Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2015: 15

# Final health and environmental risk assessment of genetically modified carnation Moonlite 123.2.38

**Scientific opinion on genetically modified carnation Moonlite 123.2.38 from Florigene with modified petal colour for import as cut flowers for ornamental use under Part C of Directive 2001/18/EC (Application C/NL/04/02)**

**Opinion of the Panel on Genetically Modified Organisms of the Norwegian Scientific Committee for Food Safety**

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26.10.2015

ISBN: 978-82-8259-170-6
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Suggested citation: VKM (2015) Final health and environmental risk assessment of genetically modified carnation Moonlite 123.2.38. Scientific opinion on genetically modified carnation Moonlite 123.2.38 from Florigene with modified petal colour for import as cut flowers for ornamental use under Part C of Directive 2001/18/EC (Application C/NL/04/02). Opinion of the Panel on Genetically Modified Organisms of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-170-6, Oslo, Norway.

# Final health and environmental risk assessment of genetically modified carnation Moonlite 123.2.38. Scientific opinion on genetically modified carnation Moonlite 123.2.38 from Florigene with modified petal colour for import as cut flowers for ornamental use under Part C of Directive 2001/18/EC (Application C/NL/04/02)

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## Assessed and approved

The opinion has been assessed and approved by Panel on Genetically Modified Organisms. Members of the Panel are: Åshild Andreassen (chair), Per Brandtzæg, Knut Helkås Dahl, Knut Tomas Dalen, Hilde-Gunn Hoen-Sorteberg, Olavi Junttila, Richard Meadow, Kåre M. Nielsen, Monica Sanden, and Rose Vikse.

## Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed the Panel on Genetically Modified Organisms (GMO) to answer the request from the Norwegian Food Safety Authority and the Norwegian Environment Agency. Project leaders from the VKM secretariat have been Anne Marie Bakke, Nana Asare, Anne-Marthe Jevnaker, Ville Erling Sipinen and Merethe Aasmo Finne.

## Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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# Abstract

Genetically modified carnation (*Dianthus caryophyllus* L.) line 123.2.38 with product name Moonlite™, expresses three introduced traits. The *dfr* and *f3'5'h* (*Hf1*) genes from *Petunia x hybrida* coding for dihydroflavonol 4-reductase (DFR) and flavonoid 3',5'-hydroxylase (F3'5'H), respectively, lead to the biosynthesis of anthocyanin pigments, which confer the desired violet colour to the flowers. A mutated *als* gene from *Nicotiana tabacum* has also been inserted, coding for an acetolactate synthase (ALS) variant protein and thereby conferring tolerance to the active, ALS-inhibiting, herbicidal substances chlorimuron, thifensulfuron and sulfonylureas, used to facilitate the selection of GM shoots during genetic transformation. Bioinformatics analyses of the inserted DNA and flanking sequences in carnation 123.2.38 have not indicated a potential production of putative harmful proteins or polypeptides caused by the genetic modification. Genomic stability of the functional insert and consistent expression of the *dfr* and *f3'5'h* (*Hf1*) genes, have been shown over several generations of carnation 123.2.38. Data reported from several field trials show that carnation 123.2.38 petals contain higher levels of the anthocyanins delphinidin and cyanidin compared to the non-GM (conventional) carnation counterpart 123. Other morphological traits were reported and along with differing petal colour, carnation Moonlite 123.2.38 differed significantly in one trait compared to conventional carnation counterpart 123. An acute toxicity study in mice and two *in vitro* studies, both employing aqueous extracts from leaves or petals, showed no adverse effects. DFR, F3'5'H and ALS proteins do not show sequence resemblance to known toxins or IgE-dependent allergens, nor have they been reported to cause IgE-mediated allergic reactions. The anthocyanins delphinidin and cyanidin are present in numerous foods and are also approved food additives. Carnations are cultivated in Norway, but since 1) the intended uses includes import of cut flowers for ornamental use only, 2) the spread and viability of pollen from the cut flowers is low, 3) seed formation in cut flowers is unlikely to occur, and 4) spread of inserted genes to target or non-target organisms is either unlikely to occur or is not of biological relevance, the VKM GMO Panel does not consider that carnation 123.2.38 represents an environmental risk in Norway.

Considering that carnation Moonlite 123.2.38 is not intended for cultivation or use as food or feed, the VKM GMO Panel considers that comparative analysis of the newly synthesised anthocyanin pigments delphinidin, cyanidin and petunidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonlite 123.2.38 and its conventional carnation counterpart 123 do not raise safety concerns. It is unlikely that the DFR, F3'5'H or ALS proteins, or the delphinidin or cyanidin pigments, will introduce a toxic or allergenic potential in Moonlite 123.2.38.

Based on current knowledge and information supplied by the applicant, and considering the intended uses, which exclude cultivation and use as food and feed, the VKM GMO Panel concludes that Moonlite 123.2.38 is as safe as its conventional counterpart 123.

Based on the current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that it is unlikely that carnation Moonlite 123.2.38 will have any adverse effects on the biotic or abiotic environment in Norway.

# Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Environment Agency (formerly Norwegian Directorate for Nature Management) and the Norwegian Food Safety Authority (NFSA) to conduct final health and environmental risk assessments of all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Agency and NFSA requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The genetically modified carnation (*Dianthus caryophyllus* L.) Moonlite 123.2.38 (Unique Identifier FLO-40644-4) with modified flower colour is approved under Directive 2001/18/EC for import of cut flowers for ornamental use since 23 May 2007 (Application C/NL/04/02, Commission Decision 2007/364/EC). The scope of the application is restricted to flowers produced by vegetative propagation, and do not cover progeny derived from sexual crosses with Moonlite 123.2.38 cultivar. A condition for placing on the market is a label or document accompanying the product that states that it is genetically modified and the words “not for human or animal consumption nor for cultivation”.

Moonlite 123.2.38 has previously been assessed for import as cut ornamental flowers by the VKM GMO Panel. The risk assessment was commissioned by the Norwegian Environment Agency and NFSA in connection with the national finalisation of the procedure of the application C/NL/04/02 in 2008.

The current safety and environmental risk assessment of the carnation Moonlite 123.2.38 is based on information provided by the applicant in the application C/NL/04/02, relevant peer-reviewed scientific literature, and scientific opinions from EFSA (EFSA, 2006b) and VKM (VKM, 2008). Except for a synopsis of more recent literature, this draft opinion is to a large extent a summary of the above-mentioned VKM and EFSA reports, which are provided in Appendix I and II, respectively, and readers are referred to these for details.

