SECTION E

SNIF PART 2: Summary information format for products containing genetically modified higher plants (GMHPs)

GENERAL INFORMATION

E.1. Details of notification

(a)	**Member State of notification:** The Netherlands
(b)	**Notification number:** C/NL/09/01
(c)	**Name of the product (commercial and other names):** IFD-25958-3
(d)	**Date of acknowledgement of notification:** 17 - 03 - 2009

E.2. Notifier

(a)	**Name of notifier:**	Florigene Pty. Limited (Australia)
(b)	**Address of notifier:**	1 Park Drive, Bundoora, VIC 3083, Australia
(c)	**Is the notifier domestic manufacturer:** No	**Importer** Yes
(d)	**In case of an import the name and address of the manufacturer shall be given:** Florigene Pty. Limited, 1, Park Drive, Bundoora, VIC 3083, Australia.	
(e)	**Name and full address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor;** Ms. Juliette Gray Suntory Ltd. Buchanan House 3 St. James's Square, London SW1Y4JU, U.K. Phone 44-20-7839-9370 Fax 44-20-7839-9379 e-mail juliette.gray@suntory-uk.co.uk	

E.3. General Description of the product

(a)	**Name of recipient or parental plant and the intended function of the genetic modification:** Recipient plant is *Dianthus caryophyllus L.* (UK carnation, NL anjer, ESP clavel). The product consists of a carnation variety in which the flowers have a modified colour as the result of genes enabling the biosynthesis of delphinidin pigment. The flowers also carry an herbicide resistance gene to facilitate selection *in vitro*.
(b)	**Any specific form in which the product must not be placed on the market (seeds, cut-flower, vegetative parts, etc.) as a proposed condition of the authorization applied for:** None
(c)	**Intended use of the product and types of users:** The flower product will be sold in the cut flower market in the same way as other carnation flowers. Users include flower importers, flower auctioneers, flower wholesalers, retailers, and florists. Flowers will ultimately be sold to the general public.
(d)	**Any specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorization applied for:** There are no specific requirements.
(e)	**If, applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorization applied for:** None
(f)	**Any type of environment to which the product is unsuited:** None
(g)	**Any proposed packaging requirements:** No specific packaging will be used for transport or marketing of the cut-flowers. The flowers will be handled according to general practice in handling carnations.
(h)	**Any proposed labeling requirements in addition to those required by law:** Product information, including written advice and associated labeling and information will be provided with the imported flowers. Proposed wording is; "This product is a genetically modified carnation and is not for human or animal consumption nor for cultivation."
(i)	**Estimated potential demand:** The popularity of flower colour is highly sensitive to changes in consumer's taste. Currently, the estimated annual consumption in Europe is expected to be between 2.5 and 25 million flower stems.

(j) Unique identification codes(s) of the GMO(s):

Florigene codes	Unique Identifier number
25958	IFD-25958-3

E.4. Has the GMHP referred to in this product been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes ☐	No ☑

If no, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC: The transformation experiment resulting in line IFD-25958-3 was executed by Florigene Pty. Ltd. in Australia. The plants were propagated and planted in trials in Australia and Colombia under permit number NLRD 09 (AUS) and resolution 3858 (Colombia).

E.5 (a) Is the product being simultaneously notified to another Member State?

Yes ☐	No ☑

(i) If no, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC: An environmental risk assessment is provided with the application.

E.5 (b) Has the product been notified in a third country either previously or simultaneously?

Yes ☑	No ☐

(i) If yes, specify: IFD-25958-3 has approval for production in Colombia resolution 3932 and approval for import only in the USA.

E.6. Has the same GMHP been previously notified for marketing in the Community?

Yes ☐	No ☑

(i) If yes, give notification number and Member State:

E.7. Measures suggested by the notifier to take in case of unintended release or misuse as well as measures for disposal and treatment

None.

