ASSESSMENT REPORT OF THE DUTCH COMPETENT AUTHORITY IN ACCORDANCE WITH DIRECTIVE 2001/18/EC

NOTIFICATION C/NL/09/01

1. THE NOTIFICATION

The notification, submitted by Florigene Pty. Ltd, Bundoora, Australia, concerns placing on the market of imported cut flowers derived from genetically modified carnation (*Dianthus caryophyllus*) line IFD-25958-3 in accordance with Directive 2001/18/EC. The flowers of the carnation line have been modified with the *F3'5'H* (*Viola hortensis*) and *dfr* (*Petunia x hybrida*) genes, resulting in a modified flower color (purple). Line IFD-25958-3 also contains a herbicide resistance gene (*SuRB),* used to facilitate selection *in vitro*.

2. SCOPE OF THE NOTIFICATION

This notification concerns import, distribution and retailing of line IFD-25958-3 in the cut flower market in the same way as any other carnation. This notification does not include cultivation, the use as feed or as food of line IFD-25958-3.

3. HISTORY

Carnation line IFD-25958-3 has been tested in the field in Australia and has been approved for commercial production in Colombia since November 2008. Three similar transgenic varieties FLORIGENE Moonshadow™ (C/NL/97/13-1363A), Moondust™ (C/NL/96/14-11) and Moonlite™ (C/NL/04/02) have already been approved in the EU for cultivation and /or for import, distribution and retailing.

4. PROCEDURE

The Dutch competent authority (CA) received this dossier on March 17, 2009 under Directive 2001/18/EC. The dossier has been assessed with reference to Article 13 of this directive.

Additional information
During the assessment period no further information was requested.

Scientific advice
Based on the notification of March 17 (2009) the Dutch scientific advisory committee (COGEM) gave its advice on April 29 (2009) (CGM/090429-01). The COGEM concluded that the risks for the environment and human health associated with import of cut flowers of line IFD-25958-3 are negligible.

Public comments
The Summary Notification Information Format (SNIF) was initially published on the Joint Research Center (JRC) website on March 26 (2009). Public comments were received during 30 days, and originated from the Netherlands. The two public comments, one in Dutch and one in English but both originating from Dutch persons, are addressed by the Dutch CA in this assessment report, and are summarized below. No public comments originating from other member states were received during the abovementioned 30 days.

Public comments on the notification C/NL/09/01 and reaction of the Dutch CA
Public comments (anonymous) which were addressed by the Dutch CA were:

1. A member of the public notes that in The Netherlands there is general awareness against manipulated items, therefore genetic manipulated carnations should not be allowed to be introduced. Such manipulations are considered never beneficial in the real sense of the word, for any other party other than narrow minded money makers.
   Answer: According to Annex VI of Directive 2001/18/EC a notification has to be assessed on potential risks for human health and the environment only. Therefore comments not related to the environmental safety of the product are not taken into account in the assessment of this notification on genetically modified carnation.

2. A member of the public asks what would happen if bees are attracted to the genetically modified carnation flowers and would carry away modified pollen. It is known that a German beekeeper could not sell its biological honey because of the presence of genetically engineered ingredients in the honey.
Answer: The scope of the notification is for import of cut flowers for ornamental use, and excludes cultivation. Therefore bees will not come in contact with the flowers. Even if they would come in contact, potential spread of pollen will be negligible since domesticated carnations produce only a few anthers and little pollen.

3. The person asks what would happen with butterflies that are mainly attracted to purple flowers. Purple flowers seem to carry much nectar, this seems to be important for the flower since butterflies are involved in pollination of flowers.
Answer: See also the reaction above.
Butterflies that are mostly attracted to purple flowers will not be affected by the ornamental use of the carnations, since there will be no direct contact. Even in case a butterfly will come in contact with a few purple carnations, this will have no effect on populations of butterflies.

4. A member of the public asks if as a result of the changed flower color the bees, butterflies and other useful insects still recognize the color. What do we know about the function of flower color anyway? According to this person a certain color represents a certain property or functionality, which is unknown.
Answer: See also the reaction above. The scope of the notification is for import of cut flowers for ornamental use, and excludes cultivation. Therefore bees or butterflies will not come in contact with the flowers. The question whether insects still recognize the color of the flower or the question what the function is of flower color is therefore not of relevance for this notification.

