their specific cultivation location. Therefore, carnation does not adversely affect any land use or native biodiversity. Carnation is not known to affect the quality of products or services in any land use.

23. Carnation is susceptible to a range of pathogens, such as *Fusarium* wilt, and insect pests such as the *Heliothis* caterpillar, aphids, thrips and mites. However, it is mainly grown in glasshouses or polytunnels, and the likelihood of it harbouring these pests and pathogens is low because plants are monitored and pesticides applied as required.

24. Carnation has no additional adverse effect on soil salinity or nutrient levels to that caused by other commonly cultivated flowers.

### 4.1.2 Potential for spread and persistence (invasiveness)

**Establishment and management of carnation volunteers**

25. *D. caryophyllus* (either cultivated varieties or wild-type) is not naturalised in Australia (Groves et al. 2003; Lazarides et al. 1997), despite having been commercially cultivated as a flower crop since 1954. This experience demonstrates that cultivated carnations have a limited ability to invade and establish in disturbed or undisturbed areas. There are three species of *Dianthus* listed as weeds in Australia: *D. armeria* (found in NSW, Vic, Tas), *D. barbatus* (NSW) and *D. plumaris* (Tas), but each species is mainly ornamental/cultivated and all are considered to be low risk environmental weeds (Lazarides et al. 1997; Rozefelds et al. 1999; NSW Royal Botanic Gardens and Domain Trust 2015; Groves et al. 2003). Carnation is not closely related to any Weeds of National Significance in Australia (Department of Environment 2015).

26. Any carnation volunteers found in urban or rural residential areas could be killed by manual removal, or by herbicide treatment. In horticultural areas, plants are grown in glasshouses or polytunnels, and any volunteers would be removed using the same methods.

**Reproduction of carnation**

27. Carnation is a perennial. Plants under horticulture conditions are grown for two to four years before being replaced. Cultivated carnation is rarely grown from seed, because its high heterozygosity reduces the reliability of offspring with the same phenotype. In cultivation, carnations are mainly reproduced by cuttings. However, no evidence has been found to suggest that carnation would reproduce vegetatively in the wild. Carnation does not produce organs...
such as stolons, rhizomes, root-borne shoots, tubers, bulbs, corms or runners and hence cannot easily disperse by vegetative propagation (OGTR 2015).

28. Within six months of planting, flowers develop and seed production can be initiated. When flowers are pollinated, up to 100 seeds can develop in each short-stalked capsule, although the average is 40 seeds (Sparnaaij & Beeger 1973). It takes at least four weeks after pollination before a mature seed is formed (Gatt et al. 1998; Sparnaaij & Beeger 1973). No published information on seed dormancy in nature has been found, however one report suggests that the seeds will remain viable for several years if stored in a cool and dry place (Sparnaaij & Beeger 1973).

29. Any carnations grown as ornamentals in gardens are unlikely to set seed owing to a highly modified flower morphology that makes natural pollination difficult. The reproductive organs of the flowers of cultivated carnation may be completely enclosed in the petals thus restricting access for potential pollinators, especially those without a long proboscis. Hence, in cultivated flowers, pollination is usually by manual methods.

30. Flowers for floristry are harvested when they are in tight bud or closed bud stage to ensure a satisfactory vase life. The flowers are then dry packed in sleeved bunches in cardboard boxes before being distributed. This process virtually eliminates the chance of flowers being pollinated under commercial growing conditions.

**Dispersal of carnation**

31. Carnation is deliberately spread by people and its seeds are sold for growing in gardens as ornamental plants. Although the seeds of carnation are small and might get caught in equipment and clothing, they are unlikely to be accidentally spread by humans as it requires considerable effort to produce seeds and as such they are treated as a desirable commodity.

32. There is no evidence that animals play a role in the dispersal of carnation seeds. Carnation seeds do not possess adaptations for dispersal via the fur or feathers of animals such as hooks or spines.

33. Dispersal of viable seed by water may be possible, e.g. through flooding or irrigation run-off; however, the seeds are not specifically adapted for water dispersal.

34. Wild-type carnation seed is spread by wind moving the seed heads to release the seeds (Bird 1994).

**4.2 Sexually compatible plants**

**4.2.1 Pollen dispersal and pollination**

35. Carnations are predominantly outcrossing because of the temporal separation of anther dehiscence and pistil receptivity: the stigma is not receptive to pollen grains until one week or more after anthesis. Floriculture carnations require hand pollination to set seed because they have dense petals which make it difficult for pollinators to access pollen, and because they are mostly grown in greenhouses or polytunnels that preclude access by potential pollinators (Bird 1994). Technicians remove petals to expose the reproductive parts of the flower, and then dip the stigma in pre-collected pollen from other carnation plants (Sparnaaij & Beeger 1973). The optimal temperature for pollen production in glasshouse plants is approximately 23°C, with temperatures lower than 17°C suppressing stamen production completely (Kho & Baer 1973).

36. Carnation pollen is not wind-dispersed as it is heavy and sticky. It has low viability (germination for some lines is less than 10%), although this is somewhat cultivar dependent (Kho & Baer 1973; Sparnaaij & Beeger 1973). Floriculture carnations do not produce much...
pollen. Selection has been for flower characteristics, and as a result of the widespread use of vegetative propagation, pollen production has not been important (Galbally & Galbally 1997). Seed set in currently cultivated carnations is low or absent compared with wild type carnation.

4.2.2 Intraspecific crosses

37. There is no information on natural intraspecific gene transfer of ornamental carnations in Australia. Most gene transfer is performed in the context of generating novel phenotypes for flower display.

38. Insect pollinators can contribute to gene transfer as they help outcrossing between individual plants. However, this is more relevant to wild-type *D. caryophyllus* rather than its domesticated cultivars as discussed in Section 4.2.1.

4.2.3 Interspecific and intergeneric crosses

39. The flowers we know as carnations are interspecific hybrids. The perpetual flowering carnations used globally in floriculture are descended from hybridisation between *D. caryophyllus* and *D. chinensis* (Galbally & Galbally 1997). Little is known about how readily species of *Dianthus* interbreed naturally, because most focus is on the cultivated varieties in ornamental or horticultural contexts (OGTR 2015).

40. Three species of *Dianthus* (namely *D. armeria*, *D. barbatus* and *D. plumarius*) are present as weeds in parts of eastern Australia (Lazarides et al. 1997; Rozefelds et al. 1999; Groves et al. 2003). Carnation (*D. caryophyllus*) has been recorded as hybridising with *D. barbatus*, but this was under experimental conditions only (Umiel et al. 1987). The likelihood of crossing with cultivated carnations is extremely low, due to pollination reasons outlined above.

41. There are 21 introduced genera in the family Caryophyllaceae present as weeds in Australia (NSW Royal Botanic Gardens and Domain Trust 2015). Within the Caryophyllaceae, the genus *Dianthus* is most closely related to the genera *Acanthophyllum*, *Gypsophila*, *Vaccaria*, *Petrorhagia* and *Saponaria* (Harbaugh et al. 2010; Greenberg & Donoghue 2011). Each of those genera, except *Acanthophyllum*, has been recorded as weedy in Australia (Lazarides et al. 1997; Rozefelds et al. 1999; Groves et al. 2003). However, most hybridisation in the Caryophyllaceae occurs within, rather than between, genera (Greenberg & Donoghue 2011). This means that the likelihood of gene transfer between carnations and species of related genera is low.

Section 5 The GM carnations

42. The carnations have been genetically modified to produce mauve, purple or violet coloured flowers by manipulating the anthocyanin biosynthesis pathway. Non-GM carnations do not contain the enzymes needed to produce delphinidin-3-glucosides, which are responsible for blue flower colours in other plants. In addition, the GM carnations contain the marker gene *SuRB* which results in tolerance to sulphonylurea-type herbicides.

5.1 Introduction to the anthocyanin biosynthesis pathway

43. In general, flower colour is attributed to the presence of carotenoids and anthocyanins. The carotenoids are responsible for yellow and orange colours. Three important types of coloured anthocyanins are pelargonidin-3-glucosides leading to orange, pink or brick red flower colour, cyanidin-3-glucosides leading to red or magenta flower colour and delphinidin-3-glucosides resulting in blue or purple flower colour (Zuker et al. 2002). Carnations do not
naturally have blue or mauve flowers, because they lack the part of the anthocyanin biosynthetic pathway that results in the production of blue pigments (Figure 2).

![Figure 2 The anthocyanin biosynthesis pathway (Source: IFD).](image)

Abbreviations; CHS – chalcone synthase; CHI – chalcone isomerase; F3H – flavanone 3β-hydroxylase; F3’H – flavonoid 3’-hydroxylase; F3’5’H – flavonoid 3’, 5’-hydroxylase; DFR – dihydroflavonol 4-reductase; ANS – anthocyanidin synthase; FLS – flavonol synthase; 3GT – UDP-glucose: anthocyanidin 3-O-glucosyltransferase.

44. The anthocyanin biosynthesis pathway is an intermediate of the phenylpropanoid pathway and an early critical enzyme is chalcone synthase, which catalyses the biosynthesis of 4, 2’, 4’, 6’-tetrahydroxychalcone. This compound is converted to naringenin by the enzyme chalcone isomerase.

45. Naringenin can be converted to dihydrokaempferol (DHK) by the enzyme flavanone 3-hydroxylase (F3H). DHK can be hydroxylated by the enzyme flavonoid 3’- hydroxylase (F3’H) to produce dihydroquercetin (DHQ) or by the enzyme flavonoid 3’, 5’-hydroxylase (F3’5’H) to produce dihydromyricetin (DHM). DHK, DHQ and DHM are colourless.

46. DHK can be converted to pelargonidin-3-glucosides, DHQ to cyanidin-3-glucosides and DHM to the purple-blue delphinidin-3-glucosides.
47. The activity of the “blue gene” *F3’5’H* is therefore necessary for biosynthesis of the delphinidin-based anthocyanins. In non-GM carnation, this gene is not naturally present.

### 5.2 The introduced genetic modifications

#### 5.2.1 The introduced genes and genetic elements

48. Tables 1 and 2 detail the genes, partial gene and regulatory sequences introduced into the GM carnations proposed for import and distribution.

