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

NOTIFICATION C/NL/97/13-01

1. THE NOTIFICATION

The notification, submitted by Florigene Pty Ltd, Bundoora, Australia, concerns the renewal of marketing consent C/NL/97/13 for placing on the market of imported cut flowers derived from genetically modified carnation (*Dianthus caryophyllus*) line 1363A in accordance with Directive 2001/18/EC. The flowers of this carnation line have been modified with the *F3'5'H* and *dfr* genes, resulting in a modified flower color (purple). Line 1363A also contains an herbicide resistance gene (*SuRB),* used to facilitate selection *in vitro*. The commercial name of the product is FLORIGENE Moonshadow ™.

2. SCOPE OF THE NOTIFICATION

This notification for renewal concerns import, distribution and retailing of line 1363A (FLORIGENE Moonshadow) 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 1363A.

3. HISTORY

The genetically modified carnation FLORIGENE Moonshadow has been authorized in Europe for cultivation, import and distribution since 1998. On October 20, 1998 a consent was granted under Directive 90/220/EC for cultivation, import and distribution of six lines (including line 1363A) of carnation FLORIGENE Moonshadow (C/NL/97/13). FLORIGENE Moonshadow was grown in Europe (Spain) from December 1998 to July 1999. From 2001 FLORIGENE Moonshadow was imported from countries outside Europe, primarily from Ecuador. FLORIGENE Moonshadow has been grown in Colombia, Ecuador and Australia and has been imported into the United States of America, Canada and Japan.

4. PROCEDURE

The application for renewal of the consent had to be sent in before October 17, 2006 according to Article 17(2) of Directive 2001/18/EC. The Netherlands received the renewal application on October 16, 2006. The application for renewal limits the scope to only import (no cultivation) of one of the 6 lines, line 1363A.
The dossier has been assessed with reference to Article 17 of Directive 2001/18/EC.

5. ARTICLE 17

According to article 17 of Directive 2001/18/EC a renewal notification has to contain:

(a) a copy of the consent to the placing on the market of the GMOs;
(b) a report on the results of the monitoring which was carried out according to article 20;
(c) any other new information which has become available with regard to the risks of the product to human health and/or the environment;
(d) as appropriate, a proposal for amending or complementing the conditions of the original consent, *inter alia* the conditions concerning future monitoring and time limitation of the consent.

Ad (a) Copy of the consent

A copy of the consent of C/NL/97/13 was provided as Attachment 1.

Ad (b) Report of the results of monitoring
In June 2006 the applicant has sent a letter to fifty-three research institutions (herbaria and other botanical collections) to ask them whether any collections made or received by these institutions in recent years are likely to have contained FLORIGENE Moonshadow or possible hybrids. The applicant received four replies, all of which indicated no specimens were in the recipients collections. Also five importers of Florigene Pty Ltd flowers were contacted in August 2006 with a questionnaire. The applicant received three replies, each answering “no” to all the three questions about harmful or unexpected effects of the flowers.
In September 2006 the applicant surveyed a number of European-based online floral databases. Cut flower carnation proved to absent in the databases surveyed.

Ad (c) Any other new information which has become available with regard to the risks of the product to human health and/or the environment
With regard to new information the applicant makes a distinction between information on genetic stability and gene expression and information on biosafety.

New technical information on genetic stability and gene expression
The following information has become available since the market introduction of FLORIGENE Moonshadow (1363A):
(1) Revertant-sports to white colored flowers of FLORIGENE Moonshadow (1363A) occurred at a very low-frequency, indicating that the event is genetically stable;
(2) Northern analysis confirms the expression of the *SuRB*, *dfr* and *F3’5’H* genes.

New technical information with regard to biosafety
Since the introduction to the market of FLORIGENE Moonshadow a number of experiments have been carried out which according to the applicant have led to additional information with regard to biosafety. These experiments are:
(1) Morphological measurements were taken to apply for Plant Breeders Rights (PBR) registration in Europe. From the data collected from a trial carried out in Australia the most significant morphological characteristics in comparison to the parental line were: a significantly lower petal count, a significantly lower petal width and smaller flowers with fewer lobes per calyx.
(2) An Ames/*Salmonella* test has been carried out with leaf extracts to study the possible mutagenic activity of FLORIGENE Moonshadow (1363A). FLORIGENE Moonshadow (1363A) as well as the parental line showed no mutagenic activity in this test.
(3) An acute toxicity study was conducted with mice, using extracts from petals of the transgenic line FLORIGENE Moonshadow (1363A) in comparison to extracts from petals of the control (recipient) line at a concentration of 2 g freeze-dried petals/kg bodyweight. No toxic effects or apparent abnormalities were observed throughout the experimental period and during autopsy.
(4) Seed germination-based bioassays were used to determine whether FLORIGENE Moonshadow (1363A) exhibited any phytotoxic effects. Lettuce seed germination and seedling growth was measured in a) soil from pots in which the transgenic or recipient plants have been grown and b) in fresh soil to which leaf material from the transgenic or recipient line had been added. In addition, microbial counts were determined in soil in which transgenic or recipient plants had been grown, using three different growth media. Based on results from these tests there was no evidence of phytotoxicity of FLORIGENE Moonshadow (1363A).