5. A member of the public notes that carnation flowers may form roots or may be otherwise propagated. Therefore it can not be ruled out that the material will be propagated by third parties.
Answer: The Committee on Genetic Modification (COGEM) has reviewed this aspect in her advice CGM/090429-01. Carnation is not able to spread vegetatively and cut flowers are not able to form roots. It cannot be completely ruled out that buyers will propagate material to plant in their gardens. However, carnation has no weedy characteristics. Although carnation has been cultivated for decades, it has never been found outside the cultivation areas. The introduced traits (modified flower color and herbicide tolerance) do not introduce a potential for weediness. It is therefore highly unlikely that the genetically modified carnation line IFD-259558-3 will spread in the environment after potential propagation by third parties.

6. A member of the public asks what happens with the function of the aromatic substances, flavorings and toxic substances when the flower color is changed?
Answer: Potential effects of color changed carnation lines on incidental human consumption, allergenicity and (soil) toxicity were assessed using several assays. The results of these assays did not indicate any differences in comparison with the parental strains and are sufficient to conclude on the environmental safety of the product (cut flowers). Potential effects on other aspects like aromatic substances and flavorings of these flowers are not considered to be of importance for the safety assessment since the notification only covers import of cut flowers and no cultivation.

7. A member of the public asks if Florigene also used human embryonic intestinal cells, just like the genetically engineered carnation C/NL/04/02, to determine the toxicity of the flower for humans.
Answer: No, an Ames/*Salmonella* test was performed. The results of this test and all other assays performed with similar carnation lines are sufficient to conclude on the safety of cut flowers of this carnation line for human health and the environment.

8. A member of the public protests against this market approval on ethical grounds. This person requests to take into account ethical considerations of the European consumers. Respect for ethical principles recognized in a Member State is particularly important. According to this person, Member States may take into consideration ethical aspects when GMOs are deliberately released or placed on the market as or in products
Answer: According to Annex VI of Directive 2001/18/EC a notification has to be assessed on potential risks for human health and the environment only. Therefore comments not related to the environmental safety of the product are not taken into account in the assessment of this notification on genetically modified carnation.

9. A member of the public protests against the genetic manipulation of cut flowers (as well as all other forms of genetic manipulation) for ethical reasons. It is not necessary, undesirable and not entirely without risks.
Answer: Like stated before, according to Annex VI of Directive 2001/18/EC a notification has to be assessed on potential risks for human health and the environment only. Therefore comments not related to the environmental safety of the product are not taken into account in the assessment of this notification on genetically modified carnation.

Confidentiality
The notification does not contain any information which the applicant regards as Confidential Business Information.

5. LIST OF DOCUMENTS

The dossier consists of:
- Technical information required according to Annex III B of Directive 2001/18/EC;
- Environmental risk assessment according to Annex II of Directive 2001/18/EC;
- Information according to Annex IV of Directive 2001/18/EC;
- Monitoring plan according to Annex VII of Directive 2001/18/EC;
- Summary notification format;
- Information about previous releases of the genetically modified plant;
- Eighteen attachments.

6. PARENTAL OR RECIPIENT CROP

Carnation is a crop with a long history of safe use. Cultivation of carnation in the field is mainly conducted in Italy and Spain. In northern European countries as Germany, France and the Netherlands carnation is grown in greenhouses, due to the less favourable climate. Within Europe wild carnation is only found in the Mediterranean area in Italy, Greece, Sicily, Sardinia and Corsica.
Carnation, an annual plant, does not form vegetative reproductive structures such as stolons, rhizomes, root-borne shoots, tubers, etc. Carnation is semi-winter hardy and can not survive in areas where temperatures occur below - 5 °C. The genetic material of carnation can only be disseminated via pollen and seeds.
Carnation is highly domesticated by generations of breeding aimed at improvement of flower size and colour variation. As result of domestication, dissemination through pollination is much less effective in carnation than in wild *Dianthus* species. In general, production of viable pollen by carnation is much lower than that of wild *Dianthus* species.
In the unlikely event that pollination should occur, no seed set will occur in cut flowers as the process of seed development (at least 5 weeks) overruns the time cut flowers will remain in consumers hand before dying (at most three weeks).
Wild relatives which can give viable progeny after hybridisation with carnation are absent in large areas of Europe. The only possible hybridization partners are other cultivated carnations and in the Mediterranean area wild carnation. There has never been any evidence of hybridization between carnation and wild *Dianthus* species.
Carnation is not a weed. 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.