**Table 1. Genes/partial genes introduced into the GM carnations lines proposed for import and distribution.**

<table>
<thead>
<tr>
<th>Carnation variety</th>
<th>Gene</th>
<th>Protein</th>
<th>Source organism</th>
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<tbody>
<tr>
<td>Moonaqua</td>
<td>vF3’5’H</td>
<td>Flavonoid 3’5’-hydroxylase</td>
<td>Viola hortensis</td>
</tr>
<tr>
<td></td>
<td>pDFR</td>
<td>Dihydroflavonol reductase</td>
<td><em>Petunia X hybrida</em></td>
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<tr>
<td>Moonberry</td>
<td>vF3’5’H</td>
<td>Flavonoid 3’5’-hydroxylase</td>
<td>Viola hortensis</td>
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<tr>
<td></td>
<td>pDFR</td>
<td>Dihydroflavonol reductase</td>
<td><em>Petunia X hybrida</em></td>
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<td></td>
<td>DFR gene-silencing construct (ds Carn DFR)</td>
<td>- *</td>
<td><em>Dianthus caryophyllus</em></td>
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<tr>
<td>Moonvelvet</td>
<td>pF3’5’H</td>
<td>Flavonoid 3’5’-hydroxylase</td>
<td><em>Petunia X hybrida</em></td>
</tr>
<tr>
<td></td>
<td>pCyt b5</td>
<td>Cytochrome b5</td>
<td><em>Petunia X hybrida</em></td>
</tr>
<tr>
<td>All GM carnations</td>
<td>SuRB</td>
<td>Acetolactate synthase</td>
<td><em>Nicotiana tabacum</em></td>
</tr>
</tbody>
</table>

*DFR gene-silencing construct does not encode a protein, but reduces the expression of endogenous DFR gene*
Table 2. Regulatory sequences introduced into the GM carnation lines proposed for import and distribution.

<table>
<thead>
<tr>
<th>Element</th>
<th>Description</th>
<th>Function</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>35S</td>
<td>Cauliflower mosaic virus (CaMV) promoter</td>
<td>Constitutive</td>
<td>Cauliflower mosaic virus</td>
</tr>
<tr>
<td><strong>Cab 5’utr</strong></td>
<td>Leader sequence of chlorophyll a/b binding protein gene</td>
<td>Improved expression</td>
<td><em>Petunia X hybrida</em></td>
</tr>
<tr>
<td>SuRB</td>
<td>Terminator</td>
<td>Stop signal</td>
<td><em>Nicotiana tabacum</em></td>
</tr>
<tr>
<td>DFR</td>
<td>Promoter</td>
<td>Promotes strong</td>
<td><em>Petunia X hybrida</em></td>
</tr>
<tr>
<td></td>
<td>Terminator</td>
<td>expression in petals</td>
<td></td>
</tr>
<tr>
<td>CHS</td>
<td>Promoter</td>
<td>Floral specific</td>
<td><em>Antirrhinum majus</em></td>
</tr>
<tr>
<td>D8</td>
<td>Terminator</td>
<td>Transcription stop</td>
<td><em>Petunia X hybrida</em></td>
</tr>
<tr>
<td></td>
<td>signal</td>
<td>signal</td>
<td></td>
</tr>
<tr>
<td>ANS</td>
<td>Promoter</td>
<td>Promotes strong</td>
<td><em>Dianthus caryophyllus</em></td>
</tr>
<tr>
<td></td>
<td>Terminator</td>
<td>expression in petals</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transcription stop</td>
<td>signal</td>
<td></td>
</tr>
</tbody>
</table>

**F3’5’H gene**

49. The F3’5’H gene encodes the enzyme F3’5’H, a microsomal cytochrome P450-dependent monoxygenase (Seitz et al. 2007). This enzyme occurs in a wide range of plant species (Brugliera et al. 2004) and plays a key role in the synthesis of 3’,5’-hydroxylated anthocyanins associated with the expression of blue or purple colour of plant parts, particularly flowers and fruits (Ainsworth 2006; Seitz et al. 2007). There are three of these anthocyanins – delphinidin, petunidin and malvidin (Shimada et al. 2008), with delphinidin being the most common in blue flowers (Harborne & Williams 2000). The enzyme has broad substrate specificity and is also able to catalyze the hydroxylation of flavanones, dihydroflavonols, flavonols and flavones (Ainsworth 2006). All putative F3’5’H amino acid sequences found to date share high sequence similarity, and homologues of the *Petunia X hybrida* F3’5’H gene have been found in edible plants including *Solanum melongena* (eggplant) and *Brassica oleracea* (red cabbage), and flowers including *Eustoma russellianum* (lisianthus) and *Campanula* (bellflower) (Holton & Cornish 1995; USDA 2007).

**DFR gene**

50. The DFR gene codes for dihydroflavonol reductase which generally acts on dihydroflavonols such as dihydrokaempferol (DHK), dihydroquercetin (DHQ) and dihydromyricetin (DHM) to produce leucoanthocyanidins. 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, the DFR of *Zea mays* (maize) cannot reduce DHM whereas the DFR of *Petunia X hybrida* (which is the gene used in the GM carnations proposed for release) has the highest activity with DHM as a substrate but does not reduce DHK (Meldgaard 1992). This specificity of DFR from Petunia X hybrida ensures that most or all of the anthocyanidin produced in the GM carnation flowers is delphinidin.
**DFR gene-silencing construct**

51. The *DFR* gene-silencing construct (ds Carn DFR) acts to suppress expression of the endogenous *DFR* gene by a natural regulatory mechanism known as RNAi or gene-silencing (Baykal & Zhang 2010). The mechanism for silencing of genes by RNAi was discussed in detail in the RARMPs prepared for DIR 112 and DIR 117. The RNAi construct contains a fragment of the *DFR* gene repeated in both sense and antisense orientation, separated by an intron from petunia. This fragment of DNA is transcribed and processed by endogenous cellular RNAi machinery into short *DFR*-specific interfering RNAs (siRNAs). The siRNAs then direct the degradation of messenger RNA (mRNA) molecules with matching sequence, in this case mRNAs transcribed from the endogenous *DFR* gene, before they are translated into proteins. This reduces the total DFR activity, and hence the production of anthocyanin pigments (Figure 2).

52. The efficiency of gene-silencing is generally determined by the extent of homology between the silencing construct and the target gene (usually > 95% homology is required) and the length of the homologous region. In plants, introduced silencing constructs have been shown to effectively suppress expression of the target genes but can also give rise to silencing of non-target genes with closely matching sequences (Baykal & Zhang 2010). Silencing of non-target genes may lead to changes other than the intended effects. These off-target effects currently cannot be predicted by bioinformatics analysis (EFSA 2014).

53. The DFR gene-silencing construct specifically silences expression of the carnation DFR gene but does not silence expression of the petunia DFR gene when this is co-introduced, indicating a high degree of specificity. Thus when both the DFR gene-silencing construct and the petunia DFR gene are introduced into carnations, pelargonidin synthesis is blocked but cyanidin and delphinidin are produced.

**Cytochrome b5 gene**

54. Cyt b5 proteins commonly occur in animals, plants and yeast. They enhance the activity of the members of the superfamily of Cyt P450 proteins by donating electrons. In plants that produce true blue flowers, Cyt b5 augments the activity of the Cyt P450 enzyme F3’5’H. In a loss of function study, inactivation of Cyt b5 resulted in mutants bearing variegated flowers and a 30 to 50% reduction of related anthocyanins. Cyt b5 is expressed exclusively in the flower of blue-flowered plants (de Vetten et al. 1999).

**SuRB**

55. All three GM carnations contain the marker gene *SuRB* from tobacco which results in tolerance to sulphonylurea-type herbicides, and was used for *in vitro* selection during development of the GM carnations. It is under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter with the petunia Cab 5’UTR and its own terminator.

56. 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 (Mazur & Falco 1989). The herbicide works by blocking cell division in the active growing regions of stem and root tips (meristematic tissue).

57. Herbicide resistance is conferred due to the production of an altered form of the ALS enzyme encoded by *SuRB* which is less sensitive to ALS inhibiting herbicides (Chaleff & Ray 1984).
5.2.2 GM carnation Moonaqua (FLO-40689-6)

58. Moonaqua was generated by transforming a non-GM carnation variety with the transformation vector pCGP1991 which was also described in the RARMP for DIR 030/2002. Two genes of the delphinidin pathway (Figure 3) were introduced into the white flowered non-GM parent carnation to produce the mauve flowered GM carnation Moonaqua:

- the $F3'5'H$ gene from pansy ($Viola hortensis$) under the control of the snap dragon ($Antirrhinum majus$) CHS promoter and the petunia ($Petunia X hybrid$) D8 terminator
- the $DFR$ gene from petunia under the control of its own promoter and terminator.

![Figure 3 Anthocyanin biosynthesis pathway in Moonaqua](image)

59. Expression of pansy $vF3'5'H$ results in the production of DHM. Petunia $pDFR$ was introduced as the non-GM parent variety lacks $DFR$ activity. Petunia $pDFR$ has a higher specificity for DHM than for DHQ and cannot utilise DHK (Meldgaard 1992). Delphinidin-based pigments are accumulated as the introduced genes $vF3'5'H$ and $pDFR$ are expressed in combination with the endogenous genes of the anthocyanin biosynthetic pathway.

5.2.3 GM carnation Moonberry (IFD-25958-3)

60. The same two genes that were used to generate Moonaqua were also introduced into a pink flowered non-GM parent carnation. In addition, a silencing construct to reduce endogenous $DFR$ levels was inserted to produce the purple flowered GM carnation Moonberry (Figure 4). The following genetic elements were inserted:

- the $vF3'5'H$ gene from pansy ($Viola hortensis$) under the control of the snap dragon CHS promoter and the petunia D8 terminator
- the $pDFR$ gene from petunia under the control of its own promoter and terminator
• the partial carnation \( DFR \) gene-silencing construct sequence with CaMV 35S promoter and terminator sequences (ds Carn DFR) and a petunia intron as a spacer sequence.