The VKM GMO Panel has evaluated carnation Moonlite 123.2.38 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. VKM has also decided to take account of the appropriate principles described in the EFSA guidelines on the risk assessment of GM plants used for non-food/feed purposes (EFSA, 2009a), the risk assessment of GM plants and derived food and feed (EFSA, 2006a; EFSA, 2011b), the environmental risk assessment of GM plants (EFSA, 2010a), selection of comparators for the

risk assessment of GM plants (EFSA, 2011a), and for the post-market environmental monitoring of GM plants (EFSA, 2011c).

The scientific risk assessment of carnation Moonlite 123.2.38 includes molecular characterisation of the inserted DNA and expression of novel proteins and other relevant components, comparative assessment of phenotypic characteristics, toxicity and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant and target and non-target organisms, and effects on biogeochemical processes.

It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility or ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms. Likewise, the VKM mandate does not include evaluations of herbicide residues in food and feed from genetically modified plants.

Carnation Moonlite 123.2.38 expresses three introduced traits: *dfr* and *f3'5'h* (*Hf1*) genes from *Petunia x hybrida* coding for dihydroflavonol 4-reductase (DFR) and flavonoid 3',5'-hydroxylase (F3'5'H), respectively, which confer the violet colour to the flowers. A mutated *als* gene (*SuRB*) from *Nicotiana tabacum* is also inserted, which codes for an acetolactate synthase (ALS) variant protein, conferring herbicide tolerance, and used to facilitate the selection of GM shoots during genetic transformation.

**Molecular characterisation**

The molecular characterisation provided by the applicant shows that Carnation Moonlite 123.2.38 contains two transgenic loci. Locus 1 contains one functional copy of each of the *dfr*, *f3'5'h* and *als* genes, as well as some plasmid backbone sequences. Locus 2 contains a truncated *dfr* gene, the *Mas* terminator, and a partial right border (RB) region. Nothern blot analyses were used to confirm expression of the three inserted genes *dfr, f3'5'h*, and *als*, and Liquid chromatography (HPLC) was used to quantify new metabolites. Levels of the anthocyanins (pigments) delphinidin and cyanidin measured in bulked petal samples were reported as 0.093 and 0.031 mg/g fresh weight, respectively. Two new open reading frames (ORFs) were created in Locus 1 during transformation of the Carnation. General BLAST searches performed by the applicant did not return relevant sequence homologies between the ORF sequences, the transgene insert, and known toxins and allergens. No instability in the introduced trait, *i.e.* the particular flower colour, has been reported during the commercial cultivation of carnation Moonlite 123.2.38.

Based on current knowledge and the information provided by the applicant, the VKM GMO panel concludes that the molecular characterisation of carnation Moonlite 123.2.38 does not indicate a safety concern.

### Comparative assessment

Considering the intended use of carnation Moonlite 123.2.38, which exclude cultivation and use as food and feed, compositional studies were limited to the content of the three anthocyanin pigments delphinidin, cyanidin and petunidin. Compared to its non-GM parental cultivar carnation 123, carnation Moonlite 123.2.38 petals contained higher levels of delphinidin and cyanidin, and neither cultivar contained petunidin, confirming the intended effects of the genetic modification. Other morphological traits were assessed following field trials and revealed that along with differing petal colour, carnation Moonlite 123.2.38 differed significantly in one trait compared to carnation 123. None of the reported differences in compositional or morphological traits were expected to influence the risk scenario upon accidental release to the environment or intake of the GM carnation.

Based on current knowledge and information provided by the applicant, and considering the intended uses of carnation Moonlite 123.2.38, which exclude cultivation and use as food or feed, the VKM GMO Panel concludes that the comparative analysis of the newly synthesised anthocyanin pigments delphinidin, cyanidin and petunidin in its petals is sufficient for the risk assessment. The reported morphological differences between carnation Moonlite 123.2.38 and its conventional carnation counterpart 123 do not raise safety concerns.

### Food and feed risk assessment

The applicant has performed a 14 day acute toxicity study with ICR mice and two *in vitro* tests on cytotoxicity and mutagenicity (Ames test), respectively, with extracts from leaves or petals from carnation Moonlite 123.2.38. None of the experiments revealed adverse effects of the extracts. The DFR, F3'5'H and ALS proteins do not show relevant sequence resemblance to known toxins or IgE-dependent allergens, nor have they been reported to cause IgE-mediated allergic reactions. The anthocyanins delphinidin and cyanidin expressed as a result of the genetic modification are normally present in numerous plant foods and are authorised as food additives.

Based on current knowledge, information provided by the applicant, and considering the intended use which excludes cultivation and use as food and feed, the VKM GMO Panel concludes that Moonlite 123.2.38 is as safe as its conventional counterpart, carnation 123. It is unlikely that the DFR, F3'5'H or ALS proteins, or the delphinidin or cyanidin pigments, will introduce a toxic or allergenic potential in Moonlite 123.2.38.

### Environmental assessment

Considering the intended use of Moonlite 123.2.38, which excludes cultivation and use as food or feed, the environmental risk assessment is concerned with accidental release into the environment of viable seeds/pollen and rooted plants during transportation and distribution.

With the exception of herbicide-tolerance, Moonlite 123.2.38 has no altered survival, multiplication or dissemination characteristics compared to conventional carnation cultivars, and there are no indications of an increased likelihood of spread and establishment of feral

carnation plants in the case of accidental release into the environment. Carnations are cultivated in Norway but plant to plant gene flow is not considered to be an issue due to low pollen spread and viability and low likelihood of seed development from cut flowers.

Based on current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that carnation Moonlite 123.2.38 does not represent an environmental risk in Norway.

### Post-market environmental monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

Based on current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that the environmental risk assessment did not identify any potential adverse environmental effects of the transgenic line of carnation Moonlite 123.2.38. Thus, the general post-market surveillance plan is sufficient and there is no need for a specific post-market surveillance plan.

### Overall conclusion

Considering that carnation Moonlite 123.2.38 is not intended for cultivation or use as food or feed, the VKM GMO Panel considers that the comparative analysis of the newly synthesised anthocyanin pigments delphinidin, cyanidin and petunidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonlite 123.2.38 and its conventional carnation counterpart 123 do not raise safety concerns.

