Application to import cut-flowers of carnation (*Dianthus caryophyllus*) line SHD-27531-4 for distribution and retail under directive 2001/18/EC

SNIF

Contact Dr Stephen Chandler
Phone; +61 409 387 386
schandler@florigene.com.au

802/ 454 Saint Kilda Road, Melbourne, VIC 3004, Australia

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/13/01

(c) Name of the product (commercial and other names):
SHD-27531-4 (no commercial name)

(d) Date of acknowledgement of notification:
13/03/2013

E.2 Notifier

(a) Name of notifier:
Suntory Holdings Limited

(b) Address of notifier:
2-1-40 Dojimahama
Kita-ku, Osaka City, Osaka
530-8203
Japan

(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
C.I. Flores Luna Nueva C.I. (NIT 90038024-7), Sesquile, Cundinamarca, Colombia

(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. Sonya Kikuchi
Suntory Holdings Limited.
Buchanan House
3 St. James's Square,
London SW1 Y4JU, U.K.
Phone 44-20-7839-9370
Fax 44-20-7839-9379
e-mail Sonya.kikuchi@suntory.eu

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.* 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. More information is provided at the answer to question C13.

(h) Any proposed labeling requirements in addition to those required by law:
Please refer to Question C8. In compliance with article 6 of EC/1830/EC, products will be labeled. The proposed wording to be used will be;
"This product is a genetically modified carnation and is not for human or animal consumption nor for cultivation."

(i) Estimated potential demand:
The amount of annual imports will depend on demand and is difficult to estimate as the popularity of flower colour is highly sensitive to changes in consumer's taste. The estimated annual utilization in Europe for SHD-27531-4 is expected to be between 10 and 25 million flower stems once maximum market penetration is reached.

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

Suntory code	Unique Identifier number
27531	SHD-27531-4

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/EC?

Yes ☐	No ☑

If no, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC:
The risk analysis (section B, Attachments B1, B2, B3 and B4) has been carried out on the basis of the elements of Part B of Directive 2001/18/EC

E.5(a) Is the product being simultaneously notified toanother 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 (section B).

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

Yes ☑	No ☐

The product has been approved for commercial production in Colombia. Cut flowers of the product are approved to be imported into the USA and Canada.

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

Yes ☐	No ☑

If yes, give notification number and Member State:
Not applicable.

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) Sub-species:
Not applicable

(e) Cultivar/breeding line:
Product is the cultivar SHD-27531-4

(f) Common name:
UK carnation, NL anjer, ESP clavel, FR œillet, DE nelke, IT garofano

E.9 (a) Information concerning reproduction

(i) Modes(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

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 Geographic distribution of the plant

The carnation is a cultivated plant and is not found in the wild. It 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 rare and in the wild are primarily found in 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 normally widely grown in Europe

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.
An environmental risk assessment indicates no potential for harm to plant, animal or human health. There is now a long history of safe use of the products.

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.

Phenotype

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 SHD-27531-4, genes have been introduced that encode a tobacco ALS (ALS), viola F3'5'H (F3'5'H) and petunia DFR (DFR). The DFR enzyme can use either DHQ or DHM, but not DHK, as substrates. Delphinidin-based anthocyanins are thus produced as a result of the combined expression of the introduced genes DFR and F3′5′H together with other endogenous genes in the anthocyanin biosynthetic pathway. The production of delphinidin leads to a change in flower colour. The flower product of this application has a purple shade, distinct from the pink flower colour of the parental recipient variety that was transformed to generate the transgenic.

Details of the genes inserted are;

(i) The **petunia *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* 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-based pigments.

(iii) The **tobacco *ALS* gene,** 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 pCGP1991.

E.17 Nature and source of the vector used

The transformation vector pCGP1991 was developed by Florigene Pty. Ltd, Bundoora (Melbourne), Australia.