Summarised, carnation does not have any characteristic which might pose a risk to the environment or human health.

7. DESCRIPTION OF THE PRODUCT

The genetically modified carnation (*Dianthus caryophyllus* L.) line IFD-25958-3 exhibits a modified flower color (purple) resulting from expression of the *dfr* and *F3'5'H* genes. Gene expression enables the biosynthesis of delphinidin pigment in the petals. Line IFD-25958-3 also contains the herbicide tolerance gene *SuRB* (also known as *ALS)* used to facilitate selection *in vitro*. Expression of this gene confers tolerance to sulfonylurea herbicides.

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8. MOLECULAR CHARACTERISATION

The Dutch CA is of the opinion that the provided information regarding the molecular characterization of line IFD-25958-3 is sufficient to assess potential hazards for human health and the environment.

Modification

Carnation line IFD-25958-3 was obtained by *Agrobacterium tumefaciens* mediated transformation, by co-cultivating cells with strain AGL0 which contain vector pCGP3366.
The transformation vector is completely sequenced and the sequence is part of the notification.

Plasmid pCGP3366 contains the following elements in the insert:

Genetic element	**Size (kbp)**	**Origin and function in plant**
LB	0.1	T-DNA border from *Agrobacterium tumefaciens*
35S promoter	0.2	Constitutive promoter in plants from *Cauliflower mosaic virus* (CaMV)
Cab 5'utr	0.1	5'untranslated region (UTR) of the Chlorophyll a/b binding protein from *Petunia x hybrida*
SuRB	3.7	Encodes acetolactate synthase resistant to chlorsulfuron. Gene with own terminator from *Nicotiana tabacum*
CHS promoter	1.2	Petal specific promoter from a gene encoding chalcone synthase from *Antirrhinum majus*
F3'5'H cDNA	1.8	Encodes flavonoid 3'5'-hydroxylase protein from *Viola* sp.; a key enzyme in the anthocyanin biosynthesis pathway
D8 terminator	0.8	Terminator sequence from *Petunia x hybrida*
Dfr genomic clone	4.9	Encodes dihydroflavonol-4-reductase protein with its own promoter and terminator from *Petunia x hybrida*, a key enzyme in the anthocyanin biosynthesis pathway
35S promoter	0.4	Constitutive promoter in plants from *Cauliflower mosaic virus* (CaMV)
Dfr sense arm	0.3	Partial dihydroflavonol-4-reductase sequence, a key enzyme in the anthocyanin biosynthesis pathway
Dfr intron	0.3	Non-functional intron used for anchoring the two arms of the hairpin construct
Dfr anti-sense arm	0.3	Partial dihydroflavonol-4-reductase sequence, a key enzyme in the anthocyanin biosynthesis pathway
35S terminator	0.2	Constitutive terminator in plants from *Cauliflower mosaic virus* (CaMV)
RB	1.8	T-DNA border from *Agrobacterium tumefaciens*

Plasmid pCGP3366 contains the antibiotic resistance marker tetracycline on the vector backbone.

F3'5'H and *dfr*

The genes *F3'5'H* encoding flavonoid 3'5'hydrolase and *dfr* encoding dihydroflavonol 4-reductase (DFR) are both derived from *Viola* and *Petunia*, respectively. Simultaneous expression of both genes in carnation results in a modified flavonoid synthesis in flowers, and subsequent formation of the blue pigment delphinidin. Carnation lacks part of the anthocyanin biosynthetic pathway involved in the production of delphinidin, *i.e.* carnation lacks the flavonoid 3'5' hydrolase en DFR enzyme activities. Expression of both inserted genes, in combination with endogenous genes, results in a modified flower color (purple instead of cerise).