Based on current knowledge, information supplied by the applicant, and considering the intended use which excludes cultivation and use as food and feed, the VKM GMO Panel concludes that Moonlite 123.2.38 is as safe as its conventional counterpart. It is unlikely that the DFR, F3'5'H or ALS proteins, or the delphinidin or cyanidin pigments, will introduce a toxic or allergenic potential in carnation Moonlite 123.2.38.

Likewise, the VKM GMO Panel concludes that carnation Moonlite 123.2.38, based on current knowledge and the intended use as cut ornamental flowers, does not represent an environmental risk in Norway.

**Key words**: GMO, carnation (*Dianthus caryophyllus* L.), Moonlite, 123.2.38, anthocyanin, petal colour, *dfr*, *f3'5'h*, *als*, *SuRB*, health safety, environmental risk evaluation, Regulation (EC) No 1829/2003, VKM, risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Food Safety Authority/Norwegian Environment Agency

# Sammendrag på norsk

Som en del av forberedelsene til implementering av EU-forordning 1829/2003 i norsk rett, er Vitenskapskomiteen for mattrygghet (VKM) bedt av Miljødirektoratet (tidligere Direktoratet for naturforvalting [DN]) og Mattilsynet om å utarbeide endelige helse- og miljørisikovurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent under forordning 1829/2003 eller direktiv 2001/18, og som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. Miljødirektoratet og Mattilsynet har bedt VKM om endelige risikovurderinger for de EU-godkjente søknader hvor VKM ikke har avgitt endelige risikovurderinger. I tillegg er VKM bedt om å vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige helse- og miljørisikovurderingene som VKM tidligere har levert.

Den genmodifiserte, nellik (*Dianthus caryophyllus* L.) Moonlite 123.2.38 (unik kode FLO-40644-4) fra Florigene Ltd. ble godkjent til import og salg som avskårne prydblomster under EUs utsettingsdirektiv 2001/18/EC den 23. mai 2007 (jfr. Kommisjonsbeslutning 2007/364/EC). Søknad C/NL/04/02 omfatter nellikplanter som er produsert ved vegetativ formering, og omfatter ikke avledete sorter fra konvensjonelle kryssinger med Moonlite 123.2.38. En betingelse for salg er en etikett eller et dokument som følger produktet der det skal spesifiseres at det er genmodifisert og ordene «not for human or animal consumption nor for cultivation» (ikke for konsum eller for dyrking).

Moonlite 123.2.38 ble første gang vurdert av VKMs faggruppe for GMO i 2008 (VKM, 2008). Helse- og miljørisikovurderingen ble utarbeidet på oppdrag av Miljødirektoratet og Mattilsynet i forbindelse med vurdering av markedsadgang i Norge.

Risikovurderingen av den genmodifiserte nelliklinjen er basert på søkers dokumentasjon og uavhengige vitenskapelige publikasjoner, samt vitenskapelige vurderinger fra EFSA (EFSA, 2006b) og VKM (VKM, 2008). Bortsett fra gjennomgang av nylig offentliggjort publikasjoner er resten av teksten i denne vurderingen en oppsummering av de tidligere VKM (VKM, 2008) og EFSA (EFSA, 2006b) vurderingene, som er vedlagt i hhv. Appendix I og II. For utfyllende detaljer henvises leserne til disse.

Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med Matloven, miljøkravene i Genteknologiloven med forskrifter, først og fremst forskrift om konsekvensutredning etter Genteknologiloven. Videre er kravene i EU-direktiv 2001/18/EF (vedlegg 2, 3 og 3B) og veiledende notat til Annex II (2002/623/EF), samt prinsippene i EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler (EFSA, 2006a; EFSA, 2009a; EFSA, 2010a; EFSA, 2011a; EFSA, 2011b; EFSA, 2011c) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsmetoden og vektorkonstruksjonen, karakterisering og nedarving av genkonstruksjonen, komparativ analyse av antocyanin

innhold i kronbladene og andre morfologiske egenskaper, kritiske toksiner, allergener og nye proteiner. Videre er potensiale for utilsiktede effekter på fitness, genoverføring til målorganismer og ikke-målorganismer, og biogeokjemiske prosesser vurdert.

Det presiseres at VKMs mandat ikke omfatter vurderinger av etikk, bærekraft og samfunnsnytte, i henhold til kravene i den norske genteknologiloven og dens konsekvensutredningsforskrift. Disse aspektene blir derfor ikke vurdert av VKMs faggruppe for genmodifiserte organismer. Vurderinger av mulige plantevernmiddelrester i den genmodifiserte planten som følge av endret sprøytemiddelbruk faller per i dag utenfor VKMs ansvarsområde og er derfor heller ikke vurdert.

Nellik Moonlite 123.2.38 uttrykker tre nye egenskaper: *dfr* -genet som koder for dihydroksyflavonol-reduktase (DFR) og *f3′5′h* -genet (*Hf1*) som koder for flavonol 3′,5′-hydroksylase (F3′5′H); begge stammer fra *Petunia x hybrida*. Disse genene fører til endringen i produksjonen av antocyanin pigmenter i kronbladene, med fargeendring i blomsten som resultat. I tillegg, inneholder Moonlite 123.2.38 et mutert *als* (*SuRB*) gen fra *Nicotiana tabacum* som koder for en variant av acetolactatsyntase (ALS)-enzymet. De transgene plantene vil derfor tolerere høyere doser av ALS-inhiberende herbicider som klorimuron, tifensulfuron og sulfonylureaer og brukes for identifikasjon av transformerte GM planter.

### Molekylær karakterisering

Den molekylære karakteriseringen fra søker viser at Nellik Moonlite 123.2.38 inneholder to transgene locus. Locus 1 inneholder én fungerende kopi for hver av de tre nye genene *dfr*, *f3'5'h* og *als*, og i tillegg noen delsekvenser fra selve plasmidet. Locus 2 inneholder et ufullstendig *dfr*-gen, *Mas*-termineringssekvensen, og deler av den høyre grense – T-DNA-sekvensen. Northern blot ble brukt til å påvise genuttrykk av *dfr, f3'5'h*, og *als,* mens væskekromatografi (HPLC) ble brukt til kvantifisering av nye metabolitter. I partier av kronblader ble nivået av pigmentene delphinidin og cyanidin målt til henholdsvis 0,093 og 0,031 mg/g ferskvekt. Transformasjonsprosessen førte også til dannelse av to nye åpne leserammer (ORFs) i nelliken, i locus 1. Databasesøk utført av søker fant ingen relevante treff i sekvenslikhet mellom ORF-sekvensene, de innsatte genene, og kjente toksiner og allergener. Ved kommersiell dyrking har det så langt ikke blitt rapportert om ustabilitet/avvik ved de introduserte egenskapene, dvs. blomsterfargen, til nellik Moonlite123.2.38.

