Renewal of the consent for import, distribution and retail of genetically modified carnation FLO-40644-6

COGEM advice CGM/160706-03

- A consent for import, distribution and retail of FLO-40644-6 in Europe was granted in 2007;
- The present application (C/NL/04/02_001) concerns renewal of the consent for import, distribution and retail of GM carnation FLO-40644-6;
- Carnation FLO-40644-6 was produced by *Agrobacterium tumefaciens* mediated transformation;
- It expresses the *dfr*, *f3'5'h* and *suRB* genes resulting in a modified flower colour and tolerance to sulfonylurea herbicides;
- Carnation does not have weedy characteristics, there are no reports of establishment of cut flower carnation varieties in the wild, and carnation cannot fertilise wild relatives under natural conditions;
- The updated bioinformatic analyses did not include an analysis of all the putative fusion ORFs. The molecular characterization of GM carnation FLO-40644-6 does not meet the current criteria of COGEM;
- Despite the weaknesses in the molecular characterization, COGEM is of the opinion that import, distribution and retail of FLO-40644-6 pose a negligible risk to the environment because there are no reports of established cut flower carnation varieties and because cut carnation flowers cannot fertilise wild relatives;
- The authorisation of FLO-40644-6 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-40644-6 would be consumed. The introduced genes do not encode toxic or allergenic products. COGEM is not aware of any reports indicating that incidental consumption of FLO-40644-6 poses a risk to human health. COGEM is of the opinion that FLO-40644-6 poses a negligible risk to human health in case of unauthorised incidental consumption;
- There is no evidence of establishment of FLO-40644-6 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 FLO-40644-6;
- Import, distribution and retail of GM carnation of FLO-40644-6 pose a negligible risk to the European environment and human health.

1. Introduction

The present application (C/NL/04/02_001) filed by Suntory Flowers Limited, concerns the renewal of the consent for import, distribution and retail of the genetically modified (GM) carnation variety FLO-40644-6 (also known as Florigene® Moonlite™, line 123.2.38 or FLO-40644-4). This GM

variety expresses the *dfr, f3'5'h* and *suRB* genes resulting in a modified flower colour and tolerance to sulfonylurea herbicides (see Table in 2.2).

In 2005, COGEM issued a positive advice on the import, distribution and retail of this GM carnation variety.[1] In 2006, EFSA issued a positive opinion for the placing on the market of the GM carnation Moonlite.[2] A consent to place Moonlite on the market in Europe was granted in 2007. In Europe, authorisations are granted for a period of ten years. After this period an authorisation has to be renewed. The applicant has filed an application to renew the consent for FLO-40644-6. This application contains updated bioinformatic analyses and reports on the post-market environmental monitoring carried out between 2007 and 2015. The Ministry of Infrastructure and the Environment requested COGEM to assess the provided information and to advise on the renewal of this consent.

2. Environmental risk assessment

2.1 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).[3] Occasionally, *D. caryophyllus* has been found naturalised on old castle walls in the United Kingdom.[4]

Wild *Dianthus* species occur worldwide.[5] In Europe, *Dianthus* species are found in mountainous areas in the alpine region, the Balkan and the Mediterranean area.[6,7,8] In the Netherlands, some rare *Dianthus* species occur: *Dianthus deltoides* (Steenanjer; maiden pink), *Dianthus armeria* (Ruige anjer; Deptford pink), *Dianthus superbus*, (Prachtanjer; large pink) and *Dianthus carthusianorum* (Kartuizer anjer; Carthusian pink).[9] The species *Dianthus barbatus* (Duizendschoon; sweet William) is commonly grown as a garden plant and has established itself in the wild.[9]

It is theoretically possible for carnation to cross-hybridise with other *Dianthus* species and interspecific crossings have been made manually by breeders to introduce new traits into carnation varieties.[5,6,10] However, spontaneous hybridisation between cultivated carnation and wild *Dianthus* species has never been reported, despite decades of cultivation in gardens and parks.

