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

NOTIFICATION C/NL/04/02

1. THE NOTIFICATION

The notification, submitted by Florigene Ltd, Melbourne, Australia, concerns placing on the market of imported cut flowers derived from genetically modified carnation (*Dianthus caryophyllus*) line 123.2.38 in accordance with Directive 2001/18/EC. The flowers of the carnation line have been modified with the *hf1* and *dfr* genes, resulting in a modified flower colour. Line 123.2.38 also contains an herbicide resistance gene (*SuRB),* used to facilitate selection *in vitro.* The commercial name of the product is Florigene Moonlite™.

2. SCOPE OF THE NOTIFICATION

This notification originally concerned import, distribution and retailing of lines 123.2.2 and 123.2.38 in the cut flower market in the same way as any other carnation. On October 4 (2004) the notifier has withdrawn line 123.2.2 from the notification and thus the notification concerns only line 123.2.38 (Moonlite). This notification does not include cultivation, the use as feed or as food of line 123.2.38.

3. PROCEDURE

The Netherlands competent authority received this dossier on September 3, 2004 under Directive 2001/18. The dossier has been assessed with reference to Article 13 of this directive.

Additional information
During the assessment period further information was requested on the following aspects:

Potential chimeric ORFs generated at the junctions of the insert and the plant DNA
Information regarding the expression of potential chimeric ORFs generated at the junctions of insert / plant DNA which might give rise to potential adverse effects. The notifier submitted information based on the assumption that novel ORFs were indeed generated in line 123.2.38, following a worst case scenario. The notifier has addressed this aspect satisfactorily.
Clock stopped September 20 (2004) till November 30 (2004).

Absence of tetracycline resistance gene (tetA)
Information regarding the absence of *tetA* in line 123.2.38. The notifier submitted information concerning a PCR test which demonstrates the absence of an intact *tetA* gene in line 123.2.38. The notifier has addressed this aspect satisfactorily.
Clock stopped September 20 (2004) till November 30 (2004) and from December 3 (2004) till December 23 (2004).

Scientific advice
Based on the notification of September 3 (2004) and the additional information of November 30 (2004) and December 23 (2004) the Dutch scientific advisory committee (COGEM) gave its advice on February 7 (2005) (CGM/050207-01). Based on an evaluation of the possible risks for human health and the environment, the COGEM concluded that the risks for the environment and human health associated with import of cut flowers of line 123.2.38 are negligible.

Public comments
The Summary Notification Information Format (SNIF) was initially published on the Joint Research Center (JRC) website on September 29 (2004). Public comments were received during 30 days, and originated from the Netherlands (3), Italy (1), Ireland (1) and the UK (1). On December 2004, a revised SNIF was published on the JRC website due to the limitation of the scope, *i.e.* the withdrawal of line 123.2.2. Only public comments originating from Dutch persons are addressed by the Netherlands CA in this assessment report, and are summarized below, under points 1 to 3. Public comments originating from other member states are to be addressed by the relevant CA's under the 2001/18/EC, during their national assessment in the 60-day period.

Public comments on the notification C/NL/04/02 and reaction of the Netherlands CA
Public comments which were addressed by the Netherlands CA were submitted by:
- Ms. Van Dort, Den Haag, The Netherlands;
- Mr. Schöttelndreier, Groenekan, The Netherlands;
- Mr. Stellingwerf, Ede, The Netherlands.

1. Ms. Van Dort is of the opinion that nature is beautiful enough and therefore sees no reason for colour modification.
   Answer: Comments of an ideological nature fall outside the scope of the legal framework of Directive 2001/18/EC. A notification under Directive 2001/18/EC is assessed on possible risks for human health and the environment.

2. Mr. Schöttelndreier objects to the notification because the long term ecological consequences of introducing GMO's in nature have not been fully assessed.
   Answer: The scope of the notification is import only, so no cultivation will take place in the EU. Therefore, there is no reason to assess long term ecological effects as a result of cultivation of the colour modified carnation within the territory of the EU.

3. Mr. Stellingwerf is of the opinion that consents for the deliberate releases of GMO's should be based on a 'no, unless..'-basis and regards a colour modification of flowers not a legitimate ground. He also states that other alternatives are available.
   Answer: A notification under Directive 2001/18/EC is assessed on possible risks for human health and the environment. If no risks are identified, there is no legal basis to withhold admission of GMO's to the European market.
   A consideration of alternatives is beyond the scope of Directive 2001/18/EC.

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

4. 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;
- Three attachments.

5. 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 green houses, 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 hybridisation 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.

6. DESCRIPTION OF THE PRODUCT

The genetically modified carnation (*Dyanthus caryophyllis* L.) line 123.2.38 exhibits a modified flower colour (violet) resulting from expression of the *dfr* and *hf1* genes. Gene expression enables the biosynthesis of delphinidin pigment in the petals. Line 123.2.38 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.