Hairpin RNAi dfr sequences

The hairpin RNAi *dfr* sequences are derived from *Dianthus caryophyllus*. The production of short interfering RNAs (siRNA) by this hairpin construct leads to down regulation or silencing of endogenous DcDFR via post-transcriptional gene silencing (PTGS) pathway. 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* DFR enzymes.

SuRB
The *SuRB* gene from *Nicotiana tabacum* encodes a mutated acetolactate synthase. Expression of the mutation confers tolerance to sulfonylurea herbicides. According to the applicant, this tolerance was only included to allow selection *in vitro*.

Molecular characterization
Inserts
The T-DNA between the left and right borders of PCGP3366 remains in the genetically modified plant and is present in one locus.
Genomic DNA isolated from the transgenic line IFD-25958-3 and non-transformed lines were compared using Southern analysis to identify integrated sequences and copy number of the introduced genes. Southern analysis with *EcoR*I digested DNA and *Bgl*II digested DNA indicates that the inserted sequences are present at a single locus in the carnation genome. A schematic of the arrangement of inserted T-DNA is provided in Attachment A7. The sequence of the locus, including the flanking regions, is provided in Attachment A8.

The genetically modified carnation has been vegetatively propagated since April 2005 (approximately 12 generations) and since 2007 plants have been in a field trial in Colombia. There has been no incidence of flower color change during this time.

Flanking sequences
The flanking sequences of both ends of the locus are sequenced (150 bp). The flanking sequences were analyzed for putative open reading frames (ORFs). All ORFs were included (no minimal size, starting with or without a methionine). Twelve ORFs at the junctions insert/plant were identified. None of the ORFs showed homology to known toxins or allergens.

Absence of tetracycline resistance gene (tetA)
Southern analysis was conducted to demonstrate the absence of backbone vector sequences. The results conclusively prove the absence of any backbone vector sequences, including *tetA* sequences encoding a resistance gene to the antibiotic tetracycline. In addition, PCR analysis with primers directed against the *tetA* gene confirmed the absence of this gene.

Gene expression
Northern analysis conducted on RNA isolated from petal leaves showed that all three genes are expressed in IFD-25958-3 whereas no signals could be detected in parental line Cerise Westpearl CW.
Except for flowers, delphinidin production has not been observed in other tissues of the transgenic plant, such as stems, nodes, leaves and roots. Due to the petal specific promoter (CHS), production of delphinidin is confined to the petals. Moreover, the biochemical pathway leading to anthocyanin biosynthesis is induced to coincide with flower development.
The concentration of delphinidin and other anthocyanins was determined in flower samples of line IFD-25958-3 and of the non-transformed recipient strain by TLC and HPLC. The delphinidin concentration amounts 0.54 mg/g fresh weight petal. Besides delphinidin IFD-25958-3 produces anthocyanin (0.02 mg/g fresh weight petal) as a result of the genetic modification. Cyanins and derivatives are produced by several plant species. The concentration produced by IFD-25958-3 is 28-228 times lower than produced by other carnation varieties.

9. ENVIRONMENTAL RISK ASSESSMENT

The Dutch CA is of the opinion that the provided information regarding the environmental safety of line IFD-25958-3 is sufficient to assess potential hazards for human health and the environment.

The environmental risk assessment of the carnation with a modified flower colour was restricted to issues that are relevant within the scope of the notification: import, distribution and retailing of cut flowers. In this respect, only the probability of gene dispersal and weediness were assessed. Furthermore, the potential risks to consumers due to incidental consumption were assessed.

Selective advantage and potential for increased weediness or persistence

Dfr, hairpin RNAi dfr sequences and F3'5'H genes

There is no reason to assume that carnation plants from spilled or discarded carnation exhibit an increased potential to survive, as a result of the modified colour of flowers by expression of the *dfr* and *F3'5'H* genes. The gene products of the *dfr* and *F3'5'H* genes are involved in the biosynthesis of the pigment delphinidin in petals. Accumulation of these pigments in petals results in a purple flower colour. This accumulation results in a modified flower colour and does not alter the biological characteristics of carnation. Therefore it is highly unlikely that the genetically modified carnation line IFD-25958-3 exhibits a selective advantage over non-modified carnation, based on the presence of the *dfr* and *F3'5'H* genes.