![Anthocyanin biosynthesis pathway in Moonberry](image)

**Figure 4 Anthocyanin biosynthesis pathway in Moonberry**

Pansy vF3’5’H and petunia pDFR have been introduced (in bold) and endogenous DFR expression is suppressed (strike through) through introduction of ds Carn DFR.

61. The function of \( DFR \) gene-silencing construct (ds Carn DFR) is to suppress the expression of the endogenous DFR, which is able to use DHK as a substrate to synthesize pelargonidin-based pigments. The introduced petunia pDFR enzyme cannot utilize DHK as a substrate and preferentially acts on DHM over DHQ (Meldgaard 1992; Tanaka & Brugliera 2014). Therefore, the flowers of Moonberry predominantly accumulate delphinidin-related anthocyanins which are lacking in the flowers of the non-GM parent variety.

5.2.4 GM carnation Moonvelvet (IFD-26407-2)

62. Two genes were introduced into the pink flowered non-GM parent (Figure 5) to produce the violet flowered Moonvelvet:

- the \( pF3’5’H \) gene from petunia under the control of the carnation ANS promoter and terminator regions
- the petunia \( pCyt b5 \) under the control of the snap dragon CHS promoter and the petunia D8 terminator.
Expression of the petunia \textit{pF3'5'H} gene results in the production of DHM. The petunia \textit{pCyt b5} enhances the activity of petunia \textit{pF3'5'H} (de Vetten et al. 1999). In Moonvelvet, the flowers accumulate delphinidin-based anthocyanins which are absent in the non-GM parent variety.

5.2.5 Method of genetic modification

The GMOs were generated using \textit{Agrobacterium tumefaciens}-mediated transformation. This transformation method has been widely used in Australia and overseas for introducing genes into plants. More detailed information on methods of genetic modification can be found in the document \textit{Methods of plant genetic modification} available on the OGTR website.

5.3 Toxicity and allergenicity of the products of the introduced genes

GM carnations modified for flower colour have an established safety record. There have been no reports of adverse effects on human health and safety or the environment as a result of the release of similar GM carnations in Australia in 1995, and which were later placed on the GMO Register in 2007. In addition, the GM carnations proposed for sale and distribution have been released in other countries since 1997 (Moonaqua) and 2008 (Moonberry and Moonvelvet), and no reports of adverse effects have been found (see this chapter, section 7).

5.3.1 Flavonoid 3’5’-hydroxylase and dihydroflavonol reductase proteins

The inserted genes express enzymes that are intermediates in the anthocyanin biosynthetic pathway. These enzymes are found in common purple flower and food plants, such as blackberries, blueberries, black beans, red grapes and red onions. All introduced anthocyanin synthesis genes have been sourced from common ornamental plants, i.e. petunia...
and pansy. The expressed proteins are not known to cause toxicity or allergenicity. GM carnations containing the introduced proteins \( F3'5'H \) and \( DFR \) were placed on the GMO Register in Australia in 2007 because there were no adverse effects in humans or on the environment as a result of previous authorised releases in Australia or other countries.

67. The introduced protein sequences were not homologous to any known allergenic proteins found in Food Allergy Research and Resource Program, University of Nebraska-Lincoln (FAARP) databases. Similarities measured in the FARRP database did not yield any sequence matches with known allergens, indicating a lack of immunological relevance.

### 5.3.2 Acetolactate synthase protein

68. The acetolactate synthase (ALS) enzyme encoded by \( SuRB \) is not a known toxin or allergenic. Similar ALS enzymes are present in a wide variety of edible plants as they are essential in the synthesis of aromatic amino acids. No new by-products are anticipated to be produced as a result of the genetic modification in the GM carnation as the resistance is based on the inability of the ALS-inhibiting herbicides to bind the active site of the introduced ALS enzyme.

69. In a recent review on validity of using animal feeding studies for determining the safety of GM crops, Bartholomaeus et al. (2013) summarised several studies including studies on ALS genes with mutations analogous to those of \( SuRB \). Proteins encoded by the mutated ALS genes from other plants that were used in the animal trials did not indicate any biologically relevant adverse effects. Acute toxicity studies with a maize ALS protein containing the same amino acid changes known to confer herbicide tolerance in tobacco ALS indicate that it was not acutely toxic (APHIS 2009).

70. Despite its presence in all plants, which creates wide exposure, unmodified ALS has not been reported as an allergen. An online search on FAARP database did not list ALS as a food allergen, aero-allergen or a contact allergen. These factors together indicate that the protein is unlikely to be allergenic.

71. GM carnations containing the same introduced \( SuRB \) gene and ALS protein have been previously assessed for release (DIR 030/2002), and later placed on the GMO Register in Australia because there were no adverse effects in humans or on the environment as a result of previous authorised releases.

### 5.3.3 Altered anthocyanin composition and anthocyanin levels

72. A variety of anthocyanins, including delphinidin- and cyanidin-derived anthocyanins, occur in many plant species, including food crops. Humans are commonly exposed to these compounds in fruits and vegetables via ingestion without reported toxic effects. For example, raw black currants contain approximately 181.1 mg per 100 g fresh weight, fresh blueberries 47.4 mg, raw eggplant 13.9 mg, black beans 12.0 mg, red grapes 3.7 mg and purple sweet potatoes 0.9 mg. The daily dietary intake of anthocyanins in the United States of America (USA) population was estimated to be approximately 12.5 mg per person per day (Wu et al. 2006). Cyanidin- and delphinidin-based pigments accounted for approximately 45% and 21%, respectively, of this total anthocyanin intake, with delphinidin consumption estimated at 2.6 mg per person per day (Wu et al. 2006).

73. In a recent review on safety of flavonoids to human health, Corcoran et al. (2012) did not identify any potential hazards associated with excess dietary intake of anthocyanins. The intrinsic toxicity of anthocyanins used as food colouring agents is considered to be low by the EU scientific committee for food (European Commission 1997) and concluded that anthocyanins are safe for consumption by young children above 12 months old. Anthocyanins
are authorised food additives used for colouring in Australia (FSANZ 2014). Anthocyanins
have a low acute toxicity in rodents and a very low order of toxicity (WHO 2001).

74. A review of allergic reactions to food colourings found that there are no reports of
allergic reaction to either grape skin extract or grape colour extract, both of which are widely
used food colorants that contain high levels of anthocyanins (Lucas et al. 2001). Allergies to
berries which are rich in anthocyanins are rare. However, cases of allergic reactions to
lingonberry and red currants have been reported (Matheu et al. 2004; Zollner et al. 2000).
Lingonberry primarily produces cyanidin-based pigments. In a study of self-reported food
hypersensitivity in northern European countries where consumption of berries is typically high,
with an estimated anthocyanin intake of approximately 80 mg per person per day, Eriksson et
al. (2004) did not find any correlation between anthocyanin intake and reported
hypersensitivity.

75. Positive health effects of anthocyanins have been reviewed by several authors (Heinonen
2007; Manach et al. 2005; Nichenametla et al. 2006). They include improved brain function
and cardio-vascular performance as well as anti-obesity, anti-inflammatory and anti-cancer
properties.

76. The GM carnations with modified colour flower contain introduced proteins similar to
those that occur in purple-coloured ornamental flowers. Some examples of widely grown
ornamental plants that contain delphinidin-based pigments include pansy and hibiscus with
delphinidin levels of 390 mg (source: IFD Pty Ltd) and 100 – 1000 mg (Puckhaber et al. 2002)
per 100 g fresh weight of petals, respectively. The delphinidin levels detected in the GM
carnation flowers are within the range found in these widely cultivated ornamental plants.
Similarly, cyanidin is found in common ornamental plants with pink, red and mauve coloured
flowers.

5.4 Characterisation of the GM carnations

5.4.1 Genetic stability

77. The applicant has assessed the genetic stability of the three proposed GM carnations and
reported that the frequency of off-types does not exceed 1%, which is typical for non-GM
carnation. As flower colour is a trait of commercial value, selection processes and vegetative
propagation ensures that plants sent to the flower production beds are true to type.

- Moonaqua was commercialised in July 2000 in Colombia. Since its commercialisation,
  39.4 million flowers were grown, 0.03% of which were off types.
- Moonberry was commercialised in September 2009 in Colombia. Since its
  commercialisation, 3.2 million flowers were grown, 0.28% of which were off types.
- Moonvelvet was commercialised in September 2009 in Colombia. Since its
  commercialisation, 2.9 million flowers were grown, 0.15% of which were off types.

78. These data are up to, and including, July 2014.

5.4.2 Detection methods

79. The applicant has developed a unique identification protocol for Moonaqua, Moonberry
and Moonvelvet. These PCR-based protocols have been validated by the European Union
reference laboratory for GM Food and Feed. The relevant documents are available on the Joint
Research Centre of European Commission Institute for Health and Consumer Protection
website (Moonaqua, Moonberry and Moonvelvet).
5.4.3 Molecular characterisation

80. The applicant carried out Southern blot analysis on genomic DNA isolated from the GM and non-GM carnations to identify integrated sequences, complexity of the integrated DNA and copy number of the introduced genetic modifications.

**Moonaqua**

81. Moonaqua contains a single copy each of SuRB and pDFR and three copies of vF3’5’H. There was no integration of sequences from the plasmid backbone. Sequencing analysis indicates that Moonaqua has a relatively complex integration pattern, with inserts at three discrete locations. None of the inserted sites contain the complete T-DNA from plasmid pCG1991, though locus 1 represents a nearly complete integration. Locus 2 contains a functional vF3’5’H gene only while locus 3 does not have any complete gene. No open reading frames (ORFs) were found within 150 base pairs of the genomic DNA on either side of each integration site.

**Moonberry**

82. Moonberry contains a single copy each of SuRB, vF3’5’H pDFR and ds Carn DFR. No hybridisation was detected with probes outside the T-DNA region of the vector. Moonberry has a simple integration pattern with a single, functionally intact, insert of the T-DNA from vector pCGP3366.

**Moonvelvet**

83. Moonvelvet contains a single copy each of SuRB, pF3’5’H and pCyt b5 from the vector pCGP2355. No hybridization was detected with probes outside the T-DNA region of the vector.

