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Onderwerp
Marktdossier C/NL/04/02

Geachte heer Van Geel,

Naar aanleiding van het dossier C/NL/04/02, 'Application to import carnation varieties Florigene Moonlite (123.2.38) en Florigene Moonshade (123.2.2)', door Florigene Ltd., Melbourne Australia, en het voorblad dat door het Bureau GGO is opgesteld deelt de COGEM u het volgende mee.

Samenvatting:
De COGEM is gevraagd te adviseren betreffende een vergunningaanvraag voor de import en verkoop van snijbloemen van een genetisch gemodificeerde (gg) anjervariëteit in Europa. Van nature bestaan er geen blauwe anjers omdat zij bepaalde genen missen. Door in een witte anjervariëteit een tweetal genen uit petunia in te bouwen vormt de gg-anjer blauwe bloemen. Productie van de bloemen vindt plaats in Zuid-Amerika en Australië. De aanvrager heeft overigens al sinds 1997 een vergunning om een andere vergelijkbare blauwe anjer te verbouwen en te verkopen in Europa.

Anjer heeft geen kruisbare wilde verwanten en is niet in staat tot verwildering. De ingebrachte genen leiden niet tot verandering van de biologische eigenschappen van de plant. Derhalve is verspreiding van de genen of de gg-anjer in de natuur uitgesloten. Bloemblaadjes van anjer worden soms gebruikt als garnering van voornamelijk desserts. Toelating van de gg-anjer als voedsel wordt niet aangevraagd maar mogelijkerwijs kan incidentele consumptie plaatsvinden. Aangezien de gg-anjer een lange geschiedenis van veilig gebruik heeft in andere werelddelen, de aanvrager gegevens heeft overlegd waaruit blijkt dat de anjer niet giftig is, en de mogelijke inname door consumenten zeer gering is, is de COGEM van mening dat de mogelijke risico's voor de menselijke gezondheid verwaarloosbaar zijn.

De COGEM acht derhalve de risico's voor mens en milieu bij import van snijbloemen van de genetische gemodificeerde blauwe anjer verwaarloosbaar klein.

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De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

Prof. dr. ir. B.C.J. Zoeteman
Voorzitter COGEM

c.c. Dr. ir. B.P. Loos
Dr. I. van der Leij

Title: Import of cut flowers of the genetically modified carnation variety 'Florigene Moonlite' (C/NL/04/02)

COGEM advice: CGM/050207-01

The application concerns the commercial import of cut flowers of a genetically modified carnation (Dianthus caryophyllus) variety with a modified flower colour. The cut flowers are produced outside the EU member states. A similar transgenic carnation variety has previously been approved for commercial production within the EU in 1997.

Carnation does not have weedy characteristics and although carnation is grown for centuries it has never been found in the wild. The introduced traits, flower colour and herbicide tolerance do not alter the biological characteristics of the plant. Carnation is not able to outcross with wild relatives and the risk of transfer of the introduced traits to related species is absent.

Petals of carnation are occasionally used as garnishing. The genetically modified carnation variety has a history of safe use and results of toxicity tests indicate that detrimental effects are absent. Moreover, the exposure to potential harmful proteins caused by incidental consumption of garnish elements is too low to evoke adverse effects like allergic reactions.

In view of the aforementioned COGEM is of the opinion that the risks for the environment and human health associated with import of cut flowers are negligible.

1. Introduction

The present application concerns the commercial import, distribution and sale of a genetically modified carnation (*Dianthus caryophyllus*) variety with a modified flower colour. The cultivar involved ('Moonlite') also contains an herbicide tolerance gene.

'Moonlite' is admitted and commercially grown in Australia, Colombia and Ecuador. A similar transgenic carnation variety 'Florigene Moondust' has been previously approved for commercial production within the EU in 1997 (C/NL/96/14-11). The present application seeks to extend this permit with import of cut flowers of the cultivar 'Moonlite'.

Carnations, like roses or chrysanthemums, do not produce a blue pigment called delphinidin since these plants lack part of the anthocyanin biosynthetic pathway. Consequently, it is impossible to create blue carnations by traditional breeding methods. However, genetic modification offers a means to introduce a violet or blue colour in these plants. This can be achieved by insertion of the DFR and F3'5'H genes

in the genome of carnation varieties with white flowers. These genes produce enzymes which convert certain flavonoids into delphinidin. Accumulation of delphinidin in petals results in different shades of violet flowers depending on the level of accumulation.

