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

NOTIFICATION C/NL/13/02

1. THE NOTIFICATION

The notification, submitted by Suntory Holdings Limited, Osaka, Japan, concerns placing on the market of imported cut flowers derived from genetically modified carnation (*Dianthus caryophyllus*) line FLO-40685-1 in accordance with Directive 2001/18/EC. The flowers of the carnation line have been modified with the *dfc* gene from petunia (*Petunia x hybrida*) and the *f3'5'h* gene from *Viola* sp., resulting in a modified flower colour (dark purple). Line FLO-40685-1 also contains a herbicide tolerance gene (*suRB*) from *Nicotiana tabacum*, used to facilitate selection *in vitro*.

2. SCOPE OF THE NOTIFICATION

This notification concerns import, distribution and retailing of line FLO-40685-1 (FLORIGENE@Moonvista™) 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 FLO-40685-1.

3. HISTORY

Carnation line FLO-40685-1 is approved for commercial cultivation in Columbia, Ecuador and Australia and for import in the USA, Japan, Australia and Canada. This genetically modified carnation variety is in production and trade since 2000. Carnation varieties modified with the same vector (pCGP1991), FLORIGENE@Moonlite™ (C/NL/04/02) and FLORIGENE@Moonaqua™ (C/NL/06/01), have already been approved in the EU for import, distribution and retailing.

4. PROCEDURE

The Dutch competent authority (CA) received this dossier on October 21, 2013 under Directive 2001/18/EC. The dossier has been assessed with reference to Article 13 of this Directive.

Additional information

During the assessment period further information was requested on November 1, 2013.

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Our ref.
C/NL/13/02/00.ar.1

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Scientific advice

Based on the notification of October 18, 2013 and the additional information received on November 2, 2013 the Dutch scientific advisory committee (COGEM) gave its advice on December 17, 2013 (CGM/131217-01). COGEM concluded that the risks for human health and the environment associated with import of cut flowers of line FLO-40685-1 are negligible.

Public comments

The Summary Notification Information Format (SNIF) was published on the website of the Joint Research Center (JRC) on November 12, 2013. Public comments were received during 30 days and originated only from the Netherlands. These comments are addressed below.

Public comments on the notification C/NL/13/02 and reaction of the Dutch CA

Public comments which were addressed by the Dutch CA were:

1. A member of the public states that the people do not want GMOs in the environment and he does not understand why politicians do not listen to the public. Is it because the big companies have the power?
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. The filed comments are not related to the environmental safety of the import of the genetically modified carnation but are related to ethical concerns about GMOs in general. Therefore these comments are not taken into account in this assessment report.

A member of the public states that recently a number of publications on genetically modified food appeared in the media, which reflected different opinions on GMOs. This member of the public wants to critically discuss a couple of the arguments that were used in the media.
2. The person is of the opinion that one of the arguments used in the debate concerns the statement that genetic modification can contribute to food security. This person does not agree with this argument and states that there is no proof for increased yields of GM crops. This person also states that there is a tendency towards a changed food crop production instead of an increases production. The person claims that increased food production can be obtained by growing other crops than wheat, such as cabbage and potato and by using traditionally grown crops. Another argument that often is used in the media is the claim that genetic modification is very precise, targeted and predictable and that effects are only due to one specific introduced gene. The person has doubts whether this argument is valid and refers to genetic modification of potatoes to obtain an amylose-free phenotype, for which a gene construct was used and phenotypic differences were observed between transformants. It is also claimed by this person that this GM potato is not needed since this trait can also be obtained by traditional breeding.
Answer: See also the answer on comment 1. According to Annex VI of Directive 2001/18/EC a notification has to be assessed on potential risks for human health and the environment. The filed comments are not related to the environmental safety of the import of the genetically modified carnation, but related to general arguments with relation to food production in general. Therefore these comments cannot be taken into account in this assessment report.
3. The person concludes that the health of people is not considered an important issue in the media and refers to a Dutch debate on GM food which was meant

**Directorate-General for
the Environment and
International Affairs**
Directorate-General for the
Environment and
International affairs

Date
17 april 2014

Our ref.
C/NL/13/02/00.ar.1

to create support for GM food among people with the intention to minimize uncertainties on risks and make an inventory of the benefits of GM food. The person does not understand how uncertainties can be reduced and states that the consumer wants a complete set of information on cultivation of GMO. It is further stated that this set of information is minimal and that there is no attention for this aspect in the media. Reference is made to potential transfer of Bt genes whereafter the soil fauna may be adversely affected.

