Renewal of the authorisation for import, distribution and retail of genetically modified carnation FLO-40689-6

COGEM advice CGM/180613-02

- The present application (C/NL/06/01) concerns the renewal of the authorisation for import, distribution and retail of cut flowers of genetically modified (GM) carnation FLO-40689-6 (Florigene Moonaqua) in the EU;
- A consent for import, distribution and retail of cut flowers of GM carnation FLO-40689-6 in Europe was granted in 2009. Since the market approval ~12 million flowers have been imported in the EU;
- COGEM advised positively on the import, distribution and retail of cut flowers of GM carnation FLO-40689-6 in the EU in 2007;
- GM carnation FLO-40689-6 expresses petunia dihydroflavonol 4-reductase (DFR) and *Viola* flavonoid 3'5'hydroxylase (*F3'5'H*), enzymes involved in the production of flower pigments. FLO-40689-6 also expresses a mutated *SuR*B gene from tobacco, which confers tolerance to sulfonylurea herbicides;

- The updated bioinformatic analyses and molecular characterisation of GM carnation FLO-40689-6 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 FLO-40689-6 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 FLO-40689-6;

- The authorisation of FLO-40689-6 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 FLO-40689-6 would be consumed. The introduced genes are derived from petunia and viola, flowers that are also sometimes used as garnishes, these genes do not encode toxic or allergenic products. COGEM is not aware of any reports indicating that incidental consumption of FLO-40689-6 poses a risk to human health. COGEM is of the opinion that FLO-40689-6 poses a negligible risk to human health in case of unauthorised incidental consumption;

- Import, distribution and retail of cut carnation flowers of GM FLO-40689-6 pose a negligible risk to the European environment and human health.

1. Introduction

The present application (C/NL/06/01), 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*) FLO-40689-6 (also known as Florigene Moonaqua, or line 123.8.12). This GM carnation expresses the *dfr, F3'5'H* and *SuR*B genes resulting in a modified flower colour and tolerance to sulfonylurea herbicides (see Table in 3.2). Consent to place Moonaqua on the market in Europe was granted in 2009. 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 2007, COGEM issued a positive advice on the import, distribution and retail in the EU of the GM carnation Moonaqua, FLO-40689-6 (123.8.12).[1] COGEM also issued several positive opinions on import, distribution and retail of similar GM carnations with altered flower colours (e.g., Moonshadow and Moonlite).[2,3,4,5,6,7,8]

3. Environmental risk assessment

3.1 Characteristics of carnation

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).[9] In Europe, wild *Dianthus* species are found in mountainous areas in the alpine region, the Balkan and the Mediterranean area.[10,11,12] 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).[13,14] Some *Dianthus* species that are commonly grown as garden plants have established themselves in the wild.[13,14] Interspecific crossings between *Dianthus* species have been made manually by breeders to introduce new traits into carnation varieties.[10,15,16]

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 presently are 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. In horticulture, propagation involves the use of mother plants.[17] Carnation does not spread vegetatively spontaneously, and it does not produce vegetative organs like bulbs, stolons or rhizomes.[17]

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.[9]

In nature, pollination of *D. caryophyllus* occurs exclusively by lepidopteran insects. *Dianthus* carnation pollen cannot be spread by wind. Any pollen produced is heavy and sticky and deeply buried in the flower.[18,19] Although the Netherlands has a large carnation handling industry, carnation pollen is not detected in the atmosphere.[20,21,22]

Transfer of viable transgenic pollen by butterflies to wild *D. caryophyllus*, could theoretically happen when GM carnation is for instance brought to cemeteries (as bouquet flowers) close to the natural habitat of wild *D. caryophyllus*, However, domesticated carnation produces little pollen with reduced viability.[10,23] 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.[10] 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.[10] 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 FLO-40689-6 was developed by *Agrobacterium*-mediated transformation, using the transformation vector pCGP1991.

