Renewal of the authorisation for import, distribution and retail of genetically modified carnation IFD-26407-2 (Moonvelvet)

COGEM advise CGM/240502-02

- The present application (C/NL/09/01_001) concerns the renewal of the authorisation for import, distribution and retail of cut flowers of genetically modified (GM) carnation (*Dianthus caryophyllus*) IFD-26407-2 (Florigene® Moonvelvet™);
- GM carnation IFD-26407-2 has been authorised import for, distribution and retail since 2015. Since the market approval ~1.6 million flowers have been imported in the EU;
- COGEM advised positively on the import, distribution and retail of carnation IFD-26407-2 in the EU in 2009;
- GM carnation IFD-26407-2 expresses the petunia flavonoid 3'5'hydroxylase gene (*f3'5'h*) which encodes enzymes involved in the production of flower pigments, and the petunia cytochrome b5 (*difF*) which encodes a protein that enhances F3'5'H activity. Furthermore, IFD-26407-2 expresses a mutated *SuR*B gene from tobacco, which confers tolerance to sulfonylurea herbicides;

- The updated bioinformatic analyses and molecular characterisation of GM carnation IFD-26407-2 meet the criteria of COGEM;
- Carnation does not have weedy characteristics and cut carnation flowers cannot fertilise wild relatives;
- There is no evidence of establishment of IFD-26407-2 in the wild, or of introgression of the transgenes into wild *Dianthus* species. There are no indications that the introduced traits alter the fitness of cut carnation flowers of IFD-26407-2;

- The authorisation of IFD-26407-2 will not include the use of the GM carnation as food. Carnation petals are sometimes used to garnish dishes. Therefore, it is likely that in case of unauthorised incidental consumption, only a small amount of IFD-26407-2 would be consumed. The introduced genes are derived from petunia and viola, flowers that are also sometimes used as garnishes, and these genes do not encode toxic or allergenic products. COGEM is not aware of any reports indicating that incidental consumption of IFD-26407-2 poses a risk to human health. COGEM is of the opinion that IFD-26407-2 poses a negligible risk to human health in case of unauthorised incidental consumption;

- COGEM is of the opinion that import, distribution and retail of cut carnation flowers of GM IFD-26407-2 poses a negligible risk to the European environment and human health.

1. Introduction

The present application (C/NL/09/01_001), filed by Suntory Flowers Ltd. concerns the renewal of the authorisation for import, distribution and retail of cut flowers of genetically modified (GM) carnation (*Dianthus caryophyllus*) IFD-26407-2 (also known as Florigene® Moonvelvet™. This GM carnation expresses the *difF* (petunia), *f3'5'h* (petunia) and *suR*B (tobacco) genes resulting in a modified flower colour and tolerance to sulfonylurea herbicides (see Table in paragraph 3.2). Authorisation for the production, import, distribution and retail of Moonvelvet™ in the EU was granted in 2015. Since import and processing authorisations remain valid for a period of 10 years, the applicant filed an application for the renewal of the authorisation.

2. Previous COGEM advices

In 2009, COGEM issued a positive advice on the import, distribution and retail in the EU of this GM carnation Moonvelvet™, IFD-26407-2.[1] COGEM also issued several positive opinions on import, distribution and retail of similar GM carnations with altered flower colours (e.g., Moonshadow, Moonlite and Moonaqua).[2,3,4,5,6,7,8,9,10]

3. Environmental risk assessment

3.1 Aspects of the wild-type crop

Carnation belongs to the species *Dianthus caryophyllus* of the widely cultivated genus *Dianthus*. The non-horticultural single-flower form of *D. caryophyllus* (the clove pink) is native to Southern Europe where it grows on walls, in rock crevices and on dry stony slopes in Mediterranean coastal regions (up to more than 200 km inland).[11] Wild *Dianthus* species are found throughout Europe, mainly in mountainous areas in the alpine region, the Balkan and the Mediterranean area.[12,13,14] In the Netherlands, several native *Dianthus* species occur: *Dianthus deltoides* (Steenanjer; maiden pink), *Dianthus armeria* (Ruige anjer; Deptford pink), and *Dianthus carthusianorum* (Kartuizer anjer; Carthusian pink).[15,16] Some *Dianthus* species that are commonly grown as garden plants have established themselves in the wild.[15,16] Interspecific crossings between *Dianthus* species have been made manually by breeders to introduce new traits into carnation varieties.[12,17,18]

