NOTIFICATION C/NL/97/12

Summary of the evaluation carried out by the Netherlands competent authority.

1. THE NOTIFICATION

The notification, submitted by Florigene Europe B.V. Rijnsburg, the Netherlands, concerns the placing on the market in the European Union of genetically modified carnation line 66 (*Dianthus caryophyllus* L.). The line exhibits an increased vase life due to the introduction of the *acc* gene.

The scope of the notification is the use in horticulture.

The notification is registered under number C/NL/97/12.

2. DEFINITIONS

In this document the following terms are used:

a. the Minister: the Minister of Housing, Spatial Planning and the Environment, in agreement with the Minister of Agriculture, Nature Management and Fisheries;
b. the GMO Decree: the Genetically Modified Organisms Decree pursuant to the Chemical Substances Act (Bulletin of Acts and Decrees 16 August 1993, 435);
c. the COGEM: the Committee on Genetic Modification, as established by the Environmental Management Act;
d. the Directive: the Council Directive of the European Union of 23 April 1990 on the deliberate release of genetically modified organisms to the environment (nr. 90/220/EEC, PbEG L117/15);

3. PROCEDURE

In the Netherlands, the production, transport, use, possession, supply to third parties and disposal of genetically modified organisms are subject to the Environmental Management Act and the GMO Decree. The permit requirements as laid down in these regulations do not apply for products placed on the market according to Directive 90/220/EEC. In the Netherlands the administrative procedure followed for notifications concerning placing on the market of genetically modified products is, to the extent possible, in line with the general legal procedure for authorizations. Upon receipt of a notification and after acknowledgement of its completeness, copies are sent to:

- the COGEM for scientific advice;
- the RIKILT-DLO for their opinion on the feed safety and related aspects;
- other relevant ministries.

In the evaluation of a notification for placing genetically modified organisms on the market, the following is taken into consideration: the notification, the advice from the COGEM, the opinion of the RIKILT-DLO when applicable and comments from other relevant parties, other aspects relevant to the notification such as considerations on potential effects of incidental consumption, the use as feed and possible weediness. Data from comparable notifications in other countries inside or outside the EU are also considered. On the basis of this evaluation, a draft opinion is published, regarding the notification and the considerations on which that draft opinion is based. The draft opinion is open for comments for a period of four weeks. The draft opinion regarding the notification is then reviewed in the light of any comments received, and the final opinion - when favourable - is sent to the European Commission, together with the dossier.

4. TECHNICAL SCIENTIFIC ADVICE

On July 14, 1997 the COGEM has given its advice on the notification. On the basis of an evaluation of the possible risks, the COGEM concludes that with respect to human health and the environment there are no objections to the proposed placing on the market of the product described.

5. RISK ANALYSIS AND EVALUATION

A consent to placing on the market of products which contain or consist of genetically modified organisms, can - on the basis of the Directive - only be withheld with regard to the protection of human health and the environment. For this purpose, a risk analysis and evaluation is carried out.

This risk analysis is based on the characteristics of the genetically modified organism and of its intended use. In this respect, the following general questions are important:

1. Are there reasons to assume that the genetically modified organisms or its progeny, due to the genetic modification, will become hazardous to human health or the environment?
2. Could the genetic material inserted into the genetically modified organisms be transferred to other organisms, and are there reasons to assume that those organisms, as a result, will become hazardous to human health or the environment?

In the analysis of this notification the following specific aspects have been taken into account:

1. characteristics of the host organism;
2. the inserted sequences and traits;
3. the use of the product.

These points are addressed below.

1. Characteristics of the host organism

The host organism in this notification, the carnation (*Dianthus caryophyllus* L.) cultivar "Ashley", belongs to the genus *Dianthus*. The genus *Dianthus* is indigenous in parts of North-Africa, Asia and Europe. The genus consists of a large number of different species, of which many are bred and grown for their ornamental value. *Dianthus* species, amongst which carnation, are cultivated for several centuries all over the world. Within Europe wild carnation is only found in the mediterranean area in Italy, Greece, Sicily, Sardinia and Corsica. The cultivation of carnation in the field is mainly done 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.