Introduced genes	Encoded proteins	Traits
*SuR*B	Acetolactate synthase (ALS) enzyme derived from *Nicotiana tabacum*[1,24]	Confers tolerance to ALS inhibiting (sulfonylurea) herbicides which are used to select transformants
F3'5'H	Flavonoid 3'5' hydroxylase (F3'5'H) enzyme derived from *Viola* sp.[1,25]	Modified flower colour
dfr	Dihydroflavonol-4-reductase derived from *Petunia x hybrida*[1,26]	Modified flower colour
For a detailed description of the introduced gene and trait, see references		

3.3 Updated bioinformatics analyses and molecular characterisation

FLO-40689-6 was resequenced in 2017. Comparison of newly generated sequence data of FLO-40689-6 to the originally submitted sequence data in 2007 identified a one nucleotide difference in one of the three insertion loci. The correction required the removal of one nucleotide from the original sequence. This nucleotide was located in the non-coding polylinker sequence of the

transformation vector. Because of this nucleotide deletion, the applicant carried out a complete bioinformatics analysis of the inserted element and the sequences spanning the 5' and 3' junctions of the insert and its flanking regions, which were screened to identify all open reading frames (ORFs) between stop codons. According to the applicant, analysis of the putative products of the ORFs spanning the 5' and 3' junctions of the inserts did not generate any protein sequence similarity with known allergens or toxins.

A carnation nuclear genome sequence database, not available in 2007, was used to look for possible insertion sites of the transgenic loci in event FLO-40689-6 in the carnation genome. Locus 3 was shown to be in a non-coding region of the carnation genome. According to the applicant, the insertions in loci 1 and 2 may be in protein encoding regions, though the function of the hypothetical proteins could not be identified from the information available in databases. FLO-40689-6 has a history of safe use and there are no indications that FLO-40689-6 has different biological characteristics apart from the introduced traits.

Considering the above, COGEM is of the opinion that the molecular characterisation has been performed correctly and meets the requirements of COGEM.[27] No new elements that would invalidate the conclusions of the initial risk assessment were identified.

Conclusion: The molecular characterisation of carnation FLO-40689-6 is adequate and no indications for potential environmental risks were identified.

3.4 Incidental consumption

Petals of carnation are sometimes used in dishes and as garnishing.[28,29,30] The genes introduced in FLO-40689-6 do not encode toxic or allergenic products. The authorisation of FLO-40689-6 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 FLO-40689-6 is unlikely to lead to an adverse effect.

FLO-40689-6 has been authorised for import, distribution and retail in Europe since 2009. COGEM is not aware of any reports indicating that incidental consumption of FLO-40689-6 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 FLO-40689-6 poses a negligible risk to human health.

Conclusion: FLO-40689-6 poses a negligible risk to human health in case of unauthorised incidental consumption.

3.5 Literature review

The applicant performed annual literature searches from 2010 to 2017 as part of the monitoring process for FLO-40689-6 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. In addition, the applicant supplied copies of three literature reviews carried out in 2013 and 2014 that were performed for other GM carnations, since the original application in 2007 using a broad collection of up to date bibliographic databases. 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 FLO-40689-6.

Conclusion: The annual literature searches and three literature reviews do not give indication of potential environmental risks resulting from import, distribution and retail of cut carnation flowers of FLO-40689-6.

3.6 Annual monitoring reports

The applicant supplied monitoring reports for FLO-40689-6 on an annual basis between July 2010 and July 2017. Several monitoring activities were conducted by the applicant. The applicant obtained questionnaire feedback from the importer at least once a year. The applicant established an expert monitoring group, comprising of breeders and research experts, and contacted individual scientists and several institutions (herbaria, plant protection services, conservation groups etc.) to, among other things, obtain information on illegal propagation of GM carnation in Europe. This was also done to ensure that unexpected establishment of GM carnation in the wild, or introgression of the transgenes into wild *Dianthus* species would be reported to the applicant. The applicant also inspected the composting areas of the production sites in Colombia (24 times) and Ecuador (9 times) and investigated whether GM carnation established at these areas. No evidence of the establishment of FLO-40689-6 nor introgression of the transgenes into wild *Dianthus* species was obtained.

Conclusion: There is no evidence of the establishment of FLO-40689-6 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 FLO-40689-6. Import, distribution and retail of cut carnation flowers of GM FLO-40689-6 pose a negligible risk to human health and the European environment.

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