Answer: The notification concerns the environmental risk assessment of import of carnation (cut flowers), genetically modified for a change of flower colour. A summary of the dossier and the complete assessment report will be published on the JRC website. The full dossier (notification) will be made available on the Dutch website (<http://www.ggo-vergunningverlening.nl>) after the dossier and the Dutch assessment report is sent to the European Commission and all European Member States for their consultation. The assessment report includes a full assessment of all potential environmental risks that may occur as a result of import of the colour-changed carnation, following the requirements of Directive 2001/18/EC. This set of information is considered to be sufficient as information to the public.

4. It is further stated that people do not want colour-changed carnations, since the modification has no purpose and does not increase food production. The person is against commercial release of the carnation.

Answer: See also the answer on comment 1 and 2. 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. The filed comments are not related to the environmental safety of the import of the genetically modified carnation but are related to ethical concerns about GMOs in general. Therefore these comments are not taken into account in this assessment report.

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;
- Additional 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 (A1-A12, B1-B5 and C1).

6. PARENTAL OR RECIPIENT CROP

Carnation (*Dianthus caryophyllus*) has a long history of safe use. Carnation does not have any weedy characteristics and despite hundreds of years of cultivation and plantings in parks and gardens, it has not become a weed or escaped from cultivation anywhere in the world.

Carnation is mainly produced in Italy, Spain and the Netherlands. Carnation is an annual plant, is semi-winter hardy and cannot survive in areas where temperatures occur below - 5 °C. Carnations are sold as cut flowers, cuttings or plants.

**Directorate-General for
the Environment and
International Affairs**
Directorate-General for the
Environment and
International affairs

Date
17 april 2014

Our ref.
C/NL/13/02/00.ar.1

Cultivated carnation is not propagated by seed but is propagated vegetatively by cuttings and tissue culture. In horticulture, propagation involves the use of mother plants. Cuttings of these mother plants are used for the production of flowers for a period of two years.

Carnation does not form vegetative reproductive structures such as bulbs, stolons or rhizomes. The genetic material of carnation can be disseminated via seed and by pollen. Seed dispersal can theoretically occur from plants, but seed formation is impossible for cut flowers. Carnation pollen can only be dispersed by lepidopteran insects as moths. Pollen is not wind dispersed. 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.

Wild *Dianthus* species which can give viable progeny after hybridisation with carnation are mainly found in mountainous areas in the alpine region, the Balkan and the Mediterranean area. Other possible hybridization partners are cultivated carnations. However, there has never been any evidence of spontaneous hybridization between cultivated carnation and wild *Dianthus* species, despite decades of cultivation in gardens and parks.

In summary, carnation does not have any characteristic which might pose a risk to human health and the environment.

7. DESCRIPTION OF THE PRODUCT

The genetically modified carnation (*Dianthus caryophyllus* L.) line FLO-40685-1 exhibits a modified flower colour (dark purple) resulting from expression of the *df*r and *f3'5'h* genes. Gene expression enables the biosynthesis of delphinine pigment in the petals. Line FLO-40685-1 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.

8. MOLECULAR CHARACTERISATION

The Dutch CA is of the opinion that the provided information regarding the molecular characterization of line FLO-40685-1 is sufficient to assess potential hazards for human health and the environment.

Modification

Carnation line FLO-40685-1 was obtained by transformation with *Rhizobium radiobacter* (previously known as *Agrobacterium tumefaciens*), by co-cultivating cells with *R. radiobacter* strain AGL0 that contains vector pCGP1991. The transformation vector is completely sequenced and the sequence is part of the notification.

Plasmid pCGP1991 contains the following elements in the T-DNA:

Genetic element	Size (kbp)	Origin and function in plant
LB	0.8	Left T-DNA border from <i>R. radiobacter</i>
35S promoter	0.2	Constitutive promoter from <i>Cauliflower mosaic virus</i> (CaMV)
<i>Cab 5'utr</i>	0.1	5'untranslated region (UTR) from the chlorophyll a/b binding protein gene, derived from <i>Petunia x hybrid</i> cDNA

Directorate-General for the Environment and International Affairs
Directorate-General for the Environment and International affairs