Risk assessment
(1) Based on an overview of the available literature on potential gene dispersal from import of cut-flowers of carnation, the applicant concludes that there is no indication for gene dispersal from the transgenic cut flowers.
(2) The potential toxicity or allergenicity of the compounds present in FLORIGENE Moonshadow (1363A) has been reviewed. The applicant concludes that since the introduction onto the market of FLORIGENE Moonshadow(1363A) no new information has

become available that points at potential risks to human health or the environment.
- FLORIGENE Moonshadow (1363A) has a history of safe use within and outside the EU and is genetically stable;
- There is no evidence that FLORIGENE Moonshadow (1363A) has become established outside of cultivation, or has hybridized to wild *Dianthus* species;
- There is no evidence that carnation flowers in general, and FLORIGENE Moonshadow (1363A) specifically, have any phytotoxic, toxic or allergenic properties.

Ad (d) Proposal for amending or complementing the conditions of the original consent, *inter alia* the conditions concerning future monitoring and time limitation of the consent
The applicant reports the following:

(1) The scope of the application is limited to import (no cultivation) into the EU of cut-flowers of FLORIGENE Moonshadow (1363A) and does not include progeny derived through crosses with traditionally bred carnation.
(2) The unique identifier of FLORIGENE Moonshadow (1363A) is FLO-11363-1.
(3) The applicant refers to monitoring and labeling conditions that would be adopted for C/NL/04/02. The applicant has provided a PCR method for identification of FLORIGENE Moonshadow (1363A). This PCR method is not yet approved by the JRC.

Request for additional information in October 2006
Based on the above application the applicant was requested to provide additional information on October 30, 2006. The applicant was requested to give an update of the molecular characterization of FLORIGENE Moonshadow (1363A). In addition, the applicant was asked to provide a monitoring plan in accordance to Directive 2001/18/EC and to propose a time limit for the renewed consent.

Additional information received in February 2009
As the molecular analysis of FLORIGENE Moonshadow (1363A) proved difficult, the applicant requested to postpone the deadline for additional information several times. During the time the clock was stopped (from October 30, 2006 to February 24, 2009) the applicant provided progress reports to the Dutch CA on a regularly basis.
The applicant submitted the requested additional information on February 24, 2009 as a complete stand alone dossier, including a monitoring plan in accordance to Directive 2001/18/EC. The proposed time limit for extension of the consent is 10 year.

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

Scientific advice
Based on the notification of October 16, 2006 and the additional information of February 24, 2009 the Dutch scientific advisory committee (COGEM) gave its advice on April 7, 2009 (CGM/090407-08). COGEM concluded that the risks for the environment and human health associated with import of cut flowers of FLORIGENE Moonshadow (1363A) are negligible.

6. MODIFICATION AND UPDATE OF MOLECULAR CHARACTERIZATION

Modification
Carnation FLORIGENE Moonshadow (1363A) was obtained by *Agrobacterium tumefaciens*-mediated transformation, by co-cultivating cells with strain AGL0 which contain vector pCGP1991. The same vector is also used for the construction of line FLORIGENE Moonaqua™ (C/NL/06/01). This carnation event was recently admitted to the EU market for import (Commission decision of March 16, 2009).

The insert in plasmid pCGP1991 contains the following elements:

Genetic element	Size (kbp)	Origin and function in plant
LB	0.9	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	4.0	Encodes acetolactate synthase resistant to chlorsulfuron. Gene with own terminator from *Nicotiana tabacum*
dfr genomic clone	5.0	Encodes dihydroflavonol-4-reductase protein with its own promoter and terminator from *Petunia x hybrida*, a key enzyme in the anthocyanin biosynthesis pathway
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*
RB	1.8	T-DNA border from *Agrobacterium tumefaciens*

Plasmid pCGP1991 contains the antibiotic resistance marker tetracycline (*TetA*) 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 derived from *Viola* and *Petunia*, respectively. Simultaneous expression of both genes in carnation results in 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 and DFR enzyme activities. Expression of both inserted genes, in combination with endogenous genes, results in a modified flower color (purple instead of white).