Basert på dagens kunnskap og informasjonen fra søker, konkluderer VKMs faggruppe for GMO at den molekylære karakteriseringen ikke tilsier noen økt risiko ved nellik Moonlite 123.2.38 sammenliknet med konvensjonelle nelliksorter.

### Komparative analyser

Med hensyn til tiltenkt bruksområde som ekskluderer dyrking og bruk i mat og fôr, og fordi innhold av næringsstoffer, antinæringsstoffer og andre biologisk aktive komponenter i konvensjonelle nelliker er lite kjent, ble kun innhold av de tre antocyanin pigmentene

delfinidin, cyanidin and petunidin i kronblader fra nellik Moonlite 123.2.38 rapportert av søker. Sammenlignet med den konvensjonelle motpart nellik 123 inneholder kronbladene fra nellik Moonlite 123.2.38 høyere nivåer av delfinidin og cyanidin, mens petunidin ikke kunne detekteres i noen av nelliktypene. Dette bekreftet de tilsiktede effektene av genmodifiseringen. Andre morfologiske egenskaper ble også rapportert fra feltforsøk og avslørte at i tillegg til endret kronbladfarge var det variasjon mellom nelliktypene i en egenskap. Ingen av de rapporterte forskjellene i sammensetning eller morfologiske egenskaper er forventet å ha innvirkning på risikoscenarier ved utilsiktet miljøeksponering eller inntak av nellik Moonlite 123.2.38.

Ut i fra dagens kunnskap og informasjon tilsendt av søker, og tatt i betraktning tiltenkt bruksområde som ekskluderer dyrking og bruk i mat og fôr, konkluderer VKMs faggruppe for GMO at de komparative analysene som er begrenset til de nysyntetiserte anthocyanin pigmentene delfinidin, cyanidin og petunidin i kronbladene er tilstrekkelig for risikovurderingen av nellik Moonlite 123.2.38. De rapporterte morfologiske forskjellene mellom Moonlite 123.2.38 og dens konvensjonelle motpart nellik 123 medfører ikke en økt sikkerhetsrisiko.

### Helserisiko

En 14 dagers akutt toksisitetsstudie med ICR mus, samt to *in vitro* forsøk for henholdsvis test av cytotoksisitet og mutagenisitet (Ames test), har blitt utført av søker med ekstrakter fra frosne kronblad og blomsterblad fra Moonlite 123.2.38. Ingen av forsøkene viste negative effekter av ekstraktene. Proteinene DFR, F3′5′H og ALS har ingen relevante sekvenslikheter med kjente toksiner eller IgE-avhengige allergener, og er heller ikke rapportert å ha forårsaket IgE-medierte allergiske reaksjoner. Antocyaninene delfinidin og cyanidin, uttrykt som et resultat av genmodifiseringen, er normalt til stede i mange frukt og grønnsaker og er godkjente tilsetningsstoffer i mat.

Ut i fra dagens kunnskap, informasjon tilsendt av søker, og tatt i betraktning tiltenkt bruksområde som ekskluderer dyrking og bruk i mat og fôr, konkluderer VKMs faggruppe for GMO at Moonlite 123.2.38 er like trygg som dens konvensjonelle motpart, nellik 123. Det er usannsynlig at DFR, F3'5'H eller ALS proteinene, eller delfinidin eller cyanidin pigmentene, vil føre til et toksisk eller allergent potensiale i Moonlite 123.2.38.

### Miljørisiko

Miljørisikovurderingen av nelliklinjen Moonlite 123.2.38 er avgrenset til mulige effekter av utilsiktet spredning av pollen og spiredyktige frø i forbindelse med transport og bruk som avskårne prydblomster. Faggruppen har ikke vurdert mulige miljøeffekter knyttet til dyrking av nelliklinjen.

Med unntak av herbicidtoleranse har genmodifiseringen av nelliklinjen Moonlite 123.2.38 ikke medført endringer i egenskaper knyttet til overlevelse, oppformering eller spredning sammenlignet med konvensjonell nellik, og det er ingen indikasjoner på økt sannsynlighet for

spredning og etablering av viltvoksende nellikplanter fra utilsiktet frøspill av nelliklinjen. Hagenellik dyrkes i Norge, men det er lite risiko for spredning av gener grunnet manglende mulighet og tid for pollen- og frøutvikling i de avskårne blomstene. Det er derfor ikke risiko for utkrysning med dyrkede sorter, ville planter eller andre organismer i Norge.

Ut i fra dagens kunnskap og med bakgrunn i tiltenkt import, distribusjon og bruksområde som avskårne prydblomster, konkluderer VKMs faggruppe for GMO at nelliken Moonlite 123.2.38 ikke vil medføre en miljørisiko i Norge.

**Samlet vurdering**

Tatt i betraktning tiltenkt bruksområde som ekskluderer dyrking og bruk i mat og fôr, konkluderer VKMs faggruppe for GMO at den komparative analysen begrenset til de nysyntetiserte antocyaninpigmentene delfinidin, cyanidin og petunidin i kronbladene til nellik Moonlite 123.2.38 er tilstrekkelig for risikovurderingen. De rapporterte morfologiske forskjellene mellom Moonlite 123.2.38 og dens konvensjonelle motpart nellik 123 medfører ikke en økt sikkerhetsrisiko.

Ut i fra dagens kunnskap, informasjon tilsendt av søker, og tatt i betraktning tiltenkt bruksområde, som ekskluderer dyrking og bruk som mat og fôr, konkluderer VKM's GMO Panel at Moonlite 123.2.38 er like trygg som dens konvensjonelle motpart. Det er usannsynlig at DFR, F3'5'H eller ALS proteinene, eller delfinidin eller cyanidin pigmentene vil føre til et toksisk eller allergent potensiale i Moonlite 123.2.38.

Likeledes finner faggruppen, ut i fra dagens kunnskap, at den omsøkte bruken av Moonlite 123.2.38 som avskårne prydblomster ikke vil medføre en miljørisiko i Norge.