NATURE OF THE GMHP CONTAINED IN THE PRODUCT INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

E.8. Complete name

(a)	**Family name:**	Caryophyllaceae
(b)	**Genus:**	*Dianthus*
(c)	**Species:**	*caryophyllus*
(d)	**Subspecies:**	Not applicable
(e)	**Cultivar/breeding line:**	Recipient variety CW
(f)	**Common name:**	Carnation

E.9 (a) Information concerning reproduction

(i) **Mode(s) of reproduction:** The cultivated carnation is vegetatively propagated and to produce plants for cut flower production cuttings are taken from vegetative 'mother plants' which are continually pruned to produce a high number of vegetative cuttings from axillary buds. These cuttings are rooted in conditions of high humidity after treatment with rooting powder.

Rooted plants may be planted in soil or grown hydroponically, and are kept for 1 to 2 years. Flowers are produced in flushes beginning 3-5 months after rooted cuttings are planted. Picking of all flowers is essential and flowers must be harvested in tight bud (or closed bud for spray types) for distribution and marketing.

Carnation is not reproduced by seed and seed cannot form during cultivation. Carnation pollen can only be dispersed by lepidopteran insects such as moths. Pollen is not wind dispersed.

(ii) **Specific factors affecting reproduction, if any:** Imported cut-flowers have no capacity for gene dispersal by seed formation or pollen dispersal.

(iii) Generation time: Cultivated carnation is grown for 1 to 2 years. The application is for import of cut-flowers only.

E.9 (b) Sexual compatibility with other cultivated or wild plant species

Carnations are double-flowered cultivars and in the general trade and botanical and horticultural literature carnation cultivars are considered to belong to the species *Dianthus caryophyllus.* The common name for *Dianthus caryophyllus* is carnation. However, the exact taxonomic and breeding history of carnation is not known and it is almost certain that carnation is a hybrid involving two or more *Dianthus* species, one of which is *Dianthus caryophyllus.*

Whilst there are wild *Dianthus* species in Europe, there is no compatibility between these plants and imported carnation flowers. No report exists of spontaneous hybridization between carnation cultivated in Europe and either wild *Dianthus* types or species of other genera. There is no potential for hybridization.

E.10. Survivability

(a) Ability to form structures for survival or dormancy: The survival structures carnation can produce are seeds and pollen, though it is impossible for imported carnation flowers to form seed.

(b) Specific factors affecting survivability, if any: Imported carnation flowers will not survive more than three weeks in the hands of the consumer. During this time seed set is impossible.

Discarded carnation flowers have no vegetative propagation ability.

E.11. Dissemination

(a) Ways and extent of dissemination: Genetic material from cultivated carnation plants could theoretically be disseminated through seed or insect pollination or vegetative propagation. None of these avenues are realistic avenues for gene dispersal in the case of the carnation flowers imported into Europe.

(b) Specific factors affecting dissemination, if any: Not applicable.

E.12. Geographical distribution of the plant

The carnation is a cultivated plant and is not found in the wild, but is grown worldwide. In Europe, main production countries are Italy, Spain and the Netherlands. Carnation flowers are imported into

the EU from Africa, South America and the middle East. Wild *Dianthus caryophyllus* is very rare and can only be found in specific coastal regions of Corsica, Sardinia, France and Italy.

E.13. In the case of plant species not normally grown in the Member States(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

Carnation is cultivated and has no natural habitat. Carnation flowers are routinely imported into the EU from Africa, South America and the middle East and are also widely grown in Europe. Several billion carnation flowers are distributed within the EU per annum.

E.14. Potentially significant interactions of the plant with other organisms in the ecosystem where it is usually grown, including information on toxic effects on humans, animals and other organisms

The product is imported cut flowers and the receiving environment is the commercial environment of airports, warehouses, trucks and shops, and the home. The product will not be grown in Europe. Discarded flowers will be dead, or soon die and have no ability to survive after use and will not enter human or animal food chains.

Carnation has been used safely by humans for ornamental purposes for centuries. The modification in the GMHP (production of delphinidin) is novel for carnation, but there are many flowers and other ornamental species that produce delphinidin. Delphinidin is also present in many common foods.

Direct tests of potential toxicity indicate no potential for harm to plant, animal or human health.

There is now a long history of safe use of the products. Carnation is not reported to be a poisonous plant, and there is no evidence that the transgenic line has, or could, cause an adverse reaction.