E.18 Size, source of the vector used

The transformation vector pCGP1991 is 27,488 bp. The table below lists the elements of the transformation vector.

Position (nt)	Genetic element	Origin	Function	Encompasses open reading frame
1 - 697	LB	*Ti* plasmid *A. tumefaciens* Octopine strain	Defines junction between T-DNA and plant genomic DNA. Utilized in transfer of insert to the plant.	No
698 - 705	polylinker	pBluescript/pUC series *E. coli* vectors	Residual sequences from vectors used in assembling transformation vector.	No
706 - 896	35S promoter	Cauliflower Mosaic Virus	Constitutive promoter in plants.	No
897 - 957	Cab 5'utr	*Petunia* X *hybrida*	5' untranslated region (utr) of the Chlorophyll a/b binding protein gene	No
958 - 4719	*Su*RB (ALS)	Tobacco, *Nicotiana tabacum*	Encodes Acetolactate Synthase. Chlorsulfuron-resistance gene with terminator. Chlorsulfuron is only used during the tissue culture process.	Yes
4720 - 4985	polylinker	pBluescript/pUC series *E. coli* vectors	Residual pBluescript and/or pUC series vector DNA used for making the construct. Cloning & ligation sites.	No
4986 - 9943	*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 .	Yes
9944 - 9984	polylinker	pBluescript/pUC series *E. coli* vectors	Residual pBluescript and/or pUC series vector DNA used for making the construct. Cloning & ligation sites.	No
9985 - 11142	*CHS* promoter	*Antirrhinum majus*	Flavonoid pathway promoter from a gene encoding chalcone synthase.	No
11143 - 11186	polylinker	pBluescript/pUC series *E. coli* vectors	Residual pBluescript and/or pUC series vector DNA used for making the construct. Cloning & ligation sites.	No
11187 - 12963	*F3'5'H* cDNA	*Viola* sp.	Encodes the flavonoid 3', 5'hydroxylase protein. A key enzyme in the anthocyanin biosynthesis pathway leading to the biosynthesis of delphinidin.	Yes
12964 - 12975	polylinker	pBluescript/pUC series *E. coli* vectors	Residual pBluescript and/or pUC series vector DNA used for making the construct. Cloning & ligation sites.	No
12976 - 13794	'D8' terminator	*Petunia* X *hybrida*	Terminator sequence from petunia 'D8', a gene encoding a phospholipid transfer protein.	No
13795 - 13981	polylinker	pBluescript/pUC series *E. coli* vectors	Residual pBluescript and/or pUC series vector DNA used for making the construct. Cloning & ligation sites.	No
13982 - 14261	RB	*Ti* plasmid *A.tumefaciens* Octopine strain	Defines junction between T-DNA and plant genomic DNA. Utilized in transfer of insert to the plant.	No
14262 - 23796	pVS1 replicon	*Pseudomonas aeruginos*	For replication in *A. tumefaciens*. 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.	No
23797 - 25770	Tetracycline resistance gene complex	*Escherichia 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.	Yes
25771 - 27489	Modified pACYC184 replicon	*Escherichia coli*	This low copy replicon allows replication in *E. coli* only. Includes flanking sequences either side of origin of replication.	No

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 SHD-27531-4 produce delphinidin-based pigments whilst petals of non-transgenic carnations 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 pink flowers of the line from which the transgenic line was derived.
An herbicide resistance gene confers resistance to sulphonylurea-type herbicides for selection *in vitro*.

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 27,488 bp. The size and structure of the inserted sequence has been determined by Southern blot analysis and sequencing. Sequence has been inserted in a single locus and only T-DNA occurs between the left and right border sequences of this insert.
No carrier (*Agrobacterium tumefaciens;* AGL0) 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 chromosome.

(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) as summarized in the table below.