5.4.4 Phenotypic characterisation

84. The GM carnations differ from the non-GM parent varieties in the novel production of delphinidin-derived anthocyanins, an increased level of cyanidin-derived anthocyanins and tolerance to sulphonylurea type herbicides. The expression and activity of the genetic modifications is demonstrated by a) the tolerance to sulphonylurea herbicides and b) the change in flower colour.

**Moonaqua**

85. The applicant assessed the concentration of delphinidin and cyanidin in flower petal samples. Whereas the non-GM parent variety contains neither delphinidins nor cyanidins,
Moonqua (Figure 6) contains 7 mg of delphinidins and 2 mg of cyanidins per 100 g fresh weight of petals.

86. The applicant took measurements for 18 morphological characteristics to evaluate any significant differences between Moonqua and its non-GM parent variety. These experiments were carried out in Netherlands in 2000 and Australia in 2005. Characteristics measured were plant height, number of internodes per stem, length of 5th internode, thickness of 5th node, flower diameter, leaf length at 3rd node from top, height of corolla, calyx length, calyx diameter, number of lobes per calyx, number of petals per flower, petal length, petal width, number of stamens, number of styles, number of anthers, style length and stamen length. A 10% difference between the means of control and GM lines with a P-value of <0.05 was considered as significant. The study demonstrated that there was variation between the GM and non-GM carnations. Moonqua has fewer internodes per stem, thinner stem at the 5th node, a shorter leaf at the 3rd node, longer styles, less viable anthers, more filaments and shorter filaments.

87. The applicant measured anther number and pollen viability in two separate trials conducted in Netherlands in 2000 and in Australia in 2005. Flowers from Moonqua had a significantly lower number of anthers than that of the parent flowers. Out of eight flowers that were dissected for each variety, only one Moonqua flower and seven non-GM parent flowers had anthers. Moreover, for those flowers that carried anthers, average anther number per GM flower was significantly lower at one compared to 2.4 for the parent variety. The low number of anthers observed precluded any meaningful comparative assessment of pollen viability. However, results based on 2000 pollen from one anther, each placed on germinating medium, gave a low germination percentage of 8% for GM carnation and 6% for parent variety. Overall, it was observed that Moonqua produces smaller flowers than the parent variety and they have reduced reproductive capacity measured by a reduced number of anthers, styles and stamens. The styles and stamens were also significantly shorter in the GM line.

**Moonberry**

![Moonberry flowers](image)

Figure 7  The change in flower colour is shown with the non-GM parent variety on the left and Moonberry on the right.

88. The applicant assessed the concentration of delphinidin and cyanidin in flower petal samples. The non-GM parent variety contains no delphinidins and only 1 mg cyanidins per 100 g fresh weight of petals, whereas Moonberry (Figure 7) contains 54 mg delphinidins and 10 mg cyanidins per 100 g fresh weight of petals.

89. The applicant took measurements for 18 morphological characteristics (as detailed for Moonqua above) to evaluate any significant differences between Moonberry and its non-GM parent variety. These experiments were carried out in Australia in 2007/2008. The study demonstrated that there was little variation between the GM and non-GM carnations; however, the following statistically significant differences were recorded: Moonberry had more petals...
per flower with 61 petals compared to 51 for non GM variety and on average a thinner stem at the 5th node at 8 mm compared to 10 mm. It also had shorter filaments per flower at 20 mm compared to 23 mm for non-GM flowers, but a larger number of filaments at 12 per flower as compared to 8 for a non-GM flower. However, only a small percentage of these filaments have anthers. A study conducted by the applicant indicates that there was no significant pollen size difference between parental control (48 ± 10 µm) and GM carnation (50 ± 4 µm) and there was no difference in pollen viability (90% in both). Mean germination rates per anther were higher for parental control (14 ± 8) than for GM carnation pollen (10 ± 8).

**Moonvelvet**

![Image of Moonvelvet]

Figure 8 The change in flower colour is shown with the non-GM parent variety on the left and Moonvelvet on the right.

90. The applicant assessed the concentration of delphinidin and cyanidin in flower petal samples. The non-GM parent variety contains no delphinidin and only 1 mg cyanidin per 100 g fresh weight of petals, whereas Moonvelvet (Figure 8) contains 287 mg delphinidin and 37 mg cyanidin per 100 g fresh weight of petals.

91. The applicant took measurements for 18 morphological characteristics (as detailed for Moonaqua above) to evaluate any significant differences between Moonvelvet and its non-GM parent variety. These experiments were carried out in Australia in 2007/2008. The following statistically significant differences were recorded: Moonvelvet had a thinner stem at the 5th node at 8 mm compared to 10 mm for non-GM stem, a shorter leaf at the 3rd node at 58 mm compared to 64 mm for non-GM carnation, longer styles at 21 mm compared to 15 mm. The average number of anthers per GM carnation flower (0.75) was significantly less than that of the non-GM parent variety (4). The GM flowers also had shorter filaments measuring 18 mm compared to that of 23 mm for non-GM. However, the non-GM flowers had 10 filaments compared to 8 for non-GM flowers. A study conducted by the applicant indicates that there was no significant pollen size difference between parental control (48 ± 10 µm) and GM carnation (50 ± 4 µm) and pollen viability was higher for parental control (n=78/87; 90% in both) than for GM carnation pollen (n=117/155; 75%). Mean germination rates per anther were higher for parental control (14 ± 8) than for GM carnation pollen (10 ± 9).

**Section 6 The receiving environment**

92. The receiving environment includes: any relevant biotic/abiotic properties of the geographic regions where the release would occur; intended agricultural practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2013).

93. The applicant is seeking approval to import the GM carnations as cut flowers and then for distribution to occur Australia-wide. Cut GM carnation flowers would be imported in the same way as other GM and non-GM carnations. Once imported, the cut flowers would enter
the retail chain in the floristry industry and be sold for ornamental purposes. There is no intention to grow these GMOs in Australia.

6.1 Relevant industry practices

94. Several species of *Dianthus* have been cultivated for hundreds of years for ornamental purposes (Ingwerson 1949). Since then the carnation has become one of the most common cut-flower ornamental crops world-wide. Carnation has been grown commercially in Australia as a flower crop since 1954 (OGTR 2015).

6.1.1 Treatment of cut-flowers entering Australia

95. Imported cut-flowers, both authorised GM and non-GM, capable of propagation must undergo a glyphosate dip treatment for devitalisation at the border. This is a quarantine requirement by Department of Agriculture. The treatment protocol for carnations specifies 20 minutes stem immersion to at least 35 cm from the cut end or to within 5 cm of the flower head in a 0.5% glyphosate solution (Department of Agriculture 2012).

96. The applicant carried out a study on the viability of similar GM cut-flower carnation varieties after treatment with a lower concentration (0.18%) of glyphosate solution for 20 minutes. Twelve cut-flowers of each variety were immersed in glyphosate solution. Following the treatment, flower heads were removed and the twelve flower stems were cut into three segments thus making up 36 replicates per variety. These cut segments were planted in a rooting bed. None of the glyphosate-treated stem segments grew compared to an average of four plants per variety in the water-treated control.

6.1.2 Cultivation and import – non-GM and approved GM carnations

97. More than 10 billion carnation flowers are produced around the world each year (Agricultural Biotechnology Council of Australia 2012). The main carnation producing countries are Colombia, the United States, Israel, Kenya and Spain. Australia imports an estimated 20 million carnation flowers annually (2010 to 2013 data), mainly from China and Colombia (UN Comtrade 2015).

98. In 2006, the Australian carnation industry produced approximately 140 million cut-flowers across a total of 100 ha in Victoria, South Australia, Western Australia and New South Wales.

99. Carnations are imported to supplement the domestic supply, and this includes both non-GM and authorised GM carnations. The applicant reported that between January and July 2014, 376 220 GM carnation flowers were imported into Australia. These imports consist of GM varieties initially approved for environmental release, including growing, in Australia in 1995 under GMAC (the former voluntary system for GMO regulation) and were placed on the GMO Register in 2007. More than 4.5 million GM carnation flowers have been sold in Australia since 1995 (Agricultural Biotechnology Council of Australia 2012). The genetic modifications of these previously approved GM carnations are very similar to those proposed for import and distribution.

6.1.3 Transport and distribution in Australia

100. Carnation flowers are usually dry packed in sleeved bunches in cardboard boxes. They are imported and forwarded for distribution by wholesalers in the major cities or to supermarket chains. Flowers are then distributed to the floristry industry and end-customers.
6.2 Relevant consumer practices

101. Once bought by the consumer, the vase life of carnations is up to three weeks. The flowers may be disposed of in general waste (landfill) or composted.

Section 7 Previous releases

7.1 Australian approvals of the GM carnation proposed for release

102. The GM carnations proposed for release have not been released previously in Australia.

7.2 Approvals by other Australian agencies

103. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. However, dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products. For example, the GM carnations are subject to import regulation by the Department of Agriculture as cut flowers (Section 6.1).

7.3 International approvals

104. The GMOs proposed for release have been commercialised in several other countries as stated below. There have been no reports of adverse effects to human health and safety or the environment from these commercial releases.

Ecuador

105. Moonaqua was approved for environmental release in 1997. There are no requirements for product labelling or monitoring.

Colombia

106. Moonaqua was approved for environmental release in 2000, with approval of the other two GM carnations occurring in 2008. Production is restricted to the State of Cundinamarca, and waste material is not allowed to be used in animal feed. There are no requirements for product labelling.

Japan

107. Moonaqua was authorised to be imported as cut flowers in 2009, with approval of the other two GM carnations occurring in 2013. There are no requirements for product labelling or monitoring.

United States of America

108. Animal and Plant Inspection Service determined that cut flowers of GM carnation destined for import are not regulated articles. Imported cut flowers of carnation are not considered capable of self-propagation. There are no requirements for product labelling or monitoring.

Canada

109. All three GM carnations were exempted from import requirements in 1999. There are no requirements for product labelling or monitoring.
European Union

110. In 2008, Moonaqua was approved for import as a cut flowers for an initial 10 years; the other two varieties received similar approval in 2015. There are labelling and monitoring requirements.

Malaysia

111. In 2013, the three GM carnations were approved for import. Labelling requirements were imposed. Any new or additional scientific information on adverse effects must be reported.