# Abbreviations and glossary

| | |
|---|---|
| **ALS** | Acetolactate synthase |
| **DFR** | Dihydroflavonol 4-reductase |
| **DNA** | Deoxyribonucleic acid |
| **EC** | European Commission |
| **EFSA** | European Food Safety Authority |
| **ERA** | Environmental risk assessment |
| **EU** | European Union |
| **F3'5'H** | Flavonoid 3',5'-hydroxylase |
| **Fitness** | Describes an individual's ability to reproduce successfully relative to that of other members of its population. |
| **GM** | Genetically modified |
| **GMO** | Genetically modified organisms |
| **GMP** | Genetically modified plants |
| **mRNA** | Messenger RNA |
| **MS** | Member states |
| **MT/NFSA** | Norwegian Food Safety Authority (Mattilsynet) |
| **OECD** | Organisation for Economic Co-operation and Development |
| **PCR** | Polymerase chain reaction, a technique to amplify DNA by copying |
| **PMEM** | Post-market environmental monitoring |
| **VKM** | Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet) |

# Background

In September 2004, an application (Reference C/NL/04/02) covering import of cut flowers of the genetically modified carnation Moonlite 123.2.38 (Unique Identifier FLO-40644-4) for ornamental use was submitted by Florigene Ltd. to the competent authority of the Netherlands. The scope of the application was restricted to flowers produced by vegetative propagation, and did not cover progeny derived from sexual crosses with Moonlite 123.2.38 cultivar.

On 9 December 2005, the European Commission received the full application and an assessment report from the Netherlands. In accordance with Directive 2001/18/EC (EC, 2001), the application was transmitted to the competent authorities of the other Member States for a 60-day public hearing.

The EFSA GMO Panel published its scientific opinion on application C/BE/96/01 27 June 2006 (EFSA 2006), and carnation Moonlite 123.2.38 was approved for import and ornamental use 23 May 2007 (Commission Decision 2007/364/EC). A condition for placing on the market is a label or document accompanying the product that states that it is genetically modified and the words “not for human or animal consumption nor for cultivation”.

Carnation Moonlite 123.2.38 has previously been assessed by the VKM GMO Panel commissioned by the Norwegian Environment Agency and the Norwegian Food Safety Authority in connection with the national finalisation of the procedure of the application in 2008. Due to the publication of new scientific literature and updated guidelines for risk assessment of genetically modified plants, the VKM GMO Panel has decided to deliver an updated risk assessment of Moonlite 123.2.38.

# Terms of reference

The Norwegian Environment Agency (formerly the Norwegian Directorate for Nature Management) has the overall responsibility for processing applications for the deliberate release of genetically modified organisms (GMOs). This entails inter alia coordinating the approval process, and to make a holistic assessment and recommendation to the Ministry of the Environment regarding the final authorisation process in Norway. The Agency is responsible for assessing environmental risks upon the deliberate release of GMOs, and to assess the product's impact on sustainability, benefit to society and ethics under the Gene Technology Act.

The Norwegian Food Safety Authority (NFSA) is responsible for assessing risks to human and animal health upon the deliberate release of GMOs pursuant to the Gene Technology Act and the Food Safety Act. In addition, NFSA administers the legislation for processed products derived from GMO and the impact assessment on Norwegian agriculture according to sector legislation.

**The Norwegian Environment Agency**

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency, by letter dated 13 June 2012 (ref. 2008/4367/ART-BI-BRH), requests VKM, to conduct final environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act.

The Norwegian Environmental Agency has also requested VKM, by letter dated 19 May 2015 (ref. 2015/4151), to conduct a final environmental risk assessment of genetically modified carnation Moonlite 123.2.38 for import of cut flowers for ornamental use (Application C/NL/04/02).

The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Norwegian Environment Agency requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The basis for evaluating the applicants' environmental risk assessments is embodied in the Act Relating to the Production and Use of Genetically Modified Organisms etc. (the Norwegian Gene Technology Act), Regulations relating to impact assessment pursuant to the Gene Technology Act, the Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, Guidance note in Annex II of the Directive 2001/18 (2002/623/EC) and the Regulation 1829/2003/EC. In addition, the EFSA guidance documents on risk assessment of genetically modified plants and food and feed from the GM plants (EFSA, 2010a; EFSA, 2011b), the risk assessment of GM plants used for non-

food/feed purposes (EFSA, 2009a) and OECD guidelines will be useful tools in the preparation of the Norwegian risk assessments.

The risk assessments' primary geographical focus should be Norway, and the risk assessments should include the potential environmental risks of the product(s) related to any changes in agricultural practices. The assignment covers assessment of direct environmental impact of the intended use of pesticides with the GMO under Norwegian conditions, as well as changes to agronomy and possible long-term changes in the use of pesticides.

**The Norwegian Food Safety Authority**

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency has requested NFSA to give final opinions on all GMOs and products containing or consisting of GMOs that are authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC within the Authority's sectoral responsibility. The request covers scope(s) relevant to the Gene Technology Act.

NFSA has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested VKM to carry out final scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorised in the European Union.

NFSA has also requested VKM, by letter dated 26 August 2015 (ref. 2015/176539), to conduct a final risk assessment of carnation Moonlite 123.2.38 for import of cut flowers for ornamental use (Application C/NL/04/02).

The assignment from NFSA includes food and feed safety assessments of GMOs and their derivatives, including processed non-germinating products, intended for use as or in food or feed.

In the case of submissions regarding genetically modified plants (GMPs) that are relevant for cultivation in Norway, VKM is also requested to evaluate the potential risks of GMPs to the Norwegian agriculture and/or environment. Depending on the intended use of the GMP(s), the environmental risk assessment should be related to import, transport, refinement, processing and cultivation. If the submission seeks to approve the GMP(s) for cultivation, VKM is requested to evaluate the potential environmental risks of implementing the plant(s) in Norwegian agriculture compared to existing varieties (e.g. consequences of new genetic traits, altered use of pesticides and tillage). The assignment covers both direct and secondary effects of altered cultivating practices.

VKM is further requested to assess risks concerning coexistence of cultivars. The assessment should cover potential gene flow from the GMP(s) to conventional and organic crops as well as to compatible wild relatives in semi-natural or natural habitats. The potential for establishment of volunteer populations within the agricultural production systems should also be considered. VKM is also requested to evaluate relevant segregation measures to secure

coexistence during agricultural operations up to harvesting. Post-harvest operations, transport and storage are not included in the assignment.

Evaluations of suggested measures for post-market environmental monitoring provided by the applicant, case-specific monitoring and general surveillance, are not covered by the assignment from NFSA. In addition, the changes related to herbicide residues of GMPs as a result of the application of plant-protection products fall outside the remit of the Norwegian VKM Panel.