Probe	Estimated Copy Number in SHD-27531-4
LB	1
*Nt*ALS (*Sur*B)	1
*Vh*F3'5'H	1
*Ph*DFR	1
RB	1

SHD-27531-4 was regenerated in 2007 and has been vegetatively propagated continuously since that time. There has been no incidence of colour change since that time, measured by flower colour, indicating SHD-27531-4 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 high pressure liquid chromatography (HPLC). Flowers of SHD-27531-4 contain approximately 1.2 mg delphinidin per gram fresh weight, determined by HPLC.
The three cassettes are directed at *de novo* expression of the respective transgenes, for selection of transformed cells in tissue culture (ALS) and development of novel flower colour (F3′5′H and DFR). The transgenes driven by anthocyanin pathway promoters exhibit expression profiles similar to their endogenous counterparts.
Expression was determined by Northern analysis. Probes used were *Nt*ALS, *V*hF3′5′H and *Ph*DFR. No expression was detected in the parental line for any of the three probes.

(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 red-purple, compared to the pink flowers of the parental recipient 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 (ALS; *SuR*B) and is under the direction of a CaMV 35S promoter that generates transcripts in various plant tissues including petal tissue. *Petunia* X *hybrida* DFR (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 (F3′5′H) is under the control of a *Dianthus caryophyllus* CHS promoter, which is floral specific.

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

(a) Mode(s) and/or rate of reproduction:
There no avenues 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 SHD-27531-4 and the recipient plant is in the colour of the

flowers, because of the production of delphinidin in the GMHP. SHD-27531-4 produced significantly fewer filaments and viable anthers than the recipient, and significantly shorter filaments. Pollen from SHD-27531-4 was less viable than pollen from the parental recipient line.

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

There is no realistic potential for 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, and other transgenic carnation varieties. Delphinidin-based pigments are also present in many common foods. Consumption of delphinidin-based anthocyanins has been associated with health benefits in humans. 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

There is now an extensive history of safe use of colour-modified transgenic carnation in Europe and elsewhere in the world. 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.

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 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 from other transgenic carnation varieties using Southern analysis and PCR. A PCR based detection method has 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 and similar transgenic varieties, but not non GM carnation varieties, are 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 expected 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 and two other transgenic carnation varieties, are a commodity in the EU and several billion non-GM carnation flowers are consumed per annum in the community. There is no evidence SHD-27531-4 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 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.
- There is no evidence that carnation flowers in general and the transgenic line that is the subject of this application specifically have any pathogenic, phytotoxic, 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 SHD-27531-4 has not been notified. However, similar products have been approved under Part B of directive 90/220/EEC and flowers of two transgenic carnation varieties are currently imported into the EU;

Trade name	OECD ID No. (Unique Identifier)	EU approval & registration No.
FLORIGENE® Moonaqua™	FLO-40689-6	C/NL/06/01
FLORIGENE® Moonlite™	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. Post release monitoring of FLORIGENE® Moonlite™ and FLORIGENE® Moonaqua™ has been carried out since 2008 and annual reports have been submitted to the EU since then. The conclusions of the monitoring have consistently confirmed that wild type, unimproved *D.caryophyllus* is rare in Europe and that there is no evidence of the establishment of transgenic carnation in the wild, or of introgression to wild *Dianthus* species.

(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.

E33 History of previous releases carried out inside or outside the Community by the same notifier

(a) Inside the community:
There has been no previous release of SHD-27531-4 inside the community.
The notifier, Suntory Holdings Limited. has no previous releases inside the community.
Florigene Pty. Ltd., a fully owned subsidiary of the notifier, has two marketing approvals in the EU, both of which are for carnation (*Dianthus caryophyllus*).

Trade name	OECD ID No. (Unique Identifier)	Vector	Authorisation number	Consent date	Current status
FLORIGENE® Moonlite™	FLO-4Ø644-4	pCGP1470	C/NL/04/02	July 11 2007	Flowers are imported to EU
FLORIGENE® Moonaqua™	FLO-4Ø689-6	pCGP1991	C/NL/06/01	July 24 2009	Flowers are imported to EU

Florigene Pty. Ltd., a fully owned subsidiary of the notifier, also have two now had withdrawn marketing approvals in the EU, and two applications in progress. All four dossiers are for carnation (*Dianthus caryophyllus*).