The nomenclature of *Dianthus* is somewhat confusing. Nowadays the common name of *D. caryophyllus* is carnation. However, some carnations are known as 'pinks' and the term carnation is sometimes used to indicate other *Dianthus* species. This application concerns a cultivated double-flowered carnation variety. 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. Carnations are sold as cut flowers, cuttings or plants. 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.[11]

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.[11]

To improve flower size and generate colour variants, carnation is bred for many generations. 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.[3]

In nature, pollination of *D. caryophyllus* occurs exclusively by lepidopteran insects. The nectaries are at the base of the flowers and only insects with a proboscis (a tubular mouthpart) longer than 2.5 cm can reach them. *Dianthus* species are protandrous, which means that the anthers and pollen mature before the pistils. One week or more after the anthers of a flower have shed their pollen grains, the stigma becomes receptive.[6] *Dianthus* carnation pollen cannot be spread by wind. Any pollen produced is heavy and sticky and deeply buried in the flower.[12,13] Although the Netherlands has a large carnation handling industry, carnation pollen is not detected in the atmosphere.[14]

The domesticated carnation produces little pollen with reduced viability.[6,15] 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.[6] 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.[6]

Conclusion: Carnation does not have weedy characteristics and there are no reports of established cut flower carnation varieties. Carnation cannot fertilise wild relatives under natural conditions.

2.2 Description of the introduced genes and traits

Carnation FLO-40644-6 was produced by *Agrobacterium tumefaciens* mediated transformation using transformation vector pCGP1470.

The application for renewal of the consent contains updated bioinformatic analyses which were carried out using recent databases. In 2014, COGEM reconsidered its criteria for the molecular characterisation of GM crops (market authorisations). COGEM notes that the molecular characterisation of FLO-40644-6 does not meet COGEMs current criteria.[16] One of these criteria is that putative open reading frames (ORFs) spanning the insertion and the flanking plant genomic DNA should be bioinformatically analysed from stop codon to stop codon. If a GM crop contains two insertions, like FLO-40644-6, in theory 24 putative fusion ORFs could be generated. The applicant analysed only two theoretical ORFs, well below the number of expected putative fusion

ORFs. Apparently, not all putative fusion ORFs were analysed. Consequently, COGEM concludes that the molecular characterisation does not meet COGEMs current criteria.

Introduced genes	Encoded protein	Trait
suRB	Acetolactate synthase (ALS) enzyme derived from *Nicotiana tabacum.*[17]	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 *Petunia x hybrida*[17]	Modified flower colour
dfr	Dihydroflavonol-4-reductase hydroxylase derived from *Petunia x hybrida*[17]	Modified flower colour
For a detailed description of the introduced genes and traits, see reference 17		

Conclusion: The updated bioinformatic analyses did not include all putative fusion ORFs. The molecular characterization of GM carnation FLO-40644-6 does not meet the current criteria of COGEM.

2.3 Incidental consumption

Petals of carnation are sometimes used in dishes and as garnishing.[18,19,20] The genes introduced in FLO-40644-6 do not encode toxic or allergenic products. COGEM noted that the updated bioinformatic analyses carried out to identify similarities with known toxins and allergens did not include all putative fusion ORFs. Theoretically, these putative ORFs could be expressed and encode harmful proteins. The authorisation of FLO-40644-6 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. A carnation flower contains approximately one gram of petals. Considering the amount of petals per flower, it is likely that a person would consume only part of the petals of a flower. Consumption of such a small amount of FLO-40644-6 is unlikely to lead to an adverse effect.

FLO-40644-6 has been authorised for import, distribution and retail in Europe since 2007. COGEM is not aware of any reports indicating that incidental consumption of FLO-40644-6 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-40644-6 poses a negligible risk to human health.

Conclusion: FLO-40644-6 poses a negligible risk to human health in case of unauthorised incidental consumption.

3. General Surveillance

The applicant supplied a report on the post-market environmental monitoring that was carried out between 2007 and 2015. Several monitoring activities were conducted by the applicant. The applicant examined databases and websites, and contacted breeders, scientists, several institutions (herbaria, plant protection services, conservation groups etc.) to obtain information on the occurrence of *Dianthus* populations and unusual hybrids in the wild, and to ensure that the unexpected establishment of transgenic carnation in the wild, or the 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 (21 times) and Ecuador (6 times) and investigated whether transgenic carnation established at these areas. No evidence of the establishment of FLO-40644-6 or of introgression of the transgenes into wild *Dianthus* species, was obtained.