SuRB gene

Carnation is not considered to be a weed in Europe. Carnation plants resistant to sulfonylurea herbicides can only exhibit a selective advantage after application of such herbicide. However, sulfonylurea herbicides are not designed/registered for use with ornamentals. Sulfonylureas are not effective against grasses, the major weeds of concern in the flower industry. The notifier prohibits use of sulfonylureas on their crops by their contract growers. The herbicide is not generally used for widescale control of weeds outside agriculture.

Effects on non-target organisms

The environment 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 interaction between the genetically modified carnation and non-target organism.
Therefore it is highly unlikely that non-target organisms will be affected as a result of import of cut flowers of line IFD-25958-3.

Effects on the soil ecosystem

Because the products are to be imported as cut flowers, no cultivation takes place. As the genetically modified carnation plants have similar production requirements as other carnations, any impact is no different to that of conventional carnation. Flowers imported to the EU will eventually be discarded in domestic and commercial waste, but the volume of the flowers and the fact that the products will be widely dispersed mean the organic mass is negligible. In addition, the compounds responsible for the colouration of the flowers are natural compounds which are widely present in the environment.
Therefore it is highly unlikely that any adverse effect on the soil ecosystem will occur as a result of imported or discarded genetically modified carnation.

Toxicity and allergenicity

Delphinidin and cyanidin

Carnation has been used safely by humans for ornamental purposes for centuries. The modification in line IFD-25958-3 (production of delphinidin) is novel for carnation, but there are many flowers and other ornamental species that produce delphinidin, such as G*entiana, Petunia, Centaurea* and *Delphinium*. Delphinidin is also present in many common foods, such as red grapes, black currants, egg plant and blueberry. Toxicity studies of delphinidins and anthocyanins indicate very low levels of toxicity. Humans are commonly exposed to and ingest delphinidins in fruits and vegetables at similar or greater concentrations than are found in genetically modified carnation, without adverse effects.

DFR and F3'5'H proteins

Possible negative effects on human and animal health as a result of incidental consumption of petal leaves of carnation, for example as garnishing for food, were considered. The proteins for modified flower colour expressed in genetically modified carnation (DFR and F3'5'H) are similar to those found in purple-coloured fruits and vegetables that are commonly consumed, and in ornamental flowers. No homology was found between the inserted genes and known toxins or allergens.
An *Ames* mutagenicity test was performed and no indication of toxicity was found.
Reports of allergenicity to carnations are rare and there are no reports of allergenicity to genetically modified carnation.

SuRB protein

ALS enzymes are widely distributed among bacteria, yeast and higher plants. The *SuRB* gene codes for an alternative form of the acetolacetate synthase enzyme. This enzyme is not a known toxin or allergen and related enzymes are expressed in a variety of edible plants (e.g. soy bean and rice).
No homology was found between the *SuRB* gene and known toxins or allergens.

Based on the nature of the inserted genes, the results of the abovementioned *Ames* test and the history of safe use of similar genetically modified carnation lines, it is concluded that it is highly unlikely that the genetically modification in carnation line IFD-25958-3 will cause an adverse effect on the human health with respect to incidental human consumption or allergenicity, as compared to conventionally bred carnation.

Change in agricultural practice
Since the notification covers only import, distribution and retailing of the genetically modified carnation, possible adverse environmental effects by changes in agricultural practice are not considered of importance for the risk analysis.

Conclusion
The Dutch CA concludes that the provided information is sufficient and is of the opinion that in the context of its intended use, carnation line IFD-25958-3, is unlikely to have adverse effects on human and animal health or the environment.

10. DETECTION METHOD

The applicant has provided a detection method that is specific for line IFD-25958-3, as is obligatory under the 2001/18/EC. The Dutch CA considers the detection method as being sufficient. The detection method is not yet verified by the Community Reference Laboratory.

11. UNIQUE IDENTIFIER

The unique identifier for the carnation line is IFD-25958-3.