Singapore

112. Moonaqua and Moonberry were approved for import as cut flowers in 2013. Flowers are to undergo de-vitalisation treatment with glyphosate. Any new scientific or technical developments, and prohibition or restrictions imposed by any other authority must be reported.
Chapter 2 Risk Assessment

Section 1 Introduction

113. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 9). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

![RISK ASSESSMENT PROCESS *](image)

RISK IDENTIFICATION RISK CHARACTERISATION

* Risk assessment terms are defined in the Risk Analysis Framework 2013

Figure 9  The risk assessment process

114. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO in the short and long term. These are called risk scenarios.

115. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are used to postulate risk scenarios (Keese et al. 2013). Risk scenarios postulated in previous RARMPs prepared for licence applications of the same or similar GMOs are also considered.

116. Postulated risk scenarios are screened to identify those that are considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

117. Substantive risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.
Section 2 Risk Identification

118. Postulated risk scenarios are comprised of three components:
   i. The source of potential harm (risk source).
   ii. A plausible causal linkage to potential harm (causal pathway).
   iii. Potential harm to an object of value, people or the environment.

119. The risk context, including the following factors, is taken into account when postulating relevant risk scenarios:
   - the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
   - any proposed limits including the extent and scale of the proposed dealings
   - any proposed controls to restrict the spread and persistence of the GMOs
   - the characteristics of the parent organism(s).

2.1 Risk source

120. The source of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

121. As discussed in Chapter 1, the GM carnations have been modified by the introduction of genes or partial genes that result in altered flower colour. These introduced gene sequences are considered further as a potential source of risk.

122. In addition, the GM carnations contain a selectable marker gene ($\text{SuRB}$) that confers herbicide tolerance and was used for initial screening of transformed plant cells and plants. This gene and its product have already been extensively characterised (Chapter 1, Section 5.2 and 5.3.2) and assessed as posing negligible risk to human or animal health or to the environment by the Regulator (DIR 030/2002) in Australia and overseas (EFSA 2008). The GM carnations have been assessed for import as cut flowers and as a quarantine requirement they are subject to a devitalisation process. This limits potential for their spread and persistence. In the unlikely event of its establishment in the environment, the GM carnations will only exhibit enhanced fitness where sulfonylurea herbicides are applied. Hence the gene has not been found to pose substantive risk to either people or the environment, its potential effect will not be further assessed for this application.

123. The introduced genes are controlled by introduced regulatory sequences. The regulatory sequences are derived from plants and a plant virus. Similar regulatory sequences are naturally present in plants, and the introduced elements are expected to operate in similar ways to endogenous elements. There is no evidence that regulatory sequences themselves have toxic or allergenic effects (EPA 1996). Although the viral sequences are derived from a plant pathogen, they only constitute small fractions of the genomes and cannot themselves cause disease. Hence, potential harms from the regulatory elements will not be considered further. However, the introduced regulatory sequences control gene expression and hence the distribution and concentration of the introduced proteins and their products in the GM plants. The effects of protein levels and of their products, especially in relation to toxicity and allergenicity, will be considered below.
124. The genetic modifications have the potential to cause unintended effects in several ways including altered expression of endogenous genes by random insertion of introduced DNA in the genome, increased metabolic burden due to expression of the introduced proteins, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously and in plants generated by conventional breeding. Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al. 2015). Plants generated by conventional breeding have a long history of safe use, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al. 2013). Therefore, unintended effects resulting from the process of genetic modification will not be considered further.

2.2 Causal pathway

125. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- routes of exposure to the GMOs, introduced gene(s), gene product(s) and downstream products
- potential effects of the introduced gene(s), gene product(s) and downstream products on the properties of the organism
- potential exposure to the introduced gene(s), gene product(s) and downstream products from other sources in the environment
- the environment at the site(s) of release
- relevant floristry industry and consumer practices
- spread and persistence (invasiveness) of the GM plant, including establishment and reproduction
- dispersal by natural means and by people
- gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer (HGT) and
- unauthorised activities.

126. The potential for horizontal gene transfer (HGT) from GMOs to other organisms, and any possible adverse outcomes, have been reviewed in the literature (Keese 2008) and assessed in many previous RARMPs. HGT was most recently considered in the RARMP for DIR 108 (available from the GMO Record on the OGTR website). In previous assessments of HGT, no substantive risk was identified due to the rarity of these events and because the wild-type gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. In addition, the applicant does not propose to grow the GM carnations in Australia, thus further reducing the likelihood of HGT. Therefore, HGT will not be further considered for this application.

127. The potential for unauthorised activities to lead to an adverse outcome has been considered in many previous RARMPs, most recently in the RARMP for DIR 117 (available from the GMO Record on the OGTR website). In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise...
risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

128. Potential harms from GM plants include:

- harm to the health of people or desirable organisms, particularly toxicity/allergenicity
- reduced establishment or yield of desirable plants
- reduced products or services from the land use
- restricted movement of people, animals, vehicles, machinery and/or water
- reduced quality of the biotic environment (e.g. providing food or shelter for pests or pathogens) or abiotic environment (e.g. negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table)
- reduced biodiversity for nature conservation.

129. These harms are based on those used to assess risk from weeds (Standards Australia Ltd et al. 2006; Keese et al. 2013). Judgements of what is considered harm depend on the management objectives of the land where the GM plant may spread and persist. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

130. Two risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 1 and more details of these scenarios is provided later in this Section. Postulation of risk scenarios considers impacts of the GM carnations or their products on people undertaking the dealings, as well as impacts on people and the environment exposed to the GM carnations or their products as the result of the commercial dealings or spread and persistence of plant material, including pollen.

131. In the context of the activities proposed by the applicant and considering both the short and long term, none of the two risk scenarios gave rise to any substantive risks.

Table 3. Summary of risk scenarios from dealings with the carnations genetically modified for altered flower colour

<table>
<thead>
<tr>
<th>Risk scenario</th>
<th>Risk source</th>
<th>Causal pathway</th>
<th>Potential harm</th>
<th>Substantive risk?</th>
<th>Reason</th>
</tr>
</thead>
</table>
| 1             | GM carnations expressing genetic modifications for altered flower colour | Exposure of people or other organisms through contact with or ingestion of GM carnation flowers | Toxicity or allergenicity in people or toxicity in other desirable organisms. | No | • The introduced proteins, and anthocyanins products, are widely consumed by humans in various foods, and not known to be toxic or allergenic.  
• Adverse effects from siRNA intake are unlikely, and any effects would be transient.  
• The GM carnations are not intended for human food or animal feed.  
• The flower morphology of the GM carnation restricts pollen release.  
• The GM carnation pollen is heavy and sticky and hence... |
GM carnations expressing genetic modifications for altered flower colour

Dispersal of viable GM carnation plant material
- Establishment of GM plants or hybrid offspring
- Spread and persistence of GM plants or hybrid offspring

Increased toxicity or allergenicity in people or increased toxicity in other desirable organisms.
- Reduced establishment of desirable plants.
- Reduced biodiversity.

No

- The cut flowers would be treated with herbicide before import into Australia.
- The genetic modifications are not expected to increase weediness of the GM carnations.
- The flower morphology of floriculture carnations restricts access to pollinating insects.
- The GM carnations are highly unlikely to release pollen.
- Similar GM carnations have been grown in Australia with no reports of adverse effects.

2.4.1 Risk scenario 1

Risk source
GM carnations expressing genetic modifications for altered flower colour

Causal pathway
Exposure of people or other organisms through contact with or ingestion of GM carnation flowers

Potential harm
Toxicity or allergenicity in people or toxicity in other desirable organisms.

Risk source
132. The sources of potential harm for this postulated risk scenario are the GM carnations expressing genetic modifications for altered flower colour.

Causal pathway
133. The applicant proposes to import, transport, store and dispose of cut flowers. The applicant does not propose to grow the GM carnations in Australia. The cut flowers would be sold for display only.

134. Potential pathways of exposure to the introduced proteins, dsRNA and derived siRNA and the anthocyanin products are ingestion, inhalation or dermal contact. Workers or members of the public who pack, transport, display, store or dispose of the GM carnations would be exposed to the GM carnation plant material. Other desirable organisms could be exposed to the GM carnations whilst they are being transported, displayed or after disposal in compost heaps or landfill waste.
135. The GM carnations proposed for release are intended for display only and not for human consumption. If these plants were to be sold as food, then an approval from FSANZ would be required. No such approval has been sought. In addition, carnations have only a very limited use in food, as a garnish. Therefore, there is minimal potential for human ingestion of the introduced proteins, dsRNA, siRNA or anthocyanins.

136. GM plant material that could potentially be airborne and inhaled includes pollen. However, very little pollen is produced, carnation pollen is tightly enclosed by the flower petals and it is heavy, sticky and not dispersed by wind (OGTR 2015). Therefore, exposure through inhalation is highly unlikely.

137. Florists, other workers and members of the public would have some skin contact with the introduced proteins, dsRNA, siRNA or anthocyanins if they touched plants where cell contents have been released, such as cut stems.

138. Desirable non-human organisms may be exposed directly to the introduced proteins, dsRNA, siRNA and anthocyanins through ingesting the GM plants, or exposed indirectly through the food chain, or exposed through contact with dead plant material (soil organisms). Livestock are not expected to ingest the GM plant material as it is not used as animal feed. As mentioned in Chapter 1 Section 4.1.2, GM carnations are transported in sleeved bunches and placed in cardboard boxes, so large animals are unlikely to be exposed to the flowers during import and transport.

139. The GM carnations will be disposed of in general waste with cut flowers going to landfill or via composting. A range of desirable invertebrates, other animals and microorganisms would be expected to ingest some GM carnation plant material upon disposal. The potential for exposure of those organisms to the introduced proteins and anthocyanins is expected to be similar to their exposure to non-GM and previously approved GM carnations.

140. siRNAs fall under a general category of small RNAs that also includes microRNAs (miRNAs). siRNAs and miRNAs are common in both plants and animals and are believed to play regulatory roles in many biological processes. In some invertebrates, ingested dsRNA has been shown to lead to gene-silencing. Zhang et al. (2011) reported that natural plant miRNAs can be absorbed by mammals through food intake, and have the potential to modulate gene expression in animals. This risk has been further analysed by other researchers but remains contentious (Liang et al. 2014; Petrick et al. 2013). More detailed discussion of this issue can be found in the RARMP for DIR 131.