7. MOLECULAR CHARACTERISATION

Modification

Carnation line 123.2.38 was obtained by *Agrobacterium tumefaciens* mediated transformation, by co-cultivating cells with strain AGL0 which contain vector pCGP1470. The same vector is used for the construction of line Florigene Moondust™ (C/NL/96/14). This carnation event was admitted to the EU market for cultivation and import in 1997.

Plasmid pCGP1470 contains the following elements in the insert:

Genetic element	Size (kbp)	Origin and function in plant
35 S promoter	0.2	Constitutive promoter in plants from *Cauliflower mosaic virus* (CaMV)
SuRB	2.0	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*.
hf1	1.6	Encodes flavonoid 3'5'-hydroxylase, a key enzyme in the anthocyanin biosynthesis pathway from *Petunia x hybrida*
D8 terminator	0.8	Terminator sequence from *Petunia x hybrida*
Mac promoter	1.2	A hybrid constitutive promoter in plants (Mas promoter (*A. tumefaciens*) en 35S enhancer elements (CaMV))
dfr	1.3	Encodes dihydroflavonol-4-reductase hydroxylase, a key enzyme in the anthocyanin biosynthesis pathway from *Petunia x hybrida*
Mas terminator	0.7	Terminator sequence from *A. tumefaciens*

Plasmid pCGP1470 contains the antibiotic resistance marker tetracycline on the vector backbone.

Hf1 and *dfr*

The genes *hf1* (also known as F3'5'H) encoding flavonoid 3'5'hydrolase and *dfr* encoding dihydroflavonol 4-reductase (DFR) are both derived from *Petunia*. 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 colour (violet in stead of white).

SuRB

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

Molecular characterisation
The molecular characterisation of line 123.2.38 is sufficient to assess possible hazards for human health and the environment.

Inserts
The full sequence of the transformation vector pCGP1470 is part of the notification and the function of all genes (or parts of genes) encoded by pCGP1470 is known.
Genomic DNA isolated from the transgenic line Moonlite (123.2.38) and non-transformed lines were compared using Southern blot analysis to identify integrated sequences and copy number of the introduced genes. It is estimated that one to three copies of the inserted sequences are present in the carnation genome.
Southern blot analysis demonstrated that the integration patterns of the introduced genes remain stable and unchanged in the nuclear genome. The genetically modified carnation has been vegetatively propagated since 1998 and approximately 5 million flowers have been produced since the start of commercial production in 2000. During the production period no phenotypic aberrations were found.

Flanking sequences
The borders and flanking sequences of the insert are not fully characterised. The notification does not contain sequence data of the flanking sequences. Therefore, the possibility of putative chimeric proteins to be encoded and expressed is not excluded. However, the Dutch Competent Authority considers data on flanking sequences not essential given the nature (import of cut flower) and the proposed use of the product (ornamental use)(see below).

Absence of tetracycline resistance gene (tetA)
Based on results of the Southern blot analysis it was concluded that vector backbone sequences containing *tetA* sequences may have been inserted into the genome of line 123.2.38. Additional PCR tests demonstrated the absence of a complete functional *tetA* gene.

Gene expression
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 123.2.38 and of the non-transformed recipient strain by HPLC. The delphinidin concentration amounts 0.093 mg/g fresh weight petal.

8. ENVIRONMENTAL RISK ASSESSMENT

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, weediness and potential risks to consumers due to incidental consumption were assessed.

Flanking sequences
The notification does not contain sequence data of the flanking sequences. It is therefore unclear whether potential chimeric ORFs are generated at the junctions of the insert and the plant DNA which might give rise to potential adverse effects. In accordance with the EFSA guidance document for the risk assessment of genetically modified plants and derived food and feed (The EFSA Journal (2004) 99, 1-94; 8 November 2004), the notification needs to contain details of the organisation and DNA sequence of the flanking regions to be able to identify the possible formation of chimeric ORFs. If such ORFs are identified, the possible expression of these ORFs should be assessed.
Instead of these sequence data, the notifier has supplied a worst case scenario describing the potential adverse effects on human health and the environment in case such chimeric gene products with toxic and/or allergic properties are expressed in carnation line 123.2.38. The information provided contains experimental evidence and observations to demonstrate that the transgenic carnation does not produce toxic or allergenic compounds. The information consists of three toxicity tests, namely an Ames mutagenicity test, an acute toxicity test conducted in mice and a cytotoxicity test using human embryonic intestinal cells in vitro. Assuming a worst case scenario, these results indicate that in case novel ORFs are present and would be expressed, they do not appear to encode toxic or allergenic compounds. In addition, the genetically modified carnation line 123.2.38 has a history of safe use. The flowers of this line are produced on a large scale in Australia and South America and sold throughout the United States, Canada