Carnation, an annual plant, does not form vegetative reproductive structures such as stolons, rhizomes, root-borne shoots, tubers, etc. The genetic material of carnation can only be disseminated via pollen and seeds. The seeds, the only survival structures formed, can be kept viable up to three years under controlled climatic conditions (low temperatures and low humidity). Under natural conditions with a higher atmospheric humidity and temperatures the survival time of the seeds is reduced to 1 - 2 months. Carnation is not winter hardy and can not survive in area's where temperatures occur below - 5 ◦C.

Cultivated carnation is biologically contained in the Northern European area, such as the Netherlands, due to the climatic conditions, and its susceptibility to diseases and plant pests. The running wild of carnation has never been observed in the Netherlands.

Wild relatives which can give viable progeny after hybridisation with carnation are absent in large area's of Europe. In those area's the only possible hybridization partners are other cultivated carnations and in the mediterranean area wild carnation. Hybridisation with other Dianthus species only gives progeny after the use of embryo rescue techniques.

Via classical breeding the pollen production of carnation cultivars is reduced due to the replacement of anthers by petals. Therefore the pollen production of the carnation cultivars used is limited. Next to this the large and sticky pollen produced remain deep within the petals, and are as a consequence hardly released. Due to this low effective release of pollen the chance of hybridisation of the genetically modified carnation with wild relatives or cultivated carnation is low. The dissemination of the genetic information via seeds or pollen is further limited due to the use of the carnation lines for the production of cut flowers. The stems with the flowers still developing are cut before and sold before the phase of flowering.

2. The inserted genes and traits

The carnation plants are modified using plasmid pWTT2160. Only the sequences as situated between the T-DNA borders of the plasmid are integrated in the carnation lines. Line 66 is checked via Southern blotting for the absence of the plasmid sequences situated outside the T-DNA borders. No incomplete elements under control of eukaryotic regulation sequences or elements under control of prokaryotic regulation sequences where introduced. The integrated sequences are completely characterised. The inserted genetic material was limited to the desired transfer of the sequences located between the borders of the vector pWTT2160. This vector contained between the T-DNA borders:

- the *acc*-gene, coding for 1-amino-cyclopropane-1-carboxylic acid synthase, derived from *Dianthus caryophyllus* L., under control of the constitutive 35S promoter and the nos terminator from *Agrobacterium tumefaciens*;

- the *surB*-gene, coding for acetolactate synthase protein, derived from *Nicotiana tabacum,* under control of the constitutive 35S promoter derived from cauliflower mosaic virus and the *surB* terminator derived from *Nicotiana tabacum.*

In the following evaluation the main characteristics are considered of:
- the selection marker,
- the delay of the senescence, increased vase live.

Ad a. Selection marker
Acetolactate synthase (ALS) belongs to the group of housekeeping enzymes. It is the first enzyme in the parallel pathways for the biosynthesis of the essential branched-chain amino acids valine, leucine and iso-leucine. Sulfonylurea-type herbicides inhibit the enzyme ALS. The phytotoxicity of the sulfonylurea-type herbicides is most likely caused by the occurrence of elevated levels of free amino acids and concomitant imbalances in their relative proportions. Growth of the susceptible plants is inhibited already within a few hours after application of the herbicide.
The product of the autologous acetolactate synthase gene from carnation is sensitive to sulphonylurea herbicides. The *sur*B gene, derived from *Nicotiana tabacum,* codes for an acetolactate synthase protein that is not sensitive to sulphonylurea herbicides, such as chlorsulfuron. The introduction and expression of the *sur*B gene provides the genetically modified carnation plants with a resistance to sulphonylurea herbicides. This resistance is only used during the transformation experiments for the selection of cells harbouring the intended genetic material.
The use of the sulphonylurea products as herbicides is not approved in the Netherlands and many other European countries. Carnation plants harbouring the sulfonylurea resistance gene therefore possess no selective advantage in comparison with carnation plants that only harbour the susceptible autologous carnation acetolactate synthase gene.