This application concerns a cultivated double-flowered carnation. Cultivated carnations are almost certainly hybrids between two or more *Dianthus* species, one of which is most likely *D. caryophyllus*. Carnations have been cultured for hundreds of years and are presently amongst the most extensively grown cut flowers with more than ten billion carnations produced around the world each year. Cultivated carnation is not propagated by seed, but vegetatively by cuttings and tissue culture.[19] Carnation does not spread vegetatively spontaneously, and it does not produce vegetative organs like bulbs, stolons or rhizomes.[19]

To improve flower size and generate colour variants, carnation has been bred for centuries. As a result, carnation is highly domesticated. Carnation is semi-winter hardy, has no weedy characteristics and even after decades of cultivation has never shown to be able to establish itself in the wild.[11]

In nature, pollination of *D. caryophyllus* occurs exclusively by lepidopteran insects. *Dianthus* carnation pollen cannot be spread by wind. The pollen produced is heavy and sticky and deeply buried in the flower.[20,21] Although the Netherlands has a large carnation handling industry, carnation pollen is not detected in the atmosphere.[22,23,24]

Transfer of viable transgenic pollen by butterflies to wild *D. caryophyllus*, could theoretically happen when GM carnation is brought to cemeteries (as bouquet flowers) close to the natural habitat of wild *D. caryophyllus*, However, domesticated carnation produces little pollen with reduced viability.[12,25] Breeding has increased the number of petals present in carnation cultivars. As a result, the reproductive tissues of the flower have become enclosed, restricting access to insect pollinators.[12] Due to these factors, the chance of natural hybridisation of cultivated carnations with wild relatives is low. In case of cut flower production, the likelihood of dissemination of genetic material through pollen or seeds is limited even further because stems are cut before anthesis.[12] There has never been any evidence of spontaneous hybridisation between carnation and wild *Dianthus* species, despite the fact that carnation has been cultivated worldwide for centuries.

Conclusion: Carnation does not have weedy characteristics and there are no reports of established cut flower carnation varieties. The likelihood that carnation can fertilise wild relatives under natural conditions is limited.

3.2 Description of the introduced genes and traits

GM carnation IFD-26407-2 was developed by *Agrobacterium*-mediated transformation, using the transformation vector pCGP2355.

Introduced genes	**Encoded proteins**	**Traits**	**Regulatory elements**
*suR*B	Acetolactate synthase (ALS) enzyme derived from *Nicotiana tabacum*[1,26]	Confers tolerance to ALS inhibiting (sulfonylurea) herbicides which are used to select transformants	Enhanced CaMV 35S (e35S) promoter and terminator. *Nicotiana tabacum* ALS terminator
f3'5'h	Flavonoid 3'5' hydroxylase (F3'5'H) enzyme derived from *Viola* sp.[1,27]	Modified flower colour	Snapdragon chalcone synthase promoter. Petunia phospholipid transfer protein terminator
difF	Cytochrome b5 [1,28]	Enhanced F3'5'H activity	Snapdragon chalcone synthase promoter. Petunia phospholipid transfer protein terminator
For a detailed description of the introduced gene and trait, see references			

3.3 Updated bioinformatics analyses and molecular characterisation

By sequencing and southern analyses of IFD-26407-2, the T-DNA was confirmed to be a singular intact insertion.[29] In this renewal longer stretches of the flanking regions were sequenced (2112 and 901 bp) as compared to the original application (308 and 359 bp), which were bioinformatically analysed to determine the chromosomal location of the T-DNA. The screening for open reading frames (ORFs) from stop-to-stop codon in flanking sequences did not indicate that an endogenous gene was affected by the insertion.