Date
17 april 2014

Our ref.
C/NL/13/02/00.ar.1

<i>suRB</i>	4.0	Encodes acetolactate synthase (ALS) from <i>Nicotiana tabacum</i> , resulting tolerance towards chlorsulfuron. Contains its own terminator
<i>dfr</i> genomic clone	5.0	Encodes a key enzyme from the anthocyanin biosynthesis pathway, dihydroflavonol-4-reductase hydroxylase, from <i>Petunia x hybrida</i> , and containing its own promoter and terminator
CHS promoter	1.2	Petal specific promoter of chalcone synthase (CHS) gene from <i>Antirrhinum majus</i>
<i>f3'5'h</i>	1.8	Encodes the key enzyme of the anthocyanin biosynthesis pathway, flavonoid 3'5'-hydroxylase, from <i>Viola</i>
D8 terminator	0.8	Terminator from <i>Petunia x hybrida</i>
RB	1.8	Right T-DNA border from <i>R. radiobacter</i>

Directorate-General for the Environment and International Affairs
Directorate-General for the Environment and International affairs

Date
17 april 2014

Our ref.
C/NL/13/02/00.ar.1

Plasmid pCGP1991 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 are derived from *Viola* and *Petunia* (*Petunia x hybrida*), 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 enzyme activities. Expression of both inserted genes, in combination with endogenous genes, results in a modified flower colour. For FLO-40685-1 this results in dark purple flowers instead of cream coloured flowers of the parental line Cream Cinderella.

SuRB

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

Molecular characterization

Inserts

Genomic DNA isolated from the transgenic line FLO-40685-1 and the non-transformed parental line Cream Cinderella were compared using Southern analysis and sequencing to identify integrated sequences and copy number of the introduced genes. Southern analysis with *EcoRV* and *NdeI* digested DNA indicated that integration of the T-DNA has occurred at four loci in the carnation nuclear genome. Sequence data confirmed the integration of two copies of the LB, two copies of the *SurB* gene, four copies of the *f3'5'h* gene, two copies of the *dfr* gene and five copies of the RB.

Flanking sequences

The flanking sequences of the four inserts are sequenced (150 bp). The flanking sequences and inserts were analysed for putative open reading frames (ORFs). All ORFs were included (no minimal size, from stop to stop codon). A total of 64 new ORFs were identified in the insert/plant junctions and a total of 2996 ORFs were identified in the four inserts. None of the ORFs showed biologically significant 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 prove the absence of any backbone vector sequences, including the tetracyclin resistance gene (*tetA*). PCR analysis confirmed the absence of

this gene.

Gene expression

Northern analysis conducted on RNA isolated from petal leaves showed that all three newly introduced genes are expressed in FLO-40685-1, whereas no signals could be detected in parental line Cream Cinderella.

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 was determined in flower samples of line FLO-40685-1 and of the non-transformed recipient strain by TLC and HPLC. The delphinidin concentration amounts 1.79 mg/g fresh weight petal. Due to the genetic modification also cyanidin is produced in petal leaves with a concentration of 0.02 mg/g fresh weight.

**Directorate-General for
the Environment and
International Affairs**
Directorate-General for the
Environment and
International affairs

Date
17 april 2014

Our ref.
C/NL/13/02/00.ar.1

9. ENVIRONMENTAL RISK ASSESSMENT

The Dutch CA is of the opinion that the provided information regarding the environmental safety of line FLO-40685-1 is sufficient to assess potential hazards for human health and the environment.

Scope

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.

Carnation is not able to spread vegetatively and cut flowers are not able to form roots. However, carnation can be propagated by stem cuttings and therefore it cannot be completely ruled out that the material will be propagated in gardens. Cut flowers of carnation can also be present in vases outside houses, like on cemeteries. These aspects have been taken into account in the environmental risk assessment.

Selective advantage and potential for increased weediness or persistence

f3'5'h and dfr genes

Carnation is not considered to be a weed in Europe. There is no reason to assume that carnation exhibits an increased potential to survive as a result of the modified colour of flowers resulting from expression of the *f3'5'h* and *dfr* genes. The gene products of *f3'5'h* and *dfr* are involved in the biosynthesis of the pigment delphinidin in petals. Accumulation of these pigments in petals results in a dark purple flowers and does not alter the biological characteristics of carnation. Therefore it is highly unlikely that the genetically modified carnation line FLO-40685-1 exhibits a selective advantage over non-modified carnation, based on the presence of the *f3'5'h* and *dfr* genes.

suRB gene

Carnation plants tolerant to sulfonylurea herbicides can only exhibit a selective advantage after application of such herbicides. However, sulfonylurea herbicides are not designed nor 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 wide scale control of weeds outside agriculture. It is therefore highly unlikely that the carnation line FLO-40685-1 exhibits a selective advantage over non-modified carnation, based on the presence of the *suRB* gene.