1.2 *Previous COGEM advices*

In the past COGEM has advised positively on a similar application involving a carnation variety ('Moondust') with a modified flower colour (CGM/961122-08, CGM/961122-13 and CGM/970513-05). This application involved both the same genes and vector DNA. However, it involved a different carnation variety and production, whereas this application only deals with import of cut flowers. 'Moondust' was admitted to the European market in December 1997 and was cultivated between 1998 and 2000 in the Netherlands and Spain and sold in Europe.

Furthermore, in 1997 COGEM advised positively on an application for production of carnation with an increased shelf life in the EU (CGM/970714-01).

2. Dianthus, Aspects of the crop

Carnations are double-flowered cultivars and considered to belong to the species *Dianthus carophyllus* of the widely cultivated genus *Dianthus*. However, the exact origin is obscure, most likely cultivated carnation stems from a hybrid involving *D. carophyllus* and another *Dianthus* species.[1, 8] The non-horticultural single-flower form of *D. carophyllus* (the 'clove pink') is a rare wild species of the coastal areas of Southern Europe. [2, 3]

The nomenclature is somewhat confusing. Nowadays the common name of *D. carophyllus* is carnation. However, some carnations are known as 'pinks' and the term carnation is sometimes used to indicate other *Dianthus* species. Moreover, some cultivated carnations are hybrids with *D. plumaris*. This application concerns a cultivated double-flowered carnation (*D. carophyllus*) variety.

Carnations are cultured for many hundreds of years and are presently amongst the most extensively grown cut flowers with more than 10 billion carnations produced around the world each year. Carnations are sold as cut flowers, cuttings or plants. Cultivated carnation is not propagated by seed but vegetatively by cuttings and tissue culture. Propagation in the horticulture involves the use of so-called mother plants. Cuttings of these mother plants are used for the production of flowers for a period of two years. Carnation does not spread vegetatively spontaneously, and it does not produce vegetative organs like bulbs, stolons or rhizomes. Carnation is highly domesticated by generations of breeding aimed at improvement of flower size and colour variation. Carnation is semi-winter hardy and has no weedy characteristics and after decades of cultivation carnations has not been able to establish in the wild.[2]

Wild *Dianthus* species occur worldwide.[1,2] They are common wildflowers in the United States and Canada. Europe is the centre of biodiversity. In Europe *Dianthus* species are found in mountainous areas like the alpine region, mainly in the Balkan and the Mediterranean area. In the Netherlands occur the rare species *D. deltoides* (steenanjer), *D. armeria* (ruige anjer), *D. superbus*, (pracht anjer) and *D. carthusonarium* (Kartuizer anjer)[4]. The species *D. barbatus* (duizendschoon; Sweet William) is commonly grown as a garden plant and has established itself in the wild.[4]

Pollination of Dianthus in nature occurs exclusively by insects (Lepidoptera species). The nectaries are at the base of the flowers and only insects with a proboscis longer than 2.5 cm can reach the nectary. The number of insects visiting the carnation flower is further limited due to the fact that carnation cultivars have a long distance between the edge of the petals and the nectary, making it extremely difficult for insects to extract the nectar. Dianthus species are protrandous, the anthers and pollen mature before the pistils. Pollen shedding takes place at the opening of the flower. As the flower age the anthers fall off and the styles become receptive.

Carnation can theoretically cross hybridise with other *Dianthus* species and interspecific crossings haven been made manually by breeders to introduce new traits into carnation. However, spontaneous hybridisation between cultivated carnation and wild *Dianthus* species has never been reported, despite decades of cultivation in gardens and parks. No records of hybrids exist in the scientific literature or floras.

3. Molecular characterisation

3.1 *Origin and function of the introduced genes*

The genetically modified carnation line was produced by transformation with *Agrobacterium tumefaciens* using a disabled *Ti* plasmid. To produce violet or delphinidin derived pigment the DFR and F3'5'H genes were inserted in the carnation genome. Accumulation in the petals of these pigments results in a violet to blue flower colour.