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

Update molecular characterization
Inserts
The full sequence of the transformation vector pCGP1991 is part of the notification and the function of all genes (or parts of genes) encoded by pCGP1991 is known.
Genomic DNA isolated from the transgenic line FLORIGENE Moonshadow (1363A) and non-transformed lines were compared using Southern analysis to identify integrated sequences and copy number of the introduced genes. Southern analysis was carried out with *Pst*I and *Bgl*II digests of plant DNA. *Pst*I cuts in the insert, *Bgl*II outside the insert. The probes are specified. Based on the Southern analysis with *Pst*I, the number of copies of the RB and LB and the *SuRB*, *F3'5'H* and *dfr* genes was estimated to be four to six in FLORIGENE Moonshadow. Southern analysis with *Bgl*II indicates that there are three independent *Bgl*II loci in which pCGP1991 DNA is integrated. Based on the results the applicant concludes that there are a number of copies of the genes and the insert present in FLORIGENE Moonshadow (1363A).
The three integration loci have been sequenced. The results show that locus 1 (18566 bp), locus 2 (26229 bp) and locus 3 (31603 bp) consist of both complete as well as parts of the T-DNA sequence. Locus 1 consists of three parts of the T-DNA, of which two parts are in sense

orientation and one in anti-sense orientation. Locus 2 consists of a part of the T-DNA in anti-sense orientation and one complete T-DNA in sense orientation. Locus 3 consists of two complete T-DNAs in sense orientation and a part of the T-DNA in anti-sense orientation.

Flanking sequences
All three integration loci including 150 base pairs of their flanking regions have been sequenced. BLASTn analysis demonstrated no significant homology with known plant genes or regulatory proteins, indicating no interruption of endogenous plant genes.
All novel junctions created as a result of the genetic modification were analyzed for the presence of putative open reading frames (ORFs). Potential ORFs were determined in the insert-plant junctions of all three integration loci and in all novel junctions within the integration loci.
Sixty-one open reading frames were identified. Bioinformatic analysis demonstrated that none of the putative ORFs showed significant homology to known toxins or allergens.

Absence of tetracycline resistance gene (tetA)
Southern analysis was conducted to demonstrate the absence of vector backbone sequences. Seven probes were used which cover the whole vector backbone. The results prove the absence of any backbone vector sequences, including *tetA* sequences encoding a resistance gene to the antibiotic tetracycline. In addition, PCR analysis for the presence of the *tetA* gene was conducted. Also these results confirmed the absence of the *tetA* gene.

The Dutch CA is of the opinion that the provided information regarding the molecular characterization of FLORIGENE Moonshadow (1363A)is sufficient to assess potential hazards for human health and the environment.

7. MONITORING AND GENERAL SURVEILLANCE

The notifier has supplied a monitoring plan in accordance to Directive 2001/18/EC.

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. Amongst others, the following monitoring activities will be undertaken:

1. Florigene Pty Ltd will maintain exact records of all imports into Europe;
2. Importers will be asked to record their sales to wholesalers and supermarkets on a brand name basis;
3. On a six-monthly basis the European importers will be asked in questionnaire format for feedback;
4. The Florigene Pty Ltd website will provide a link at which European consumers will be invited to comment on Florigene Pty Ltd products with all Florigene Pty Ltd contact details;
5. After release, taxonomists and botanists with interest in *Dianthus* biology will be asked to alert Florigene Pty Ltd in case of any unusual hybrids that they might find during survey work.

The Netherlands considers this general surveillance plan as sufficient.

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

Based on the notification of October 2006, the additional information of February 24, 2009, and the above mentioned considerations, the Dutch Competent Authority concludes that no reasons have emerged on the basis of which consent to the proposed prolonged placing on the market should be withheld.

The Dutch Competent Authority therefore proposes to consent to the prolonged placing on the market of the product as described below, for which a notification for renewal has been submitted on October 16, 2006, registered under number C/NL/97/13-01 under explicit specification of:

a) The consent will be granted to Florigene Pty Ltd, Bundoora, Australia and concerns the prolonged 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 for renewal includes line 1363A, product name FLORIGENE Moonshadow
b) The product may be put to ornamental use only. This consent excludes cultivation and excludes the use as feed or as food of FLORIGENE Moonshadow (1363A).
c) The unique identification code of the product is FLO-11363-1.
d) The period of validity of the consent shall be 10 years starting from the date on which the consent for renewal 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 FLORIGENE Moonshadow (1363A) is verified by the Community Reference Laboratory.

The Hague, 23-06-2009
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