E.15. Phenotypic and genetic traits

The GMHP of the present application consists of imported flowers, which have been harvested from carnation plants that have a modified flower colour and are herbicide resistant.

<u>Phenotype</u>

Flower colour is generally the result of the relative concentration and type of two pigment classes

- carotenoids and flavonoids. Of the two, flavonoids contribute the most to flower colour. Anthocyanins are flavonoid-based coloured pigments. There are three groups of anthocyanins, those based on delphinidin that generally produce blue flower colour, those based on cyanidin that produce red or pink flower colour, and those based on pelargonidin that produce orange or brick red flower colour. Non-genetically modified carnations lack the part of the anthocyanin biosynthetic pathway that is responsible for the production of the delphinidin molecule, due to the absence of a gene encoding the enzyme flavonoid 3'5' hydroxylase (F3'5'H) that converts dihydrokaempferol (DHK) to dihydroquercetin (DHQ) and then to dihydromyricetin (DHM). In the genetically modified carnation line IFD-25958-3, four genes have been introduced that encode a tobacco ALS (*Nt*ALS), viola F3'5'H (*Vh*F3'5'H) and petunia DFR (*Ph*DFR), along with a gene designed to down-regulate endogenous *DFR* (*Dc*DFR) in flowers with the aim of producing high levels of delphinidin-based anthocyanins. The *Ph*DFR enzyme can use either DHQ or DHM, but not DHK, as substrates. Delphinidin derivatives are thus produced as a result of the combined expression of the introduced genes *Ph*DFR and *Vh*F3′5′H together with other endogenous genes in the anthocyanin biosynthetic pathway, and the down-regulation of carnation *DFR*. The production of delphinidin leads to a change in flower colour. The flower product of this application has a purple shade, distinct from the cerise flower colour of the control line that was transformed to generate the transgenic.

The genes that have been inserted are:

(i) The **petunia *DFR* (*Ph*DFR) gene**, coding for dihydroflavonol 4-reductase (DFR), derived from *Petunia* X *hybrida*. The petunia DFR enzyme is only capable of using DHQ and DHM as substrates, but not DHK. It preferentially uses DHM over DHQ. This ensures that delphinidin is the predominant anthocyanidin. The *DFR* gene is under control of its own promoter.

(ii) The **pansy *F3′5′H* (*Vh*F3'5'H) cDNA**, coding for flavonoid 3' 5' hydroxylase (F3'5'H), derived from *Viola* hortensis. F3'5'H converts the dihydroflavonols DHK and/or DHQ into the dihydroflavonol DHM. The presence of the enzyme F3'5'H allows transgenic plants normally lacking this enzyme to produce violet or blue delphinidin-derived pigments.

(iii) The **carnation DFRhp** (*Dc*DFRhp) aimed at production of short interfering RNAs (siRNA) directed at the down-regulation of endogenous *DcDFR*. The down-regulation of endogenous *DFR* reduces the biosynthesis of pelargonidin and leads to an increase in the production of delphinidin-derived pigments, via the action of the introduced F3'5'H and petunia DRF enzymes.

(iv)	The **tobacco *ALS* gene *(SuR*B; *Nt*ALS*),*** coding for a mutant acetolactate synthase protein (ALS), derived from *Nicotiana tabacum.* Expression of ALS confers resistance to sulfonylurea herbicides. The gene is included to allow selection of transgenic shoots *in vitro.*

INFORMATION RELATING TO THE GENETIC MODIFICATION

E.16. Description of the methods used for the genetic modification

Genetic material was inserted into carnation by transformation using the disarmed *Agrobacterium tumefaciens* strain AGL0 carrying the transformation vector pCGP3366, developed by Florigene Pty. Ltd. Bundoora (Melbourne), Australia.

E.17. Nature and source of the vector used

The transformation vector pCGP3366 was developed by Florigene Pty. Ltd, Bundoora (Melbourne), Australia.