141. To have any effect in animals, the GM carnations would need to constitute a large proportion of the diet, and the dsRNA or siRNA would need to be present at high levels in the material consumed, match a sequence in an animal gene and be taken up by cells expressing that gene. As only plants possess a DFR gene, sequence homology in other organisms is unlikely. In addition, both dsRNA and the derived siRNA would quickly be destroyed by RNases in dying GM carnation flowers, minimising potential for exposure. Even if dsRNA or siRNAs were acquired through eating GM carnation and did affect gene expression, it is expected that any effect would be transient as described in Zhang et al. (2011).

**Potential harm**

142. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000). Allergenicity is the potential of a substance to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).
143. Increased toxicity or allergenicity of GM plants could be due to direct expression of the introduced genes in the GM carnations. The proteins encoded by the introduced genes are widespread in many plants, including food crops, occur at varying levels and are not known to cause toxicity or allergenicity. The introduced genes lead to increased levels of the anthocyanins delphinidin and cyanidin in the petals of the GM carnations relative to the non-GM parent varieties. These anthocyanins are also widespread in the environment, including in food crops, and not known to be toxic or allergenic.

144. Non-GM carnations are known to lead to contact dermatitis in some cases and possibly to mild toxicity in dogs and cats upon ingestion (OGTR 2015). The introduced proteins in the GM carnations have well understood modes of action that would not alter other metabolic pathways. Therefore, the GM carnations are not expected to have increased levels of natural toxins.

145. There have been no confirmed reports of adverse effects as a result of the commercial release of these GM carnations in a number of overseas countries over several years. Similarly, there have been no reported adverse effects from the release of similar GM carnations in Australia.

**Conclusion**

146. Risk scenario 1 is not identified as a substantive risk because GM plant material is not proposed to be consumed in human food or animal feed, the introduced genes and proteins, and their products, are already present and widespread in the Australian environment and not known to be toxic or allergenic, and there is extensive experience with these and other very similar GM carnations. Adverse effects from siRNA intake are unlikely, and any effects would be transient. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

### 2.4.2 Risk Scenario 2

<table>
<thead>
<tr>
<th>Risk source</th>
<th>GM carnations expressing genetic modifications for altered flower colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causal pathway</td>
<td>Dispersal of viable GM carnation plant material</td>
</tr>
<tr>
<td></td>
<td>Establishment of GM plants or hybrid offspring</td>
</tr>
<tr>
<td></td>
<td>Spread and persistence of GM plants or hybrid offspring</td>
</tr>
<tr>
<td>Potential harms</td>
<td>Increased toxicity or allergenicity in people or increased toxicity in other desirable organisms or Reduced establishment of desirable plants or Reduced biodiversity</td>
</tr>
</tbody>
</table>

**Risk source**

147. The sources of potential harm for this postulated risk scenario are GM carnations expressing genetic modifications for altered flower colour.

**Causal pathway**

148. The first steps in this risk scenario are the dispersal and establishment of the GM carnations. This could occur either deliberately or inadvertently.

149. For deliberate dispersal and establishment, people may attempt to strike the cut flowers under conducive conditions. However, due to the mandatory pre-border herbicide treatment of
all propagatable cut flowers entering Australia (Chapter 1, Section 6.1), successful striking of these GM carnations is highly unlikely.

150. For inadvertent dispersal and establishment, vegetative plant material, seed or pollen would have to be dispersed. Vegetative plant material will be dispersed either by accidental dispersal during transport or by disposal. Disposal would occur either via general waste with cut flowers going to landfill or via composting. Natural striking of floriculture carnations has not been reported in either situation. This is evidenced by the lack of naturalisation of carnation in Australia despite it being grown as a flower crop for more than 50 years. The likelihood of natural vegetative propagation is further reduced by the mandatory devitalisation treatment for all imported carnations (Chapter 1, section 6.1).

151. Floriculture carnations, including the GM carnations proposed for release, produce low amounts of pollen, have low pollen viability, have dense petals which impede access by pollinators and the pollen is not dispersed by wind (OGTR 2015). This means that both pollination of the cut GM carnation flowers or pollen from the cut GM carnation flowers pollinating any other sexually compatible plant is highly unlikely. As described in Chapter 1 (Section 4.1.2), flowers are harvested when they are in tight bud or closed bud stage for marketing purposes to ensure a satisfactory vase life. The flowers are then dry packed in sleeved bunches and cardboard boxes before being distributed. This process virtually eliminates the chance of flowers being pollinated in growing conditions before they are imported into Australia.

152. Dispersal of seed would require seed formation on the cut GM carnation flowers. The formation of viable seed would require at least four weeks post-pollination whereas the vase life of cut flowers generally is less than three weeks (Gatt et al. 1998; Spenaaij & Beeger 1973). In the highly unlikely event that seed set did occur, the seeds may be dispersed, e.g. during or after disposal. If seed was dispersed, it would have to arrive in an environment which is conducive for germination and establishment. This is highly unlikely as evidenced by the experience with both non-GM and previously approved GM carnations in Australia. Carnation is not known as naturalised in Australia despite having been commercially cultivated as a flower crop since 1954. Carnation has not been reported as a weed in any of the countries that it is cultivated.

153. Finally, if the GM carnations did arrive and establish in a suitable habitat then the genetic modification is not expected to increase the spread and persistence of the GM carnations as it is not involved in pathways that would contribute to overcome the major abiotic limiting factors, including water and temperature requirements, of carnations.

Potential harms

154. The potential harms from this risk scenario are toxicity or allergenicity in people or toxicity to desirable organisms, reduced establishment or yield of desirable plants, or reduced biodiversity.

155. As discussed in Risk Scenario 1, the introduced genetic modifications in the GM carnations are not expected to increase toxicity or allergenicity to people, or toxicity to other desirable organisms. This is also expected to be the case if the introduced genetic modifications are present in hybrids with non-GM carnations.

156. If the GMOs included in this application outcrossed with commercially released GM carnations, the hybrid offspring could contain the introduced genetic modifications of this application and those in already commercially approved GM carnations. The GM carnations previously approved for commercial release and put on the GMO Register (GMO Register 001/2007) have similar traits of flower colour modification and herbicide
tolerance. Their introduced genetic modifications are either identical or are part of the same biochemical pathway. In hybrids between GMOs, it is possible that additive or synergistic effects could occur, potentially changing the level of the introduced proteins or the anthocyanins. However, there is no reasonable expectation that the hybrids could be more toxic or allergenic than the GM carnations, which are considered to pose no substantive risk to humans or other desirable organisms.

157. The GM carnations or any hybrid offspring could reduce the establishment of desirable organisms in intensive use areas such as landfill waste areas or urban areas or in nature reserves or disturbed habitats, if those plants were able to establish. However, as discussed above, carnations have a limited potential to establish without human intervention, and the introduced genetic modifications are not expected to increase their ability to spread and persist.

158. The GM carnations or any hybrid offspring could potentially reduce biodiversity through direct toxicity of the introduced genetic modifications, and indirect effects on predators and parasites that depend on susceptible organisms. However, the extensive experience with other, very similar approved GM carnations and non-GM carnations in Australia as well as with the GM carnations in various countries overseas strongly suggests otherwise.

Conclusion

159. Risk scenario 2 is not identified as a substantive risk because the biological characteristics of GM and non-GM floriculture carnations are such that it is highly unlikely that pollen, seed or other viable material will be dispersed. The introduced genetic modifications are not expected to increase weediness of the GM carnations or any hybrid offspring. No adverse effects have been reported in other countries where these GM carnations have been released, nor in Australia following release of very similar GM carnations. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

160. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis\(^2\). Uncertainty in risk assessments arises from sources such as incomplete knowledge and inherent biological variability. Uncertainty is addressed by approaches including balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

161. For commercial/general releases, where there may not be limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, uncertainty may be addressed through post-release review (Chapter 3, Section 4).

162. For the current application, no substantial areas of uncertainty have been identified in the risk assessment. Therefore, specific indicators of harm have not been identified.

Section 4 Risk evaluation

163. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed

\(^2\) A more detailed discussion of uncertainty is contained in the Regulator’s Risk Analysis Framework available from the Risk Assessment References page on the OGTR website or via Free call 1800 181 030.
dealings should be authorised, need further assessment, or require collection of additional information.

164. Factors used to determine which risks need treatment may include:

- risk criteria
- level of risk
- uncertainty associated with risk characterisation
- interactions between substantive risks.

165. Two risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the risk assessment, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 3 and include:

- the GM carnations will not be grown in Australia
- none of the GM plant material is proposed for human food or animal feed
- widespread presence of the introduced genes, their encoded proteins and pigments in the environment
- extremely limited ability of the GM carnation cut flowers to disperse and establish populations outside cultivation
- extremely limited ability of the GM carnation cut flowers to transfer the introduced genetic modification to sexually compatible plants
- extensive experience both in Australia and overseas with other, very similar, GM carnations and extensive experience overseas with the GM carnations proposed for release.

166. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM carnation cut flowers into the environment are considered to be negligible. The Risk Analysis Framework (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.
Chapter 3 Risk Management Plan

Section 1 Background

167. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator’s decision-making process and is given effect through licence conditions.

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

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

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

Section 2 Risk treatment measures for substantive risks

171. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed release of the GM carnation cut-flowers. These risk scenarios were considered in the context of the proposed Australia-wide release and the receiving environment. The risk evaluation concluded that no controls are required to treat these negligible risks.

Section 3 General risk management

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

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

3.1 Applicant suitability

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

- any relevant convictions of the applicant (both individuals and the body corporate)
any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country

the capacity of the applicant to meet the conditions of the licence.

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

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

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

3.2 Testing methodology

177. IFD is required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This instrument is required prior to conducting any dealings with the GMOs and was provided with the application. The relevant documents are available on the Joint Research Centre of European Commission Institute for Health and Consumer Protection website (Moonaqua, Moonberry and Moonvelvet).