The applicant performed annual literature searches from 2010 to 2015 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 performed literature reviews for other GM carnation applications and updated these literature reviews in May 2016. These reviews concerned the probability of gene flow, the biosafety of acetolactate synthase (ALS) and information relevant for the safety of GM carnations to humans. According to the applicant, the information obtained by the literature reviews did not change the outcome of the risk assessment carried out at the time of the marketing approval of FLO-40644-6.

COGEM noted that the applicant did not provide information on the databases, keywords and search syntaxes used to carry out the literature search. It is normal practice to provide this kind of information to allow others to repeat the literature searches. Future monitoring reports could be further improved by the addition of detailed information on the literature search that was carried out.

Conclusion: There is no evidence of the establishment of FLO-40644-6 in the wild, or of introgression of the transgenes into wild *Dianthus* species.

4. Overall conclusion

Import, distribution and retail of GM carnation FLO-40644-6 pose a negligible risk to human health and the European environment.

References

1. COGEM (2005). Import of cut flowers of the genetically modified carnation variety 'Florigene Moonlite' COGEM advice CGM/050207-01
2. EFSA (2006). Opinion of the Scientific Panel on genetically modified organisms [GMO] related to the notification (Reference C/NL/04/02) for the placing on the market of the genetically modified carnation Moonlite 123.2.38 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Florigene. EFSA Journal 362 (1-19)
3. CABI (2007). Crop Protection Compendium, 2007 Edition. Wallingford, United Kingdom
4. Clove Pink or Carnation. Internet: www.gardenherbs.org/simples/clove_pink_carnation (visited July 11th, 2013)
5. Andersson-Kottö I & Gairdner AE (1931). Interspecific crosses in the genus *Dianthus*. Genetica 13: 77-112
6. Office of the Gene Technology Regulator (2015). The biology of *Dianthus caryophyllus* L. (Carnation). Australian Government. Department of Health
7. Tela Botanica - *Dianthus caryophyllus* L. Internet: www.tela-botanica.org/page:eflore_bdtfx?referentiel=bdtfx&niveau=2&module=fiche&action=fiche&num_nom=22083&type_nom=nom_scientifique&nom=dianthus%20caryophyllus (visited July 6th, 2016)
8. Flora Italiana - *Dianthus caryophyllus* L. Internet: http://luirig.altervista.org/flora/taxa/index1.php?scientific-name=dianthus+caryophyllus (visited July 6th, 2016)
9. Van der Meijden R. (2005). Heukels' Flora van Nederland. Wolters Noordhoff Groningen, the Netherlands
10. Nimura M *et al.* (2003). Unilateral compatibility and genotypic difference in crossability in interspecific hybridization between *Dianthsus caryophyllus* L. and *Dianthus japonicus* Thunb. Theoretical and Applied Genetics 106: 1164-1170
11. Strid A & Tan K (1997). Flora Hellenica, Volume 1. Koeltz scientific books. Koningstein, Germany
12. Jennersten O (1983). Butterfly visitors as vectors of *Ustilago violacea* spores between Caryophyllaceous plants. Oikos 40:125-130
13. Keane AT (1989). Breeding new carnation cultivars. International Plant Propagation Society Combined Proceedings 39: 88-89
14. Driessen MNBM & Derksen JWM (1988). Pollenatlas van de Nederlandse Atmosfeer, Onkenhout, Hilversum, eerste druk
15. Buell KM (1952). Developmental morphology in *Dianthus* I. Structure of the pistil and seed development. American Journal of Botany 39: 194-210
16. COGEM (2014). Reconsideration of the molecular characterisation criteria for marketing authorisation of GM crops. COGEM Report CGM/140929-02
17. COGEM (2013). Import, distribution and retail of GM carnation FLO-40685-1. COGEM advice CGM/131217-01
18. Eat me – Anjer. Internet: https://www.eatme.nl/producten/anjer (visited July 6th, 2016)
19. Jardins de pareillas – art de vivre entre botanique, gastronomie et créativité. Internet: jardinsdepareillas.over-blog.com/ article-2893237.html (visited July 6th, 2016)

20. Elle à table. Internet: cuisine.elle.fr/elle/elle-a-table/recettes-de-cuisine/tartines-au-beurre-d-oeillet (visited July 6th, 2016)