E.18. Size, source of the vector used

Position (nt)	Genetic element	Origin	Function
28484 – 581 (28599)	LB	T*i* plasmid *Agrobacterium tumefaciens* Octopine strain	Defines junction between T-DNA and plant genomic DNA or vector DNA. Includes site of T-DNA processing and inside and outside border regions. Utilized in transfer of insert to the plant cell and integration into the genome.
582 - 589	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
590 - 780	35S promoter	Cauliflower mosaic virus (CaMV)	Constitutive promoter in plants.
781 - 841	*Cab* 5'UTR	*Petunia* X *hybrida*	Chlorophyll a/b binding protein 5' untranslated region (UTR) from cDNA.
842 - 2836	*SuRB* (ALS)	Tobacco, *Nicotiana tabacum*	Encodes acetolactate synthase resistance to chlorsulfuron. Gene with own terminator (no promoter).
2837 - 4603	*Su*RB (ALS)	Tobacco, *Nicotiana tabacum*	Tobacco ALS (*Su*RB) terminator.
4604 - 4621	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
4622 - 5779	*CHS* promoter	Snapdragon, *Antirrhinum majus*	Flavonoid pathway promoter from a gene encoding chalcone synthase.
5780 - 5797	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
5798 - 7574	*F3'5'H* cDNA	*Viola* hortensis	Encodes the flavonoid 3' 5' hydroxylase protein. A key enzyme in the anthocyanin biosynthesis pathway leading to the biosynthesis of delphinidin.
7575 - 7586	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
7587 - 8405	'D8' terminator	*Petunia* X *hybrida*	Terminator sequence from petunia 'D8', a gene encoding a putative phospholipid transfer protein homologue.
8406 - 8418	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
8419 - 11401	*DFR* promoter	*Petunia* X *hybrida*	Dihydroflavonol 4-reductase promoter.
11402 - 13099	*DFR* genomic clone	*Petunia* X *hybrida*	Encodes the dihydroflavonol reductase protein with its own promoter and terminator; a key enzyme in the anthocyanin biosynthesis pathway. The gene is comprised of 6 exons and 5 introns.
13100 - 13376	*DFR* terminator	*Petunia* X *hybrida*	Dihydroflavonol 4-reductase terminator.
13377 - 13423	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
13424 - 13846	35S promoter	Cauliflower mosaic virus (CaMV)	Constitutive promoter in plants.
13847 - 13852	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
13853 - 14115	DFRhp sense arm	*Dianthus caryophyllus*	Partial dihydroflavonol 4-reductase sequence. A key enzyme in the anthocyanin biosynthesis pathway.
14116 - 14302	DFR intron	*Petunia* X *hybrida*	Non-functional intron used for anchoring the two arms of the hairpin construct.
14303 - 14565	DFRhp antisense arm	*Dianthus caryophyllus*	Partial dihydroflavonol 4-reductase sequence. A key enzyme in the anthocyanin biosynthesis pathway.

Position (nt)	Genetic element	Origin	Function
14566 - 14571	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
14572 - 14794	35S terminator	Cauliflower mosaic virus (CaMV)	Constitutive terminator in plants.
14795 - 14976	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
14977- 16820	RB	*Ti* plasmid *Agrobacterium tumefaciens* Octopine strain	Defines junction between T-DNA and plant genomic DNA or vector DNA. Includes site of T-DNA processing and inside and outside border regions. Utilized in transfer of insert to the plant cell and integration into the genome.
16821 – 16876	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.
16877 – 24791	pVS1 replicon	*Pseudomonas aeruginosa*	For replication in *Agrobacterium*. This is a broad spectrum replicon, which allows plasmid replication in a wide range of bacteria. Includes flanking sequences either side of origin of replication.
24792 – 26765	Tetracycline resistance gene complex	*E. coli*	Used for the selection of bacteria carrying the transformation vector. This DNA has a known function and encodes a membrane associated protein that prevents tetracycline from entering bacterial cells.
26766 - 28483	Modified pACYC184 replicon	*E. coli*	This low copy replicon allows replication in *E. coli* only. Includes flanking sequences either side of origin of replication.

INFORMATION RELATING TO THE GMHP

E.19 Description of the trait(s) and characteristics, which have been introduced or modified

Petals of the genetically modified carnation product of this application produce delphinidin based pigments whilst petals of carnations which are not modified do not. The production of delphinidin based anthocyanins results in a change in petal colour. The flower products of this application are a shade of purple, compared to the cerise flowers of the line from which the transgenic line was derived.