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

178. Any person, including the licence holder, may conduct any permitted dealing with the GMOs.

3.4 Reporting requirements

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

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

180. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

181. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

3.5 Monitoring for compliance

182. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

183. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to the health and safety of people or the environment could result.
Section 4 Post release review

184. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator does not fix durations, but takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

185. For the current application for a DIR licence, the Regulator has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. This ongoing oversight will be achieved through post release review (PRR) activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3).

186. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

187. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), fax (02 6271 4202), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the RARMPs of future applications involving similar GMO(s).

4.2 Requirement to monitor specific indicators of harm

188. Additional specific information on an intentional release provides a mechanism for ‘closing the loop’ in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

189. The term ‘specific indicators of harm’ does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

190. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

191. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. The uncertainty associated with the proposed dealings is considered to be low and no specific indicators of harm have been identified in this RARMP for application DIR 134. However, specific indicators of harm may also be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

192. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.
4.3 Review of the RARMP

193. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s), or identified new risks to people or to the environment that needed managing, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

194. The risk assessment concludes that this proposed commercial release of GM carnation cut flowers poses negligible risks to the health and safety of people or the environment as a result of gene technology.

195. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, general conditions have been imposed to ensure that there is ongoing oversight of the release.
References


EPA (1996) Plant pesticide inert ingredient CP4 Enolpyruvylshikimate-3-D and the genetic material necessary for its production in all plants. No. 61, US Environmental Protection Agency.


References 38


References 39


Appendix A Summary of submissions from prescribed experts, agencies and authorities

The Regulator received a number of submissions from prescribed experts, agencies and authorities on matters considered relevant to the preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. The issues raised, and how they were addressed in the consultation RARMP, are summarised below.

<table>
<thead>
<tr>
<th>Sub. No.</th>
<th>Summary of issues raised</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Considers that the application has minimal impact on the operations of the city and hence will not make a submission.</td>
<td>Noted.</td>
</tr>
<tr>
<td>2</td>
<td>Has no concerns on being impacted by the importation of the GM carnations and has no comments to make.</td>
<td>Noted.</td>
</tr>
<tr>
<td>3</td>
<td>Does not have the expertise in the field to provide any further comment.</td>
<td>Noted.</td>
</tr>
<tr>
<td>4</td>
<td>Concerned that the GM carnations could spread via seed as weeds upon disposal.</td>
<td>A number of factors prevent seed formation on cut flower carnations, including self-incompatibility, harvesting the flowers at bud stage, packaging, flower morphology and a shorter shelf life than required for seed formation. Imported cut-flowers are devitalised prior to import, preventing propagation from cuttings. GM and non-GM carnations both in Australia and overseas are not considered weeds. The concern was addressed in the RARMP in Chapter 1 section 4.1 and Chapter 2 risk scenario 2.</td>
</tr>
<tr>
<td>5</td>
<td>Assumes that the likelihood of the carnations being able to propagate by seed is negligible.</td>
<td>A number of factors prevent seed formation on cut flower carnations, including self-incompatibility, harvesting the flowers at bud stage, packaging, flower morphology and a shorter shelf life than required for seed formation. Imported cut-flowers are devitalised prior to import, preventing propagation from cuttings. GM and non-GM carnations both in Australia and overseas are not considered weeds. The concern was addressed in the RARMP in Chapter 1 section 4.1 and Chapter 2 risk scenario 2.</td>
</tr>
</tbody>
</table>

Sought to know whether the florists have to dispose unused GM carnations differently to avoid them from getting into environment via landfill.  
The application proposes to dispose of the GM carnation cut-flowers in the same way as any other cut-flowers. Disposal would mainly occur either via general waste with cut flowers ending in landfill or via composting. This issue is addressed in Chapter 2 risk scenario 2 and was not identified as a substantive risk.
<table>
<thead>
<tr>
<th>Sub. No.</th>
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<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wants to know if the herbicide resistance means that the GM carnations have a potential to be a weed.</td>
<td>Risk associated with the herbicide tolerance is trait addressed in Chapter 2, section 2.1. As the imported GM carnations flowers are not able to propagate, potential weeediness is not considered a plausible risk.</td>
</tr>
<tr>
<td>6</td>
<td>Is not in a position to provide rigorous scientific comment. However, observes that extreme caution be exercised in allowing genetic modification of organisms, especially when it is not for food production, but for commercial gain.</td>
<td>The object of the Act is to protect human health and safety and the environment by identifying and managing risks posed by or as a result of gene technology. The consultation RARMP provides an assessment of risks and addresses risk management.</td>
</tr>
<tr>
<td></td>
<td>Considers genetic modification of non-food plants a folly.</td>
<td>The Regulator must evaluate every licence application that meets the criteria set out in the Act.</td>
</tr>
<tr>
<td></td>
<td>Concurs with the Canadian Association of Physicians for the Environment advocating sustainable and organic agriculture on global scale.</td>
<td>The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. Consideration of alternative farming methods is outside the Regulator’s legislative responsibility.</td>
</tr>
<tr>
<td>7</td>
<td>Envisages no local issues. Though the GMOs have resistance to specific herbicide, they can be managed with other herbicides if need arises.</td>
<td>Risk associated with the herbicide tolerance trait is addressed in Chapter 2, section 2.2. As the imported GM carnation flowers are not able to propagate potential weeediness is not considered a plausible risk.</td>
</tr>
<tr>
<td></td>
<td>Have no scientific expertise to comment on human health and safety.</td>
<td>Noted.</td>
</tr>
<tr>
<td>8</td>
<td>Does not have sufficient technical or scientific expertise to be able to provide comment.</td>
<td>Noted.</td>
</tr>
<tr>
<td>9</td>
<td>Expresses concerns over the safety of GMOs.</td>
<td>The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. The concerns are addressed in Chapter 1 and Chapter 2.</td>
</tr>
<tr>
<td></td>
<td>Expressed concerns over the potential impact on local flower growers due to competition from imported carnations. Supports the current South Australian moratorium on GM crops.</td>
<td>Marketing and trade issues are outside the Regulator’s legislative responsibility. Some areas may be designated GM, GM-free or both under State or Territory law for the purpose of preserving the identity of GM or non-GM crops (or both) for marketing purposes.</td>
</tr>
<tr>
<td>10</td>
<td>Is unable to provide information or advice regarding risks to human health and safety and the environment.</td>
<td>Noted.</td>
</tr>
<tr>
<td>11</td>
<td>Is generally opposed to the introduction of genetically modified organisms to the Shire and advocates for clear labelling to be introduced. The council was declared a GMO Free Zone in 2008.</td>
<td>The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. Marketing and trade issues are outside the Regulator’s legislative responsibility. Some areas may be designated GM, GM-free or both under State or Territory law for the purpose of preserving the identity of GM or non-GM crops (or both) for marketing purposes.</td>
</tr>
<tr>
<td>Sub. No.</td>
<td>Summary of issues raised</td>
<td>Comment</td>
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</tr>
<tr>
<td>12</td>
<td>Believes that there is minimal risk associated with the proposed dealings. Have no staff that are experienced in this field to provide formal comments.</td>
<td>Noted.</td>
</tr>
<tr>
<td>13</td>
<td>Suggests that the occurrence of <em>Dianthus</em> species be researched, so that up-to-date information is provided in the RARMP. Further, a statement on limitations on vegetative propagation of carnation be included.</td>
<td>Occurrence of <em>Dianthus</em> species and their limited potential to propagate vegetatively has been researched and information provided in Chapter 1, Section, 4.1 and Chapter 2, Section 2.4.</td>
</tr>
<tr>
<td></td>
<td>Suggests that the issue of seed dormancy be considered in the RARMP. Suggests that the length of time that seed can survive in the environment may be addressed with respect to potential unintentional spread of seed.</td>
<td>A number of factors prevent seed formation on cut flower carnations, including self-incompatibility, harvesting the flowers at bud stage, packaging, flower morphology and a shorter shelf life than required for seed formation. GM and non-GM carnations both in Australia and overseas are not considered weeds. The concern was addressed in the RARMP in Chapter 1 section 4.1 and Chapter 2 risk scenario 2.</td>
</tr>
<tr>
<td></td>
<td>Notes the use of RNA interference in the application and references articles by Zhang et al. 2012 and EFSA 2014 which discuss risk issues with the use of this technology. Although the cut-flowers are not for human consumption, suggest the issue of off-target silencing should be addressed.</td>
<td>Off-target effects associated with RNA interference are discussed in Chapter 2 risk scenario 1. The EFSA 2014 and the Zhang et al. 2012 papers are referenced is in the RARMP.</td>
</tr>
<tr>
<td></td>
<td>Suggests to explore that humans have had regular exposure to the expressed proteins over millennia and that the anthocyanins expressed due to the genetic modification are common in other (non-GM) ornamental plants and also in plants that are part of human diet. Hence, their presence in the environment is unlikely to be an issue of concern for human and animal health or the environment. This also applies to herbicide tolerance gene <em>SurB</em> which encodes a protein involved in the production of branched aliphatic amino acids.</td>
<td>This in noted in the RARMP Chapter 1 section 5.3 and Chapter 2 including risk scenarios 1 and 2.</td>
</tr>
<tr>
<td>14</td>
<td>No issues were identified relating to human health and the environment</td>
<td>Noted.</td>
</tr>
<tr>
<td>15</td>
<td>Thinks that the Regulator should consider the effect of any proposed treatment of the GM carnations for devitalisation or lack of propagation.</td>
<td>The Australian Department of Agriculture requires that all propagatable cut flowers and foliage must be treated to devitalise plant tissue prior to entering Australia. This issue is addressed in Chapter 1, Section 6.1.1 and in Chapter 2, risk scenario 2.</td>
</tr>
<tr>
<td></td>
<td>Advises that the Regulator should consider the potential for people to eat the GM carnations.</td>
<td>The cut flowers would be sold for ornamental purpose only. The RARMP addresses toxicity and allergenicity via consumption in Chapter 2 risk scenario 1.</td>
</tr>
</tbody>
</table>
Appendix B  Summary of advice from prescribed experts, agencies and authorities on the consultation RARMP³