As a selection marker in the transformation process an herbicide tolerance (suRB) gene was introduced in the transgenic line. This herbicide tolerance has no agronomic relevance.

An overview of the introduced sequences is given below:

- DFR gene, coding for dihydroxyflavonol 4-reductase, derived from *Petunia* X *hybrida*. The *DFR* enzyme uses dihydroquercetin and dihydromyricetin as a substrate to produce delphinidin;
- F3'5'H gene (*Hf1*), flavonoid 3',5' hydroxylase, derived from *Petunia* X *hybrida*. *Hf1* converts dihydrokaempferol or dihydroquercetin into dihydroflavonol dihydromyricetin;

- suRB gene, coding for a mutant acetolactate synthetase protein (*ALS*), derived from *Nicotiana tabacum. ALS* confers tolerance to sulfonylurea herbicides;
- CHS-promoter, chalcone synthase petal specific promoter derived from *Anthirrhinium majus*;
- CaMV 35S promoter, 35S promoter of *Cauliflower mosaic virus*;
- Mac promoter, constitutive hybrid promoter consisting of the *A. tumefaciens* Mas promoter and the CaMV 35S promoter;
- MAS terminator, the 3'-terminal sequence of the *A. tumefaciens* mannopine synthase gene;
- D8 terminator; terminator sequence derived from *Petunia* X *hybrida*;

The inserted genes DFR, F3'5'H and suRB all origin from plants. The proteins encoded by these genes do not share homology with known toxins or antigens.

3.2 *Molecular analysis*

Noteworthy is that the TetA gene is present on the backbone sequence of the vector used in the transformation process. This gene confers resistance to the antibiotic tetracycline and was used in the production process of the vector DNA. Backbone sequences are presumed not to be inserted in the genome of the plant. The applicant has conclusively proven by PCR that the complete TetA sequence is indeed not present in the carnation variety 'Moonlite'. However, a small part of the TetA sequence is inserted into the carnation genome. To the opinion of the experts of COGEM the chances of expression of this small fragment are negligible and the possibility of translation into a protein absent.

Insertion of foreign genes into the genome of an organism can lead to disruption of host genes and the formation of new open reading frames (ORFs) consisting of host and donor sequences. Therefore, applicants are usually required to analyse the border and bordering sequences of the insertion for the presence of chimer ORFs. The applicant did not fully characterise the insert and its bordering sequences. Using a so-called southern blot assay it is estimated that one to three copies of the inserted sequences are present in the carnation genome. The applicant did not perform sequence analysis to determine whether new ORFs were formed due to these insertions. However, it is questionable whether information on putative new ORFs is relevant to this notification as it involves the import of cut flowers of carnation.

The applicant provided information, based on southern blot analysis, that the integration patterns of the introduced genes remain stable and unchanged. The genetically modified carnation has been vegetatively propagated since 1998. Commercial production has started in 2000 and approximately 5 million of flowers have been produced in South America en Australia. To the opinion of the experts of

COGEM the genetic stability of the cultivar is sufficiently proven by the provided information and the fact that during the production period no phenotypic aberrations were found.

4. Advice

This application concerns the import of cut flowers of genetically modified carnation. This carnation variety is admitted and in commercial production in Australia[8], Colombia and Ecuador. A similar transgenic carnation variety has already been approved for commercial production within the EU.

In the environmental risk assessment the probability of gene dispersal, weediness and potential risks to consumers due to incidental consumption has to be considered. However, as it involves the import of cut flowers the number of relevant issues is limited.

Carnation is not able to spread vegetatively and cut flowers are not able to form roots. This appears to exclude the possibility that the imported material will give rise to plants and establish itself in the wild. Nevertheless, carnation can be propagated by stem cuttings and this method is used both by professionals in the flower industry and amateur gardeners. It can not be ruled out that buyers will propagate the material to plant in their gardens. However, carnation has no weedy characteristics[2] and the traits (blue pigmentation and herbicide tolerance) which are introduced in the genetically modified variety do not alter the biology of carnation to the opinion of COGEM. Although carnation is cultivated for decades worldwide, it has never been found growing in the wild.