Effects on non-target organisms

There is no reason to assume that the new traits introduced (modified flower colour, tolerance towards to sulfonylurea herbicides) will result in adverse effects on non-

target organisms. In addition, 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 minimize direct or indirect interaction between the genetically modified carnation and non-target organisms. Therefore it is highly unlikely that non-target organisms will be affected as a result of import of cut flowers of line FLO-40685-1.

Effects on the soil ecosystem

Because the products are to be imported as cut flowers, no commercial cultivation takes place. 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. Also in case of propagation of the genetically modified carnation FLO-40685-1 by stem cuttings no adverse effects are foreseen.

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

Possible negative effects on human and animal health as a result of handling flowers or as a result of incidental consumption of petal leaves of the genetically modified carnation, for example as garnishing for food, were considered.

Delphinidin

Carnation has been used safely by humans for ornamental purposes for centuries. The modification in line FLO-40685-1 (production of delphinidin) is novel for carnation, but there are many flowers and other ornamental species that produce delphinidin, such as *Gentiana*, *Petunia*, *Centaurea* and *Delphinium*. Delphinidin is also present in many common foods, such as red grapes, black currants, eggplant and blueberry. Studies with delphinidins 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.

f3'5'h and dfr proteins

The proteins for modified flower colour expressed in genetically modified carnation (*f3'5'h* and *dfr*) are similar to those found in purple-coloured fruits and vegetables that are commonly consumed, and in ornamental flowers. No significant homology was found between the inserted genes and known toxins or allergens.

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. soybean and rice).

No homology was found between the *suRB* gene and known toxins or allergens.

Based on the nature of the inserted genes 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 FLO-40685-1 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

**Directorate-General for
the Environment and
International Affairs**
Directorate-General for the
Environment and
International affairs

Date
17 april 2014

Our ref.
C/NL/13/02/00.ar.1

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 FLO-40685-1 is unlikely to have adverse effects on human and animal health or the environment.

**Directorate-General for
the Environment and
International Affairs**
Directorate-General for the
Environment and
International affairs

10. DETECTION METHOD

The applicant has provided a detection method that is specific for line FLO-40685-1, as is obligatory under the 2001/18/EC (Attachment C1). The Dutch CA considers the detection method as being sufficient. The detection method is not yet verified by the Community Reference Laboratory.

Date
17 april 2014

Our ref.
C/NL/13/02/00.ar.1

11. UNIQUE IDENTIFIER

The unique identifier for the carnation line is FLO-40685-1.

12. TRACEABILITY AND LABELLING

The notifier proposes to label flowers of the transgenic carnation line FLO-40685-1 similar to other GM carnation varieties, like FLORIGENE®Moonlite™ (C/NL/04/02) and FLORIGENE®Moonaqua™ (C/NL/06/01) which are imported 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. The notifier states that there will be 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. Suntory will maintain records of all imports into Europe;
2. The importers will be asked in a questionnaire format for feedback on unexpected effects or illegal growth, on an annual basis;
3. The Florigene website will provide a link with all Suntory contact details at which European consumers will be invited to comment on Suntory products;
4. After release, breeders and botanists with interest in *Dianthus* biology will be asked to alert Suntory in case of any unusual hybrids that they might find during survey work.
5. Each year the scientific literature is reviewed for new reports on *Dianthus* taxonomy, botany and vegetation;
6. Suntory will report the results of monitoring to the Dutch CA and the European Commission on an annual basis.

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 October 18, 2013, registered under number C/NL/13/02 under explicit specification of:

- a) The consent will be granted to Suntory Holdings Limited, Osaka, Japan 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 *f3'5'h*, *dfr* and *suRB* genes for the purpose of import, distribution and retailing. The consent includes line FLO-40685-1.
- 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 FLO-40685-1.
- c) The unique identification code of the product will be FLO-40685-1.
- 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 FLO-40685-1 is verified by the Community Reference Laboratory.

Directorate-General for the Environment and International Affairs
Directorate-General for the Environment and International affairs

Date
17 april 2014

Our ref.
C/NL/13/02/00.ar.1

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DE STAATSSECRETARIS VAN INFRASTRUCTUUR EN MILIEU,
namens deze,
de directeur Veiligheid en Risico's,



drs. ing. Peter Torbijn

**Directorate-General for
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International Affairs**
Directorate-General for the
Environment and
International affairs

Date
17 april 2014

Our ref.
C/NL/13/02/00.ar.1