Trade name	OECD ID No. (Unique Identifier)	Vector	Authorization number	Consent date	Current status
FLORIGENE® Moondust™	FLO- 07442-4	pCGP1470	C/NL/96/14-11	1 December 1997	After several years of import, now withdrawn
FLORIGENE® Moonshadow™	FLO-11363-1	pCGP1991	C/NL/97/13-1363A	October 20 1998	After several years of import, now withdrawn
FLORIGENE® Moonberry™	IFD-25958-3	pCGP3366	C/NL/09/01	Assessment report 21 October 2009	Under review
FLORIGENE® Moonvelvet™	IFD-26407-2	pCGP2355	C/NL/09/02	Assessment report 21 October 2009	Under review

(b) Outside the community:
SHD-27531-4 has commercial approval, and is grown, in Colombia. Flowers of the line have approval for export to the United States and to Canada.
The notifier and its fully owned subsidiary, Florigene Pty. Ltd. have a long and extensive history of previous releases of transgenic carnation, rose and chrysanthemum outside of the community. In terms of transgenic carnation lines similar to SHD-27531-4 i.e. that have the same selectable marker gene and have been genetically modified to accumulate delphinidin-based anthocyanins, eight varieties have been released commercially. Details of outside commercial release of commercial varieties generated with the same transformation vector (pCGP1991) are;
United States. Flowers of three transgenic varieties have been imported into the USA since 1997.
Canada. Flowers of three transgenic varieties have been imported into Canada since 1999.
Japan. A commercial variety was grown in Japan from 1998 to 2001. Flowers of three transgenic varieties have been imported into Japan since 1997.
Australia. GM carnation has been grown and sold in Australia since 1997. Two varieties generated using pCGP1991 were placed on the GMO register in 2004.
Ecuador. Three transgenic varieties have been grown and exported since 1997.
Colombia. Three transgenic varieties have been grown and exported since 2000.

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.

- Tens of million genetically modified carnation plants have been grown in South America since 2000, and nearly 200 million flowers exported. 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.
- There is experience of importing millions of flowers from three similar transgenic carnation varieties within the EU, without any reports of adverse effects.

The monitoring plan is based on general surveillance. 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. As no potentially adverse affects have been identified for the GM product described in this application, case-specific monitoring is not appropriate for SHD-27531-4 as the guideline parameters that are recommended for case specific monitoring do not apply. For example;

- Because there is no avenue for gene dispersal it is not possible to document the spread, persistence and accumulation of transgenes and recombinant proteins.
- There are no apparent organisms, food chains or habitats that are affected by conventional carnation flower imports, which could therefore be the subject of specific attention for transgenic carnation.
- It is not possible to quantify a baseline environment in the absence of the imported carnation flowers in the floral trade distribution environment and household environment.

The components of the monitoring plan are;
Importer questionnaire. On an annual basis the importers will be asked to complete the questionnaire detailing any adverse observations.
Consumer feedback.
Survey reports. Each year Suntory engages the services of botanists with interests in *Dianthus* biology to assist with identification of any wild populations or unusual *Dianthus* hybrids they find during their routine survey work.
Institutional mail out. Suntory has a mailing list of herbaria, national botanical survey networks, plant protection services and botanical gardens in Europe. These are contacted each year to seek information on any Dianthus collections or samples which come into their institutions.
Literature review. Each year, the scientific literature is reviewed for new reports on *Dianthus* taxonomy, botany and vegetation.
Web site, database and personal communication review. Each year, web sites and data bases are reviewed for new reports on *Dianthus* taxonomy, botany and vegetation.