E.20 Information on the sequences actually inserted/deleted/modified

(a) Size and structure of the insert and methods used for its characterization, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP:

The transformation vector is 28,599 bp. The size and structure of the inserted sequence has been determined by Southern blot analysis, which confirmed only T-DNA between the left and right borders of the transformation vector pCGP3366 remained in the GMHP as one locus.

No carrier (*Agrobacterium tumefaciens*) remains in the GMHP.

(b) In the case of deletion, size and function of the deleted region(s):

Not applicable.

(c) Location of the insert in the plant cells (integrated in the chromosome, chloroplast, mitochondrion, or maintained in a non-integrated form) and method for its determination:

The insert has integrated into the plant chromosome as determined by T-DNA locus cloning and sequencing.

(d) Copy number and genetic stability of the insert:

The T-DNA is present at one integration locus and contains one copy of each T-DNA component as determined by Southern blot analysis (Attachment A4 and A5) as summarized in Table 1 below.

Table 1. Estimated copy number of T-DNA components using probes that span the region within transgenic tissue of the line IFD-25958-3

Probe	Estimated Copy Number in IFD-25958-3
LB	1
*Nt*ALS	1
*Vh*F3'5'H	1
*Ph*DFR	1
RB	1

IFD-25958-3 has been vegetatively propagated since 2005. Measured by flower colour, the line is genetically stable.

(e) In case of modification other than insertion or deletion, describe function of the modified genetic material before and after modification as well as direct changes in expression of genes as a result of the modification:

Not applicable.

E.21 Information on the expression of the insert

(a) Information on the expression of the insert and methods used for its characterization:

Expression of the insert has primarily been determined by detecting delphinidin-type pigments using thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC). Flowers of IFD-25958-3 contain approximately 0.54 mg delphinidin per gram fresh weight, determined by HPLC. Only transgenic flowers can produce such pigments through the activity of the enzyme F3′5′H encoded by one of the introduced genes. The substrates on which flower colour modification enzymes act are generally only present in flower petals at the appropriate stage of development.

Total RNA was isolated from petals of transgenic carnation line IFD-25958-3 and its parent, Cerise Westpearl (CW). The RNA from both lines was hybridized sequentially with probes specific to each component of the transformation vector pCGP3366. This analysis thus provides information regarding transgene expression in petal for each introduced gene in the carnation line IFD-25958-3.

The petal material analyzed was grown in the Florigene glasshouse facility in Melbourne, Australia. Petals derived from newly opened buds were carefully harvested, immediately snap frozen in liquid nitrogen and stored at -80°C. Total RNA was extracted from petals using an RNeasy Plant Mini Kit (Qiagen, Australia). Ten µg of total RNA was separated by electrophoresis in an agarose gel containing 1.2 % formamide, and then transferred to Hybond-NX nylon membrane (Amersham Biosciences, UK). RNA was hybridized with four different probes to determine the relative levels of expression of the respective transgenes in petal tissue. DNA fragments (25-50 ng) were labelled with 50 µCi of [α-^{32}P]-dCTP (PerkinElmer Life and Analytical Sciences, USA) using a Decaprime kit (Ambion, USA).

The transformation vector pCGP3366 carries four expression cassettes:

(i) tobacco ALS *(Nt*ALS*)* driven by a CaMV 35S promoter,

(ii) viola F3'5'H (*Vh*F3'5'H) driven by an *Antirrhinum majus* CHS promoter,

(iii) petunia DFR (*Ph*DFR) driven by its own promoter and

(iv) a dsRNA::carnation DFR hairpin cassette (*Dc*DFRhp) driven by a CaMV 35S promoter.

The four cassettes are directed at *de novo* expression of the respective transgenes, for selection of transformed cells in tissue culture (*Nt*ALS) and development of novel flower colour (*V*hF3′5′H, *Ph*DFR and *Dc*DFRhp). Thus, transgenes driven by the CaMV promoter would be expressed in most tissues at most stages of development. Dividing cells would likely give rise to higher levels of expression than non-dividing cells. The transgenes driven by anthocyanin pathway promoters should exhibit expression profiles similar to their endogenous counterparts.