Carnation can only theoretically hybridize with wild relatives. Carnation is exclusively pollinated by butterflies or moths. Outcrossing during production or transport is unlikely as flowers are cut before opening and transported refrigerated. It is possible, although highly unlikely, that cut flowers in the vase are visited by butterflies and become pollinated.

This window of opportunity is small. Carnation produces few anthers and little pollen with a reduced viability. Pollen shedding only takes place at the opening of the flower. The applicant provides data that the variety ‘Moonlite’ produce significantly lower numbers of anthers compared to other carnation varieties. This further lowers the possibility of hybridisation with wild relatives.

Formation of seed on cut flowers is highly improbable. Carnations plants require five to six weeks for seed development while the vase life of carnation flowers is only three to four weeks.

Most importantly, there has never been any evidence of hybridisation between carnation and wild dianthus species, despite the fact that carnation is cultivated worldwide for decades.

Moreover, the environmental risks linked to hybridization of this genetically modified carnation variety with wild relatives are comparable with those of conventional carnation. The genetic modification involving genes which play a role in the anthocyanin pathway resulting in blue pigmentation do not alter the biological characteristics of carnation. Neither the F3'5'H gene and DFR gene nor the herbicide tolerance gene SuRB offer selectable advantages in nature. Accordingly, gene flow to wild relatives will not pose an environmental risk.

Therefore, COGEM concludes that the risk of transfer of genetic traits from the transgenic carnation variety to species in unmanaged environments is insignificant.

Cut flowers are not a food, nevertheless, the risks of incidental consumption have to be considered. Petals of carnation are occasionally used as garnishing[5,6]. In Europe they are mainly used as garnishing in desserts in low amounts. The flower petals lack a strong taste but have a strong smell of cloves. Occasionally, petals are candied used as a garnish in salads, for flavouring fruit, fruit salads etc[5]. Petals of the 'wild clove pink' are very aromatic and used in syrups. Unclear is whether recipes refer to petals of cultivated carnation or other dianthus species like 'wild clove pink'.

This notification is for the import and distribution of cut flowers and not for food purposes. Therefore, retailers will not be allowed to sell the petals of the genetically modified carnation for food purposes. However, it can not be entirely excluded that individuals will use petals of bought flowers to garnish their plates.

The introduced transgenic proteins all origin from plants and do not share homologies with known toxins or antigens. Delphinidin is present in fruits like blueberries. Thus, expression of the introduced genes or the production of delphinine will not pose a threat to human health.

The applicant did not fully characterise the borders and flanking sequence of the inserts. Therefore, it can not be excluded that chimeric ORFs have been created. These ORFs could theoretically be expressed and encode proteins with detrimental effects. It is unlikely that persons will consume large amounts of petals since it is used as garnishing and not as a food. Per flower head there is approximately one gram of petals present. Considering the amount of petals per flower head it is likely that only a part of this will be consumed by one person. The exact protein content of the petals is not known but is probably circa 20 milligram per gram dry weight. The expression of putative new ORFs will be extremely low as they lack suitable regulation signals and will make up an insignificant part of the total protein content. Therefore, the amount of intake will be in the nanogram level. Consequently, any unintended exposure to potential new proteins caused by incidental consumption of garnish elements is highly unlikely to evoke adverse effects like allergic reactions.

This is supported by results of phytotoxicity, cytotoxicity assays, and acute toxicity tests using mice. These latter tests represented the consumption of 20 flowers by a 60 kg human. No significant effects were measured, indicating that the incidental consumption of petals of genetically modified carnation do not pose a threat to human health.

Noteworthy is that genetically modified carnation has a history of safe use. The flowers are produced on a large scale in Australia and South America and sold throughout the United States, Canada and Japan.[7,8] Other genetically modified carnation varieties have been produced and sold in Europe. There have been no reports of allergic reactions or other adverse reactions.

In view of the aforementioned the COGEM is of the opinion that the potential risk to the health of potential consumers is negligible.

In recapitulation, the application involves the import of cut flowers, the genetically modified carnation has no weedy characteristics and is not able to establish itself in the wild, the risk of transfer of the introduced genes to wild relatives is nil, and the genetically modified variety does not pose a threat to the health of consumers or the environment.

In view of this COGEM is of the opinion that the proposed import of cut flowers does not pose a significant risk to human health or the environment.

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