# Assessment

# 1 Introduction

Carnation Moonlite 123.2.38 (Unique Identifier FLO-40644-4) from Florigene Ltd. is a genetically modified cultivar of *Dianthus caryophyllus* L. intended for import, distribution and retail in the European Union as cut flowers for ornamental use only. This draft opinion is to a large extent a summary of the previous scientific opinions from VKM (2008) and EFSA (2006a), and relevant peer-reviewed scientific literature. The above-mentioned VKM and EFSA reports are provided in Appendix I and II respectively, and readers are referred to these for details. The more recent assessments are performed in accordance with principles of guidance documents on risk assessment of GM plants for non-food and non-feed purposes (EFSA, 2009a) and on the environmental risk assessment of GM plants (EFSA, 2010a).

Carnation Moonlite 123.2.38 was developed for petal colour for decorative purposes. The expression of the newly introduced genes from petunia, *dfr* and *f3′5′h* coding for dihydroflavonol 4-reductase (DFR) and flavonoid 3′,5′-hydroxylase (F3′5′H), respectively, confers the violet colour to the flowers. Biosynthesis of the anthocyanin pigments cyanidin and delphinidin in the petals is enabled via interplay between introduced and endogenous genes in the anthocyanin biosynthesis pathway. In addition, carnation Moonlite 123.2.38 expresses herbicide tolerance by the introduction of a mutated *als* gene (*SuRB*) from *Nicotiana tabacum* coding for an acetolactate synthase (ALS) variant protein, used to facilitate the selection of successfully modified shoots during the genetic transformation process.

Anthocyanins are widely distributed in nature. Cyanidin and delphinidin are among the most common of a class of about 100 water soluble pigments with common biosynthetic origins. These glycosides are naturally formed by anthocyanidins and various sugars. They are stably localized in plant organs, such as petals, and are red, purple, blue, and black (Zhao and Tao, 2015). Cyanidin and delphinidin are naturally present in foods like aubergines, blueberries and blackcurrants at relatively high levels. Studies have shown that colour differences are related to the type(s) of anthocyanin present. Pink flowers contain cyanidin aglycone and pelargonidin aglycone as the core anthocyanins, and purple flowers contain mainly delphinidin aglycone and cyanidin aglycone as the core anthocyanins (Zhao and Tao, 2015).

The acetolactate synthase (ALS) enzyme is present in all plant species and catalyses the biosynthesis of branched amino acids (reviewed in (Chandler et al., 2013). ALS -inhibiting herbicides, such as chlorimuron, thifensulfuron and sulfonylureas, cause growth retardation in seedlings by impairing branch chain amino acid synthesis in treated grasses and broadleaf weeds, but not in crops such as rice, wheat, barley, soybean, maize and others due to their high endogenous ALS expression. The herbicides have potency at extremely low concentrations, but rapid resistance development in weeds has limited their application (see

review by (Tranel and Wright, 2002). However, the introduction of the mutated *als* (*SuRB*) gene in carnation Moonlite 123.2.38 with resulting tolerance to sulfonylurea herbicides was not primarily intended for plant protection purposes, but rather as a marker trait for the selection of successfully transformed plants.

Carnation Moonlite 123.2.38 has been currently evaluated by the VKM GMO Panel with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, and Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

VKM has also taken into account the appropriate principles described in the EFSA guideline on the risk assessment of GM plants used for non-food/feed purposes (EFSA, 2009a), the risk assessment of GM plants and derived food and feed (EFSA, 2011b), the environmental risk assessment of GM plants (EFSA, 2010), the selection of comparators for the risk assessment of GM plants (EFSA, 2011a), and for the post-market environmental monitoring of GM plants (EFSA, 2011c).

It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility or ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms.

# 2 Molecular characterisation

The VKM and EFSA GMO Panels (VKM, 2008 in Appendix I; EFSA, 2006b in Appendix II) have previously assessed the molecular characterisation of the event FLO-40644-4 (Moonlite 123.2.38; *dfr*, *f3'5'h* [from the *hf1* locus], and *SuRB* [mutated version of *als*] inserts) with regards to the following:

1. The transformation system and vector constructs
2. Characterisation of the transgene insertions and constructs
3. Information on the expression of the insert including quantification of new metabolites
4. Analyses of new open reading frames (ORFs)
5. Inheritance and stability of the inserted DNA

Both Panels concluded that the applicant had provided sufficient analyses to characterise the DNA insert, number of inserts, integration site and flanking sequences in the carnation Carnation Moonlite 123.2.38 contains two transgenic loci. Locus 1 contains one functional copy of each of the *dfr*, *f3'5'h* and *als* genes, as well as some plasmid backbone sequences. The backbone sequences include the modified pACYC184 sequence necessary for replication (Replication origin, *ori*) of the transformation vector in *E. coli*, and a fragment (ca. <20%) of the *tet(A)* resistance gene, an essential gene for the tetracycline repressor complex. The absence of an intact functional *tet(A)* gene was determined by the applicant by Southern blot and PCR -analyses. The second integration site, locus 2, contained a truncated *dfr* gene and the *Mas* terminator as well as a partial RB region. Expression (mRNA) of the inserted transgenes *dfr*, *f3'5'h* and *als* was demonstrated by Northern blot analysis. Quantification of new metabolites was determined by Liquid chromatography (HPLC). The levels of the anthocyanins delphinidin and cyanidin, in a single assay of bulked petal samples were reported as 0.093 and 0.031 mg/g fresh weight, respectively.

During the transformation, two new ORFs were created in Locus 1 at the junction between the inserted DNA fragment and the plant DNA. According to the applicant no relevant sequence homologies were observed between the ORF sequences or the three inserted transgenes to sequences of known toxins and allergens using General BLAST searches. When searches for sequence homologies of at least six identical contiguous amino acids were performed, various identical sequences were found in the three inserted transgenes and known allergens. None of the transgenic proteins are however considered as allergenic, and there are no further indications of allergenic properties of these proteins. Moreover, the 6-amino-acid threshold is likely to give rise to many false positives, as noted by The EFSA GMO Panel in their assessment of another genetically modified carnation (EFSA 2008, Moonaqua 123.8.12).

Carnations are propagated vegetatively. No instability in the introduced trait, *i.e.* the particular flower colour, has been reported during the commercial cultivation of the carnation Moonlite 123.2.38, which includes approximately seven generations and the production of millions of flowers.

## 2.1 Conclusions

Based on current knowledge and the information provided by the applicant, the VKM GMO panel concludes that the molecular characterisation of carnation Moonlite 123.2.38 does not indicate a safety concern.