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

**Issues raised:** Environment, import requirements, marketing and segregation.

<table>
<thead>
<tr>
<th>Sub. No.</th>
<th>Summary of issues raised</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Has not determined a formal position on GM carnation importation, but does not expect any impacts on the rural location of the Shire. Does not have sufficient scientific expertise to provide comment.</td>
<td>Noted</td>
</tr>
<tr>
<td>2</td>
<td>No impact likely in the short term on the urban council. Given that the negligible risks associated to this proposal and that the RARMP concludes that these risks can be managed, accepts the recommendation for the collection of information to verify the findings of the RARMP. Would be interested to know the outcomes of these findings in the future.</td>
<td>Noted</td>
</tr>
<tr>
<td>3</td>
<td>Does not have jurisdiction over, or decision-making powers, regarding growth, transport or sale of either GM crops or food produced from GM crops, so there is no requirement to seek its approval. Lacks scientific expertise to reach an overall conclusion on safety of GM crops to human health and the environment. Does not support the import and distribution of GM carnations. Acknowledges that a significant number of residents are opposed to GM crops and foods and are also concerned about marketing general and organic produce from the Shire.</td>
<td>Noted</td>
</tr>
</tbody>
</table>

³ Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.
<table>
<thead>
<tr>
<th>Sub. No.</th>
<th>Summary of issues raised</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Expresses concern of potential contamination of local biodiversity with insect borne GM pollen or organisms.</td>
<td>The RARMP prepared for this application concludes that the proposed commercial import and distribution of GM carnation cut-flowers poses negligible risks to the environment. A number of factors prevent pollen transfer from cultivated cut-flower carnations, including self-incompatibility, low pollen production, flower morphology and display environment where pollinating insects are absent. Imported cut-flowers are devitalised prior to import, preventing propagation from cuttings. GM and non-GM carnations are not considered weeds either in Australia or overseas. The is discussed in the RARMP in Ch 1 section 4.1 and Ch 2 risk scenario 2.</td>
</tr>
<tr>
<td>4</td>
<td>Does not have a specialist scientific expert and hence is unable to provide comment.</td>
<td>Noted</td>
</tr>
<tr>
<td>5</td>
<td>Has no issues if the GM cut-flowers have been devitalised and shown to be non-viable. Plant material should be imported according to relevant State biosecurity requirements.</td>
<td>The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. Biosecurity requirements are the responsibility of the Department of Agriculture and relevant State departments.</td>
</tr>
<tr>
<td>6</td>
<td>Has no objection to the import and distribution of GM carnation cut-flowers. Expresses confidence in OGTR’s risk assessment process and considers that risk of seed contamination is mitigated by devitalisation. Supports the local cut-flower production and imported flower distribution industry and does not want them impacted by restrictions.</td>
<td>Noted</td>
</tr>
<tr>
<td>7</td>
<td>Questions whether it is a GMO dealing as the risk of propagation seems limited. Has no concerns about this release.</td>
<td>Noted</td>
</tr>
<tr>
<td>8</td>
<td>Supports the OGTR’s conclusion that importation and distribution of GM carnations posed negligible risk of harm to human health and the environment. Noted that the GM carnations selectable marker SuRB and ALS protein are already present in GM carnations released in Australia with no adverse reports. Also noted that GM carnations are dipped in glyphosate to render them non-viable before import into Australia.</td>
<td>Noted</td>
</tr>
<tr>
<td>9</td>
<td>Is supportive of the application as the release poses negligible risks to people or the environment. Notes that licence conditions would ensure ongoing oversight and that GM carnations import would be subject to import regulation by Department of Agriculture.</td>
<td>Noted</td>
</tr>
<tr>
<td>10</td>
<td>Is satisfied with the conclusions of the draft RARMP and has no additional comments.</td>
<td>Noted</td>
</tr>
<tr>
<td>11</td>
<td>GTTAC agrees with the conclusions in the RARMP.</td>
<td>Noted</td>
</tr>
</tbody>
</table>
Appendix C  Summary of submissions from the public on the consultation RARMP

The Regulator received four submissions from the public on the consultation RARMP. The issues raised in these submissions are summarised in the table below. All issues raised in the submissions that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator’s decision to issue the licence.

**Abbreviations**

**Issues raised:** Co: Consultation; E: Environment; Et: Ethics; Hhs: Human health and safety; M: Marketing; R: Regulatory; Sc: Scope of legislation; T: Trade.

**Other abbreviations:** The *Gene Technology Act 2000*; Ch: Chapter; GM: Genetically modified; RARMP: Risk Assessment and Risk Management Plan; Regulator: The Gene Technology Regulator.

<table>
<thead>
<tr>
<th>Submission number</th>
<th>Issue</th>
<th>Summary of issues raised</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E, Hhs</td>
<td>States that environmental and human health and safety are threatened by modern society which seeks to manipulate nature and destroys natural systems.</td>
<td>The RARMP concluded that the commercial release of the GM carnation cut-flowers poses negligible risks to the health and safety of people and the environment. Its preparation included the use of information provided by the applicant, published scientific literature, and advice received from a range of Australian government authorities, agencies, experts. Ongoing oversight of the release will occur and the licence holder is required by the licence to report any unintended effects or risks.</td>
</tr>
<tr>
<td></td>
<td>Et</td>
<td>No immediate threat from GM flowers may be discerned, yet labelling them as “safe” is short sighted and misleading.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sc, Et</td>
<td>Protests against money spent approving GM crops for corporate interests. Is of the opinion that genetic modification is immoral in the present modern context.</td>
<td>The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. The matters raised are outside the legislative responsibility of the Regulator.</td>
</tr>
<tr>
<td></td>
<td>Co</td>
<td>States it is not in the public interest and not democratically debated.</td>
<td>The Regulator followed the decision making process as set out in the Act. One of the two rounds of consultation included the public.</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>Is 100% opposed to the idea.</td>
<td>Noted.</td>
</tr>
<tr>
<td>3</td>
<td>E</td>
<td>Expressed concerns that people would try to propagate the plants.</td>
<td>The Australian Department of Agriculture requires that all imported cut flowers and foliage that could be propagated must be treated to devitalise plant tissue prior to entering Australia. This issue is addressed in Ch 1, Section 6.1.1 and in Ch 2, risk scenario 2. In addition, growing the GM carnations in Australia is not permitted by the licence.</td>
</tr>
<tr>
<td>Submission number</td>
<td>Issue</td>
<td>Summary of issues raised</td>
<td>Comment</td>
</tr>
<tr>
<td>-------------------</td>
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</tr>
<tr>
<td>M</td>
<td>Also concerned about harm to local industry caused by imports.</td>
<td>When deciding whether or not to issue a licence, matters that relate to marketing and trade are outside the legislative responsibility of the Regulator. These issues are the responsibility of the States, Territories and industry.</td>
<td></td>
</tr>
</tbody>
</table>
| 4                 | Hhs   | Objects to the proposed release and has raised the following questions:  
- As the carnations are not approved for human consumption, how can this be ensured and what would be done if ingestion occurred?  
- What if animals or humans (especially little children) ingest the flowers?  
- Asks what allergic/intolerance testing is available if there is skin contact or are used in cosmetics, bridal bouquets, dining table centrepieces, flower essences, food colouring, cake decorations | The cut flowers would be sold for ornamental purposes only. The RARMP addresses toxicity and allergenicity via handling or consumption in Chapter 1 Section 5.3.3 and Chapter 2 risk scenario 1 and determined that there was negligible risk to people and the environment from these GM carnations. As discussed in Chapter 1 of the RARMP, carnations are not generally consumed. |
| E                 | What biosecurity checks are in place? Are the carnations living or dried flowers?  
How importers and retail customers would check for living organism passengers such as new soil pathogens or viable pollen and seeds. | Biosecurity risk from presence of pathogens is the responsibility of the Department of Agriculture. These are living flowers which are herbicide treated prior to entering Australia as required by the strict biosecurity requirements and procedures of the Department of Agriculture. This is addressed in Ch 1, Section 6.1.1 and in Ch 2, risk scenario 2. Growing the GM carnations in Australia was not proposed by the applicant and is not permitted by the licence. |
<p>| T                 | Asks if there was a tolerance for low level presence and adventitious presence of soil pathogens, pollen or seeds, and if those values were not zero, what would be the limits and why. | As discussed above, biosecurity requirements are the responsibility of the Department of Agriculture and relevant State departments. With regard to GM material, adventitious presence pertains to GMOs that have not been approved by any government authority. Since the GM carnations have been approved by other countries and the Regulator has decided to issue a licence for their import into Australia, adventitious presence does not apply in the case of these GM carnations. Low level presence applies when imported products carry low levels of GMOs that are not authorised in the importing country. Since the licence authorises import of GM carnation cut-flowers, the issue of low level presence does not arise. |</p>
<table>
<thead>
<tr>
<th>Submission number</th>
<th>Issue</th>
<th>Summary of issues raised</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>Asks how we could test for these GM carnations, if they escaped and grew. Asks who would own them and how they should be destroyed.</td>
<td>GM and non-GM carnations both in Australia and overseas are not considered weeds. This was addressed in Chapter 1 section 4.1 and Chapter 2 risk scenario 2. Growing the GM carnations in Australia is not permitted by the licence. This issue is addressed in Ch 1, Section 6.1.1 and in Ch 2, risk scenario 2. The risk assessment concludes that the risk from the GM carnations spreading in the environment is not greater than negligible. IFD has provided methods to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials (see Chapter 1 section 5.4.2).</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Asks who is going to do licence compliance checks.</td>
<td>The Regulator is responsible for maintaining oversight of licenced dealings. Regulatory compliance tools include monitoring, audits, practice reviews and investigations and are explained in some detail on our <a href="#">Regulatory Compliance</a> website page. The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>What happens to these flowers when they are disposed of/composted? Asks if the GM DNA would be picked up by soil organisms. Asks which studies on horizontal gene transfer are used in assessing the risk of transfer.</td>
<td>Disposal/composting of the flowers will result in them being degraded over time as is the case for any other flower, including both non-GM and other authorised GM carnations. Disposal and exposure of soil organisms to the GM carnations was addressed in Chapter 2 risk scenario 1. The potential for horizontal gene transfer from GMOs to other organisms, and any possible adverse outcomes, have been addressed in Chapter 2 section 1.</td>
<td></td>
</tr>
</tbody>
</table>