The amino acid sequences of the proteins encoded by the genes located in the T-DNA were compared with sequences in allergen databases which did not yield any significant similarity. However, not all stop-to-stop codon ORFs in the T-DNA and the ORFs spanning the junction between T-DNA and genomic DNA were fully bioinformatically analysed, a requirement set out by the COGEM.**Error! Bookmark not defined.** Given that the application does not include food or feed purposes and that IFD-26407-2 has been on the market for 10 years without reports of adverse effects, COGEM is of the opinion that the molecular characterisation is adequate in this specific case.

Conclusion: No new indications for potential environmental risks were identified. Although he molecular characterisation of carnation IFD-26407-2 does not meet COGEM requirements, in this case it is deemed adequate.

3.4 Incidental consumption

Petals of carnation are sometimes used in dishes and as garnishing.[30,31,32] The genes introduced in IFD-26407-2 do not encode toxic or allergenic products. The authorisation of IFD-26407-2 is limited to import, distribution and retail and does not allow the use of the GM carnation as food. Therefore, retailers will not be allowed to sell the petals of the GM carnation for food purposes.

People are generally advised against using flowers from flower shops or commercial growers for food purposes because these might contain residues from pesticides or other chemicals. However, it cannot be entirely excluded that individuals will use petals of bought flowers in dishes. In such a case, it is unlikely that large amounts of petals will be consumed, because these petals are used as a garnish. It is likely that a person would consume only part of the petals of a flower. Consumption of such a small amount of IFD-26407-2 is unlikely to lead to an adverse effect.

IFD-26407-2 has been authorised for import, distribution and retail in Europe since 2015. COGEM is not aware of any reports indicating that incidental consumption of IFD-26407-2 poses a risk to human health. In view of the above-mentioned considerations, COGEM is of the opinion that unauthorised incidental consumption of GM carnation IFD-26407-2 poses a negligible risk to human health.

3.5 Literature & database review

The applicant performed annual literature and database searches from 2016 to 2023 as part of the monitoring process for IFD-26407-2 to identify reports of carnation in the wild. The literature that was identified did not contain any reports of carnations occurring in the wild or evidence of

introgression of the transgenes to wild *Dianthus* species. According to the applicant, no evidence was found to indicate a change in the outcome of the risk assessment carried out at the time of the marketing approval of IFD-26407-2.

Conclusion: The annual literature and database searches do not give an indication of potential environmental risks resulting from import, distribution and retail of cut carnation flowers of IFD-26407-2.

3.6 Annual monitoring reports

The applicant supplied monitoring reports for IFD-26407-2 on an annual basis between September 2015 and June 2023. Several monitoring activities were conducted by the applicant. The applicant obtained questionnaire feedback from the importer at least once each year. The applicant established an expert monitoring group comprising of breeders and research experts to, among other things, obtain information on illegal propagation of GM carnation in Europe. This was also done to ensure that the unexpected establishment of GM carnation in the wild, or the introgression of the transgenes into wild *Dianthus* species would be reported to the applicant. Furthermore, they contacted individual scientists and several institutions (herbaria, plant protection services, conservation groups etc.) to request any reports on the identification of wild populations of carnation, *D. caryophyllus* or related *Dianthus* species. The monitoring plan was adapted once in November 2021, discontinuing the need for the so called ‘mail out’ component of the monitoring plan. COGEM previously advised positively on the discontinuation of the 'mail out' component.[33] COGEM was of the opinion that the alternative methods used by the permit holder are sufficient to allow a timely observation of any adverse effects on human health and the environment if these would occur. This change was implemented in 2023. No evidence of the establishment of IFD-26407-2 nor introgression of the transgenes into wild *Dianthus* species was obtained.

Conclusion: There is no evidence of the presence of IFD-26407-2 in the wild, nor of introgression of the transgenes into wild *Dianthus* species.

4. Overall conclusion

There are no indications that the introduced traits will alter the fitness of cut carnation flowers of IFD-26407-2. Import, distribution and retail of cut carnation flowers of GM IFD-26407-2 pose a negligible risk to human health and the European environment.

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