From the evaluation of the introduction of the *sur*B-gene into carnation, no reasons have emerged on the basis of which a consent to the proposed placing on the market should be withheld with respect to carnation lines harbouring this property.

Ad b. Delay of the senescence, increased vase live
The introduction of the constitutive expressed *acc*-synthase gene, derived from carnation, aimed at the inhibition of the senescence, to obtain an increased vase life of the cut flower.

Classical bred, non genetically modified carnation plants have a vase life on water of approximately 10 days. To be able to guarantee consumers a vase life of the carnation cut flowers of at least eight days, the carnations are pre-treated with Silver-containing solutions or with AOA (Amino-Oxyacetic Acid). Furthermore the results of these pre-treatments with these environmental harmful agents are to a large extent depending on the treatment conditions. Incorrect application may lead to a reduction of the vase life.

The senescence of the carnation flowers is closely related to the increase of the ethylene production in the flower. The product of the 1-amino-cyclopropane-1-carboxylic acid synthase-gene (*acc*-synthase gene) is as an enzyme involved in the biosynthesis of ethylene in plants. The insertion of the carnation derived petal (flower leaves) specific *acc*-sequences leads to the co-suppression of the autologous carnation *acc*-synthase genes. Due to the co-suppression the production in the petals of 1-amino-cyclopropane-1-carboxylic acid synthase is decreased, leading to a reduction in the ethylene production in the petals. The reduced ethylene production leads to a retardation of the senescence, thereby increasing the vase life of the cut flower. The insertion of the *acc*-gene does not lead to the

production of additional proteins.

The *acc* sequence as introduced in the carnation line 66, is petal specific and derived from carnation. The consequences of the introduction of the *acc* sequence is restricted to the petals. In the petals no new proteins or other substances are produced due to the introduced *acc*-sequence.
This modification does not lead to an increased dispersal or viability of the pollen. No effects of the ethylene expression was found in other parts of the carnation plants. therefore, no effects on the temperature sensitivity or the establishment of the carnation plants are to be expected.

Changes in the toxicity or allergenicity are not expected as there are no new proteins or other substances produced.

From the evaluation of the modification leading to a delay of the senescence due to a reduced ethylene production no reasons have emerged on the basis of which a consent to the proposed placing on the market should be withheld with respect to the carnation line harbouring this property.

3. The use of the colour modified carnation lines

Carnations are grown for their ornamental value. The carnation line with an increased vase life is in principle intended for world wide use in horticulture. For this purpose cutflowers will be produced by growers from cuttings of the motherplants. The cutflowers produced will be sold via flower auctions, flower wholesalers and retailers to customers.

Conclusion:

The potential effects of the introduction and expression of the selection gene *sur*B and the ethylene production influencing gene *acc*, are analysed. From this evaluation no reasons have emerged on the basis of which a consent to the proposed placing on the market of this product should be withheld.

7. CONCLUSION

Based on the notification, the appendixes and the above considerations, the Netherlands Competent Authority concludes that no reasons have emerged on the basis of which a 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 June 3, 1997, registered under number C/NL/97/12.

The product consists of carnation plants (*Dianthus caryophyllus* L.), derived from genetically modified carnation line 66, in which the following functional genes are introduced:

- the *acc*-gene, coding for 1-Amino-cyclopropane-1-carboxylic acid synthase, derived from *Dianthus caryophyllus* L., under control of the constitutive 35S promoter and the nos terminator from *Agrobacterium tumefaciens*;
- the *surB*-gene, coding for acetolactate synthase protein, derived from *Nicotiana tabacum*, under control of the constitutive 35S promoter derived from cauliflower mosaic virus and the *surB* terminator derived from *Nicotiana tabacum*.

The product includes progeny derived through vegetative reproduction as well as progeny derived through crosses of the genetically modified carnation line 66 with any traditionally bred non-genetically modified carnation plant.

The above mentioned genetically modified carnation plants derived from carnation line 66 are registered under consent registration number C/NL/97/12-66.