Probes used in northern analysis were thus based on the expression cassettes present in the transformation vector pCGP3366. The probes used were *Nt*ALS, *V*hF3′5′H, *Ph*DFR and *Dc*DFRhp (Figure 1). No expression was detected in the control line CW for probes *Nt*ALS, *V*hF3′5′H and *Ph*DFR. The *Dc*DFRhp probe hybridized to endogenous DFR sequences within

parental line CW and the hairpin cassette in transgenic line IFD-25958-3. A degradation product can also be seen in the autoradiograph for transgenic line IFD-25958-3 when using the *Dc*DFRhp probe, which suggests active post-transcriptional gene silencing of endogenous DFR sequences. Expression levels were not quantified.

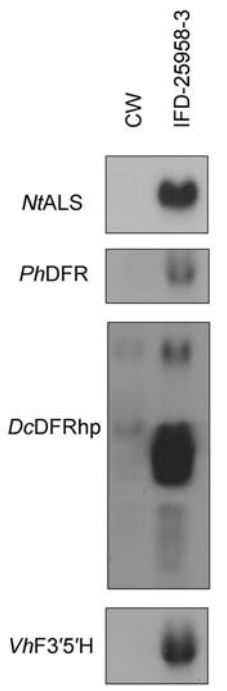

Figure 1. Autoradiographs of northern blots of RNA derived from petal tissue from line IFD-25958-3, hybridized with four probes: **Nt*ALS*, V*hF3'5'H*, Ph*DFR and* Dc*DFRhp.***

(b) Parts of the plant where the insert is expressed (e.g. roots, stem, pollen, etc): Flowers of the genetically modified carnation product produce delphinidin-based pigments whilst carnations which are un-modified do not. The production of delphinidin ultimately results in a change in flower colour. Transgenic flowers are purple, compared to the white flowers of the control line. Delphinidin-based pigments have not been observed in other tissues of the transgenic flowers and plants, such as stems, nodes, leaves and roots. Production of delphinidin-based pigments is confined to the petals as a result of the use of floral specific promoters for some genes and because the biochemical pathway leading to anthocyanin biosynthesis is induced to coincide with flower development. Thus substrates on which the introduced F3′5′H enzyme act are typically only found in flower petal tissue.

The *Nicotiana tabacum* ALS gene (*Nt*ALS; SuRB) and dsRNA::carnation DFR hairpin cassette (*Dc*DFRhp) are under the direction of a CaMV 35S promoter that generates transcripts in various plant tissues including petal tissue. Hybridization signal indicates the introduced *Nt*ALS mRNA is present in petal tissue. The *Petunia* X *hybrida* DFR (*Ph*DFR) gene used is under the

direction of its own promoter which typically directs expression through most stages of flower development. The *Viola* hortensis F3′5′H gene (*Vh*F3′5′H) is under the control of a *Dianthus caryophyllus* CHS (*Dc*CHS) promoter, which is floral specific.

E.22 Information on how the GMHP differs from the recipient plant in:

(a) Mode(s) and/or rate of reproduction: There no avenue of reproduction from imported cut flowers of either recipient or GMHP.

(b) Dissemination: There are three theoretical avenues of gene dispersal from an imported carnation flower;

1. Vegetative spread of the imported cut flowers leading to the formation of wild clonal populations.
2. Formation and dispersal of seed from the imported cut flower as a result of self fertilization or fertilization with pollen from an external source.
3. Formation of seed by a recipient plant, fertilized by pollen dispersed from the imported cut flower.

The probability of gene dispersal from a carnation flower, of recipient or GM origin, is negligible to nil.

(c) Survivability: Imported flowers of the GMHP have no greater ability to survive than flowers from any other carnation variety, including the recipient.

(d) Other differences: The primary difference between IFD-25958-3 and the recipient plant is in the colour of the flowers, because of the production of delphinidin in the GMHP. The transgenic line IFD-25958-3 produces flowers with more petals than the parental line it is derived from and a thicker stem at the 5th node. IFD-25958-3 also shows increased filaments which are significantly shorter than the parental line.