12. TRACEABILITY AND LABELLING

The notifier proposes to label flowers of the transgenic carnation line IFD-25958-3 similar to those of variety Moonshadow (C/NL/97/13-1363A), Moondust (C/NL/96/14-11) and Moonlite (C/NL/04/02) which are already imported into and sold in the EU. The flowers will be imported in cardboard boxes, within which 10-25 bunches of flowers will be packed and sleeved in plastic flower sleeves for protection. Each sleeve will contain 10-15 flowers. The notifier states that Florigene will place a label on the sleeves. The proposed wording of the label is as follows: “This product is a genetically modified carnation and is not for human or animal consumption nor for cultivation”.

13. MONITORING AND GENERAL SURVEILLANCE

Specific monitoring
Since the environmental risk analysis does not identify any potential risks, the notifier has not included a specific monitoring plan. The Dutch CA accepts this reasoning.

General surveillance
The intended use of the placing on the market of this product is import, distribution and retailing. Therefore the general surveillance plan addresses escapes of the genetically modified carnation (or its traits) to the environment, and unforeseen effects on human health by handling the product. Amongst others, the following monitoring activities will be undertaken:

1. Florigene will maintain exact records of all imports into Europe;
2. Importers will be asked to monitor their markets for any suppliers selling flowers resembling the Florigene product and which may be sold outside of the regular distribution and retail channels;
3. On a 6 monthly basis the European importers will be asked in questionnaire format for feedback;
4. The Florigene website will provide a link at which European consumers will be invited to comment on Florigene products with all Florigene contact details;
5. After release, breeders and botanists with interest in *Dianthus* biology will be asked to alert Florigene in case of any unusual hybrids that they might find during survey work.

The Netherlands considers this general surveillance plan as sufficient.

14. ADVICE OF THE DUTCH COMPETENT AUTHORITY FOR DIRECTIVE 2001/18/EC

Based on the notification and the above mentioned considerations, the Dutch competent authority concludes that no reasons have emerged on the basis of which consent to the proposed placing on the market should be withheld.

The Dutch competent authority therefore proposes to consent to the placing on the market of the product as described below, for which a notification has been submitted on March 17, 2009, registered under number C/NL/09/01 under explicit specification of:

a) The consent will be granted to Florigene Pty. Ltd, Bundoora, Australia and concerns the placing on the market under part C of 2001/18/EC of the product consisting of cut flowers of carnation (*Dianthus caryophyllus* L.) genetically modified with the *dfr, F3'5'H* and *SuRB* genes for the purpose of import, distribution and retailing. The consent includes line IFD-25958-3.
b) The product may be put to ornamental use only. This consent excludes cultivation and excludes the use as feed or as food of line IFD-25958-3.
c) The unique identification code of the product will be IFD-25958-3.
d) The period of validity of the consent shall be 10 years starting from the date on which the consent is issued.
e) The words 'This product is a genetically modified organism' or 'This product is a genetically modified carnation', and the words 'not for human or animal consumption nor for cultivation' shall appear either on a label or in a document accompanying the product.
f) The consent holder shall, whenever requested to do so, make positive and negative control samples of the product, or its genetic material, or reference materials available to the competent authorities and to inspection services of Member States as well as the Community control laboratories.
g) Throughout the period of validity of the consent, the consent holder shall ensure that the monitoring plan, contained in the notification and consisting of a general surveillance plan to check for any adverse effects on human and animal health or the environment arising from handling or use of the product, is put in place and implemented.
h) The consent holder shall directly inform the operators and users concerning the safety and general characteristics of the product and of the conditions as to monitoring, including the appropriate management to be taken in case of accidental cultivation.
i) The consent holder shall submit to the Commission and to the competent authorities of the Member States annual reports on the results of the monitoring activities.
j) The decision shall apply from the date on which the detection method specific to carnation line IFD-25958-3 is verified by the Community Reference Laboratory.

The Hague, 30-06-2009
The Minister of Housing, Spatial Planning and the Environment,
For these,
The Secretary-General Environment
o.c.,

Director environment protection office and environment safety and risk management directorate

drs. ing. Peter Torbijn