# 3 Comparative assessments

Previously, the VKM Panel (VKM, 2008 in Appendix I) and EFSA (EFSA, 2006b in Appendix II) assessed compositional and morphological data provided by the applicant. A brief summary from these reports are provided below.

Generally, carnations have no or very limited history of use in food and feed, and their content of nutrients, antinutritional factors and other components with biological activity is largely unknown. The import of carnation Moonlite 123.2.38 into the EU or Norway is not intended for food or feed use, nor for cultivation, and therefore components other than the anthocyanins delphinidin, cyanidin, petunidin and pelargonidin have not been analysed in carnation Moonlite 123.2.38 (EFSA, 2006b) or other GM carnations (EFSA, 2008; EFSA, 2014a; EFSA, 2014b). The comparative compositional assessment as defined in EFSA guidance documents for GM plants and derived food and feed (EFSA, 2006a) was therefore only partially applied and possible unintended effects of the genetic modification in carnation Moonlite 123.2.38 cannot be assessed.

## 3.1 Production of material for comparative assessment

The field trials conducted by the applicant, from which materials and morphological characteristics were gathered, were not described in detail. The VKM GMO Panel considers this a short-coming in the application and it makes a full assessment of the data difficult. However, since the carnation Moonlite 123.2.38 is not intended for cultivation or for use in food or feed, the documentation provided is most likely sufficient for the scope of the application.

For the compositional studies, the three anthocyanins – delphinidin, cyanidin and petunidin – were analysed by HPLC in freeze-dried petals of carnation Moonlite 123.2.38 and its conventional comparator (control) cultivar 123. Carnation 123 does not produce anthocyanins and therefore has white petals. Other plant tissues were not analysed.

For assessment of morphological traits, carnation Moonlite 123.2.38 and its non-GM conventional comparator 123 were grown in field trials in the Netherlands in 1999 and 2000.

## 3.2 Compositional analysis

HPLC data (Technical dossier; (Fukui et al., 2003) indicated that petals of carnation Moonlite 123.2.38 and parental cultivar 123 did not contain detectable levels of petunidin. Delphinidin and cyanidin were detected in Moonlite 123.2.38 petals at levels of 0.093 and 0.031 mg/g fresh weight, respectively, but were not detected in cultivar 123.

EFSA (EFSA, 2006b) considered that since the intended uses of carnation Moonlite 123.2.38 did not include cultivation or human or animal consumption, compositional analysis limited to

the newly synthesised anthocyanins in petals was sufficient for the risk assessment. Reported differences in anthocyanin content were not expected to influence the risk scenario upon accidental release to the environment or intake of the GM carnation. Furthermore, EFSA (EFSA, 2006b) concluded that the compositional data provided by the applicant confirmed the intended effects of the genetic modification.

## 3.3 Morphological traits and GM phenotype

According to the applicant, 13 morphological characteristics most relevant to potential gene dispersal were analysed in carnation Moonlite 123.2.38 and its conventional comparator (cultivar 123), including stem length, leaf length and width, bud shape, flower diameter and fragrance, number of petals, number of styles, and the height of the calyx and corolla.

An analysis of variance (ANOVA) showed no significant differences in any of these characteristics, except for the introduced traits and the mean height of the corolla of carnation Moonlite 123.2.38 (3.5 cm), which was higher than in the control (2.7 cm).

EFSA (EFSA, 2006b) concluded that the differences in corolla height were not considered relevant for the safety assessment of carnation Moonlite 123.2.38. Reported differences in morphological traits were not expected to influence the risk scenario upon accidental release to the environment or intake of the GM carnation.

## 3.4 Conclusion

Based on current knowledge and information provided by the applicant, and considering the intended uses of carnation Moonlite 123.2.38, which exclude cultivation and use as food or feed, the VKM GMO Panel concludes that the comparative analysis of the newly synthesised anthocyanin pigments delphinidin, cyanidin and petunidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonlite 123.2.38 and its conventional carnation counterpart 123 do not raise safety concerns.

# 4 Food and feed safety assessment

## 4.1 Previous evaluations by the VKM GMO Panel and EFSA

Carnation Moonlite 123.2.38, based on information supplied by the applicant, has previously been evaluated (VKM, 2008 in Appendix I; EFSA, 2006b in Appendix II). These assessments identified no adverse effects for use of GM carnations in relation to non-GM cultivars.

## 4.2 Product description and intended uses

The EU Commission Decision 2007/364/EC stipulates that a condition for placing carnation Moonlite 123.2.38 on the market is an accompanying label or document that states that it is genetically modified and the words “not for human or animal consumption nor for cultivation”. Yet the possibility of accidental intake of the Moonlite 123.2.38 cannot be excluded. Therefore, the VKM GMO Panel has followed principles used in the safety assessment of food and feed derived from GMOs, as described in EFSA’s guidelines (EFSA, 2011b), in the current safety assessment of carnation Moonlite 123.2.38.

The scope of the application C/NL/04/02 is restricted to the import of cut carnations for ornamental use only. As is the case for the non-GM carnations, the petals of GM carnations are highly unlikely to be processed and used as food and feed. Thus, the stability of GM carnations during processing is not considered as an issue.

## 4.3 Toxicological assessment

### 4.3.1 Toxicological assessment of newly expressed proteins

Bioinformatics analyses of the amino acid sequences of the newly expressed proteins in carnation Moonlite 123.2.38 do not show sequence resemblance to known toxins or IgE-dependent allergens, nor have they been reported to cause IgE-mediated allergic reactions.

### 4.3.2 Toxicological assessment of new constituents other than proteins

The anthocyanins, cyanidin and delphinidin are naturally present in foods like aubergines, blueberries and blackcurrants at higher levels than in the petals of carnation Moonlite 123.2.38 (Cacho et al., 1992). Notably, anthocyanins (E 163) are authorised food additives according to regulation 1333/2008 (Reference EC No. 1333/2008) on food additives. Previous evaluations of anthocyanins prepared by physical processes from natural foods identified no adverse effects or reason for concern (EFSA, 2013).

#### 4.3.2.1 In vitro *studies*

The applicant performed studies on gene mutagenicity, Ames test, employing *Salmonella typhimurium* exposed to aqueous extracts from petals and leaves of GM carnation Moonlite 123.2.38 and non-GM parental cultivar 123 as control. No mutagenic activity was observed.