E.23 Potential for transfer of genetic material from the GMHP to other organisms

There is an extremely low risk of gene dispersal for imported carnation flowers. The imported flowers from the GMHP have no enhanced ability to transfer genetic material.

E.24 Information on any harmful effects on human health and the environment, arising from the genetic modification

Carnation has been used safely by humans for ornamental purposes for centuries. The modification in the GMHP (production of delphinidin-based pigments) is novel for carnation, but there are many flowers and other ornamental species that produce delphinidin-based pigments. Delphinidin-based pigments are also present in many common foods.

Carnation is not reported to be a poisonous plant or to cause allergic reactions and there is no evidence that the transgenic line has or could cause an adverse reaction. There is now an extensive history of safe use of the product. Carnation is not used as a food but there is a slight possibility that some home consumers may decide to eat flower petals or garnish foods with flower petals. In the event that this did occur we do not believe the transgenic carnation poses any health risk because the novel products in the GMHP are found naturally in many foods. Open reading frame analysis of introduced regions of DNA reveals that the deduced amino acid sequences of the transgenic carnation line in this application are neither toxic nor allergenic. Direct tests of potential toxicity indicate no potential for harm to plant, animal or human health.

There is no potential for gene dispersal. The settings in which the imported flowers will be used, the relatively small number of flowers imported, their dispersal across Europe and the short longevity of the flowers are all factors that preclude any direct or indirect effect on the environment.

E.25 Information on the safety of the GMHP to animal health, where the GMHP is intended to be used in animal feedstuffs, if different from that of the recipient/parental organism(s)

Not applicable. The product is not intended to be used as animal feed.

E.26 Mechanism of interaction between the GMHP and target organisms (if applicable), if different from that of the recipient/parental organism(s)

Not applicable. There are no target organisms.

E.27 Potentially significant interactions with non-target organisms, if different from the recipient or parental organism(s)

The flowers from the GMHP are intended to be used for human consumption as an ornamental product in the same way as other carnation flowers. There are no changes in this interaction as a result of the genetic modification.

E.28 Description of detection and identification techniques for the GMHP, to distinguish it from the recipient or parental organism(s)

The GMHP can be distinguished using DNA based identification methods such as Southern analysis and PCR. A PCR based identification technique has also been developed that will allow the product to be distinguished from other transgenic carnation lines.

Flower colour can be used to distinguish the product from the recipient plant and biochemical tests such as thin layer chromatography may be used to determine that delphinidin based pigments are produced. The product, but no non GM carnation variety, is able to produce delphinidin.

INFORMATION ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OF THE GMHP

E.29 Potential environmental impact from the release or the placing on the market of GMOs (Annex II, D2 of Directive 2001/18/EC), if different from a similar release or placing on the market of the recipient or parental organism(s)

There is no environmental impact from the placing on the market of the GMHP which would be different to that of placing flowers from the recipient plant on the market. Carnation flowers from many varieties, including the recipient, are a commodity in the EU and several billion non-GM carnation flowers are consumed per annum in the community. There is no evidence the products would have any adverse effects;

- An analysis of the biology of carnation shows no potential for gene dispersal as a result of import of cut-flowers. The flowers are not invasive and there is no opportunity for the cut-flowers to become weeds. Carnation is not a weed in Europe and despite hundreds of years of cultivation and plantings in parks and gardens it has not become a weed or escaped from

cultivation anywhere in the world. No hybrid between carnation and any other *Dianthus* species has ever been recorded in the wild.

- Field trial results show substantial equivalence between IFD-25958-3 and the recipient plant, aside from an increase in petal number and stem thickness at the 5th node. IFD-25958-3 also shows increased filaments which are significantly shorter.

There is no evidence that carnation flowers in general and the transgenic line that is the subject of this application specifically have any pathogenic, phyto-toxic, toxic or allergenic properties.

E.30 Potential environmental impact of the interaction between the GMHP and target organisms (if applicable), if different from that of the recipient or parental organism(s)

Not applicable. There are no target organisms.

E.31 Possible environmental impact resulting from potential interactions with non-target organisms, if different from that of the recipient or parental organism(s)

(a) Effects on biodiversity in the area of cultivation: Not applicable. The products are cut flowers and will not be cultivated.