and Japan since 2000. There have been no reports of allergenic effects at either the growing location, nor by any other person exposed to the flowers. Thus the risks of adverse effects on the environment or human health occurring is negligible.
Although the data in the notification are not in line with the requirements as indicated in the abovementioned EFSA guidance document, the Dutch Competent Authority considered sequence data on flanking sequences and the identification of chimeric ORFs not essential given the nature (import of cut flowers) and the proposed use of the product (ornamental use only). Therefore, the Dutch CA considered the information as included in the notification sufficient to conclude that import of the colour modified carnation line 123.2.38 will not cause adverse effects to human health and the environment.

Selective advantage
Dfr and hf1 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 *hf1* genes. The gene products of the *dfr* and *hf1* genes are involved in the biosynthesis of the pigment delphinidin in petals. Accumulation of these pigments in petals results in a violet to blue 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 123.2.38 exhibits a selective advantage over non-modified carnation, based on the presence of the *dfr* and *hf1* gene.

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 123.2.38.

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
Carnation has been used safely by humans for ornamental purposes for centuries. The modification in line 123.2.38 (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. 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 HF1 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 HF1) 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.
Three toxicity tests were performed, namely an Ames mutagenicity test, an acute toxicity test conducted in mice and a cytotoxicity test using human embryonic intestinal cells in vitro. No indication of toxicity was

found (see also under 8. Environmental risk assessement-'flanking sequences').
Reports of allergenicity to carnations are rare and there are no reports of allergenicity to genetically modified carnation. The transgenic carnation line 123.2.38 has been in commercial production for several years and over 5 million cut flowers have been grown and distributed to the general public without having any allergenic effect been reported.

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. An acute toxicity study with a carnation line 123.2.38 was performed with mice. No indication of toxicity was found.

Based on the nature of the inserted genes, the results of abovementioned toxicity tests and the history of safe use, it is concluded that it is highly unlikely that the genetically modification in carnation line 123.2.38 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.

9. DETECTION METHOD

The applicant has provided a detection method that is specific for line 123.2.38, as is obligatory under the 2001/18/EC. The Netherlands CA considers the detection method as being sufficient.

10. UNIQUE IDENTIFIER

The unique identifier for the carnation line is FLO-40644-4.

11. TRACEABILITY AND LABELLING

The notifier proposes to label flowers of the transgenic variety Moonlite (line 123.2.38) similar to those of variety Moondust (C/NL/96/14) which are already imported into and sold in the EU. The notifier will place a label inside every box that is shipped to the EU. The wording of the label is: "These flowers are genetically modified to alter the flower colour and are only produced for use as an ornamental product". The Netherlands CA accepts this proposal if the Florigene also states the unique identifier assigned to line 123.238 on the label.

12. 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 Netherlands 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. The following monitoring activities will be undertaken:

1. 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;
2. On a 6 monthly basis the European importers will be asked in questionnaire format for feedback;
3. The Florigene website will provide a link at which European consumers will be invited to comment on Florigene products with all Florigene contact details;
4. After release, taxonomists 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.

13. ADVICE OF THE NETHERLANDS COMPETENT AUTHORITY FOR DIRECTIVE 2001/18/EC

Based on the notification, including all requested additional information, and the above mentioned considerations, the Netherlands 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 Netherlands 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 August 30, 2004, registered under number C/NL/04/02 under explicit specification of:

a) The consent will be granted to Florigene Ltd, Melbourne, Australia and concerns the placing on the market under part C of 2001/18/EC of the product consisting of carnation genetically modified with the *dfr, hf1* and *SuRB* genes, with the unique identification code FLO-40644-4, for the purpose of import, distribution and retailing. The consent includes line 123.2.38, product name Florigene Moonlite. This consent excludes cultivation and excludes the use as feed or as food of line 123.2.38.
b) The consent will be valid for a period of 10 years after approval.
c) At the first stage of the placing on the market of line 123.2.38 the company should ensure that the following information is transmitted in writing to the importer receiving the product:
   - The statement that “These flowers are genetically modified to alter the flower colour and are only produced for use as an ornamental product”;
   - The unique identifier of line 123.2.38, namely FLO-40644-4.
d) The consent holder is required to supply reference material of line 123.2.38 for detection purposes at any time to the competent authority.
e) The consent holder should carry out monitoring according to the general surveillance plan of the notification and report on the results of the general surveillance every year, during the period the consent is valid.
f) 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.

The Hague, 04-03-2005

The State Secretary of Housing, Spatial Planning and the Environment,

drs. P.L.B.A. van Geel