(b) Effects on biodiversity in other habitats: Not applicable. The imported cut flowers have no means to become established in any habitat.

(c) Effects on pollinators: Not applicable. Imported cut flowers are very unlikely to come into contact with pollinators in the environment in which they will be used.

(d) Effects on endangered species: Not applicable. The imported cut flowers have no means to become established and will be consumed in the human household environment in the same way as other carnation flowers.

INFORMATION RELATING TO PREVIOUS RELEASES

E.32 History of previous releases notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

(a) Notification number: The variety IFD-25958-3 has not been notified. However, similar products have been approved under Part B of directive 90/220/EEC and three varieties of these approved GM carnation have been imported into the EU;

Trade name	Plasmid	OECD ID No. (Unique Identifier)	EU approval & registration No.
FLORIGENE Moondust™	pCGP1470	FLO-07442-4	C/NL/96/14-11
FLORIGENE Moonshadow™	pCGP1991	FLO-11363-1	C/NL/97/13-1363A
FLORIGENE Moonlite™	pCGP1470	FLO-4Ø644-4	C/NL/04/02

(b) Conclusions of post-release monitoring: Production sites overseas have been monitored for escapes from cultivation of the transgenic carnation and none have been found. There have been no reports from growers and consumers of the product relating to harmful effects on human health.

(c) Results of the release in respect to any risk to human health and the environment (submitted to the competent authority according to Article of Directive 2001/18/EC):

The product has been released in Colombia and there have been no reports from growers of the product relating to harmful effects on human health.

E.33 History of previous releases carried out inside or outside the Community by the same notifier

(a) Inside the community:

There has been no previous releases of this line

(b) Outside the community:

Colombia

A resolution (3932) permitting commercial release was issued in November 2008. Production of IFD-25958-3 is to begin in early 2009 and first flowers for export to be ready from mid 2009.

USA

As of the 17th September 2008 applications to import genetically modified cut carnations are no longer required. A letter from APHIS dated 17th September 2008 has permitted Florigene to import any genetically modified cut carnations, it states:

"APHIS considers cut carnations as being incapable of self-propagation, and because gene flow from pollen produced from carnations during transit is not reasonably foreseeable, no permits from BRS are required for the interstate movement or importation of GE cut carnation; likewise a petition for non regulated status. Our conclusion that these plants destined for import are not regulated articles".

INFORMATION RELATING TO THE MONITORING PLAN- IDENTIFIED TRAITS, CHARACTERISTICS AND UNCERTAINTIES RELATED TO THE GMO OR ITS INTERACTION WITH THE ENVIRONMENTAL THAT SHOULD BE ADDRESSED IN THE POST –COMMERCIALIZATION MONITORING PLAN

E.34 Information relating to the monitoring plan- identified traits, characteristics and uncertainties related to the GMO or its interaction with the environmental that should be addressed in the post – commercialization monitoring plan

Transgenic carnation now has sufficient history of safe use to support the fact that the biology of the crop precludes gene dispersal and dissemination from transgenic carnation at either production locations or after import of flowers.

- In trials, no observations were made to suggest that the GMHP behaved any differently to non-genetically modified carnation.
- Several million genetically modified carnation plants have been grown in South America since 2000, and several million flowers produced. Surveys of the production sites have found no evidence of dissemination from outside of the cultivation area and there have been no adverse effect reports from any of the workers handling the plants or flowers.
- Several million genetically modified cut carnation flowers have been exported to the USA and Japan with no reports of adverse effects on distributors or end users.
- As of the 17th September 2008 any genetically modified cut carnation may be imported into the USA with no restrictions. No monitoring of these GMO's is required.
- There is experience of selling three similar transgenic carnation varieties within the EU, without any reports of adverse effects.

A general monitoring plan has therefore been proposed for this product. The environmental risk

assessment indicates no risks associated with the import of the GMHP and that issues associated with imports are the same as non GM carnation flowers. As the flowers will not be grown in the EU, there is no requirement for monitoring of production locations within the EU.

Information collected will be from general surveillance, rather than collection of specific data or tracing products to end use.