Additionally, cytotoxicity was examined using human embryonic intestinal cells *in vitro* according to a test procedure by the applicant Florigene. Results provided by the applicant indicated that carnation Moonlite 123.2.38 leaf extracts have no cytotoxic effect at the highest concentration tested.

#### *4.3.2.2 Acute toxicity study*

To evaluate the impact of accidental exposure to carnation Moonlite 123.2.38 on human or animal health, a 14-day acute toxicity study was conducted by the applicant. Four-week-old ICR male mice were fed with aqueous extracts of frozen petals (2 g petals/kg body weight) from carnation Moonlite 123.2.38 or aqueous extracts of the non-GM control cultivar 123. The extract from carnation Moonlite 123.2.38 contains delphinidin and cyanidin since anthocyanins are water soluble. Groups of five mice were employed for each exposure. No mortalities were observed. Other than a slight body weight increase of 4% was observed in the group supplied with extracts from GM carnations compared to the group supplied with extracts from non-GM carnations, no treatment related differences or adverse effects were observed between groups.

### 4.3.3 Toxicological assessment of the whole GM plant

Taking into account that carnation Moonlite 123.2.38 is not intended for human or animal consumption as food or feed but is intended for ornamental use only, the possible effects of the genetic modifications on human health in the case of accidental intake was considered according to the EFSA guideline on the risk assessment of GM plants used for non-food/feed purposes (EFSA, 2009a). Considering the assessment of the newly expressed proteins (section 4.3.1) and of the new constituents cyanidin and delphinidin (section 4.3.2 and 4.4), no adverse effects were reported or considered likely.

The applicant did not provide information from studies on the whole GM plant.

### 4.3.4 Allergenicity

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food or plant. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive

evidence for allergenicity (Codex Alimentarius, 2003; EFSA, 2006a; EFSA, 2010b; EFSA, 2011b).

#### *4.3.4.1 Assessment of allergenicity of the newly expressed proteins*

No significant similarities to known allergens were identified via bioinformatics analyses of the amino acid sequence of the newly expressed proteins in carnation Moonlite 123.2.38 using the criterion of more than 35% identity in a segment of 80 or more amino acids (Codex Alimentarius, 2003). Additionally, the applicant performed analyses searching for matches of six contiguous identical amino acid sequences between the newly expressed proteins and known allergens, which would confirm the outcome of the above-mentioned bioinformatic analyses. No such similarities to known allergens were revealed. Moreover, other safety assessments of the ALS, DFR and F3'5'H proteins in other GM carnations have not identified reason for concern (EFSA, 2008; EFSA, 2014a; EFSA, 2014b).

The ALS, DFR and F3'5'H proteins do not show sequence resemblance to known IgE-dependent allergens, nor have they been reported to cause IgE-mediated allergic reactions.

#### *4.3.4.2 Assessment of allergenicity of the whole GM plant*

As stated earlier, carnation Moonlite 123.2.38 is not intended for food or feed purposes. Although dermal and respiratory allergies to carnations in workers handling cut flowers/carnations has been described (Cistero-Bahima et al., 2000; Sanchez-Fernandez et al., 2004; Sanchez-Guerrero et al., 1999; Stefanaki and Pitsios, 2008), the source of which appears to be multifaceted. These allergies appear to be caused by the flower, mites such as *Tetranychus urticae* infesting the carnations or a combination of the two. Notably, case reports of occupational allergies to carnations are rare. Interestingly, a case report of an individual with a respiratory allergy to carnations with no occupational exposure was published recently (Brinia et al., 2013). However, according to the applicant, no adverse allergenic reactions to GM carnation cut flowers used for ornamental purposes have been reported in the human populations handling the flowers.

## 4.4 Nutritional assessment of GM food and feed

Although carnation Moonlite 123.2.38 is intended for ornamental use only and not intended for human or animal consumption as food or feed, it is worth noting that ornamental plants may become popular as foodstuff species due to their intrinsic nutritional value, antioxidant capacity and attractive appearance (Mlcek and Rop, 2011). Flower species of *Dianthus*, *Chrysanthemum* and *Viola* have been found to possess high levels of mineral elements, with potassium being the most abundant element observed (Rop et al., 2012) and as such may be considered to have health benefits (Chandler et al., 2013). Thus, the possible use of carnation Moonlite 123.2.38 as food, dietary supplements or garnish (edible decoration) in food cannot be entirely ruled out. A need for a health risk assessment associated with such occasional consumption has therefore been suggested (Chandler et al., 2013). Moreover, a

recent evaluation suggested that the release of genetically modified carnation varieties that express *f3'5'h* gene and thereby delphinidin-based anthocyanins do not pose an increased risk of harm to human or animal health (Chandler et al., 2013).

Additionally, as mentioned earlier in section 4.3.2, cyanidin- and delphinidin-based anthocyanins are naturally present in foods like aubergines, blueberries and blackcurrants, as well as some non-GM carnation cultivars and other edible flower petals, at higher levels than in the petals of carnation Moonlite 123.2.38 (Cacho et al., 1992). According to regulation 1333/2008 (Reference EC No. 1333/2008) on food additives, anthocyanins (E 163) are authorised food additives. Previous evaluations of anthocyanins prepared by physical processes from natural foods identified no adverse effects or reason for concern (EFSA, 2013).

Chemically, water-soluble anthocyanins are derived from anthocyanidins by adding sugars. Thus, an anthocyanin contains a colour component, e.g. delphinidin or cyanidin, and 1-2 glycosides (sugar derivatives). The most important anthocyanidins in plants are delphinidin and cyanidin, the same anthocyanins found in Moonlite 123.2.38 petals, as well as pelargonidin, peonidin, petunidin and malvidin (Wu et al., 2006).

In terms of theoretical anthocyanin exposure with the intake of petals from carnation Moonlite 123.2.38, a comparison to anthocyanin levels in other common foods is of value. The amount of total anthocyanins is especially high in many dark berries and has been reported to be 3.9-4.9 mg/g fresh weight in blueberries (Wu et al., 2006), 2.5-4.9 mg/g in black currents (Rubinskiene et al., 2005; Wu et al., 2006) and 4.0-6.7 mg/g in crowberry (*Empetrum nigrum* ; Koskela et al., 2010).

Wu et al. (2006) estimated a daily anthocyanin intake of 12.5 mg/day/person in the United States, in which cyanidin and delphinidin contributed 45 and 21%, respectively. EFSA (2013) estimated that the mean exposure of anthocyanins in adults ranges from 0.7 to 1.9 mg/kg body weight per day and high level exposure to be in the range of 1.1 and 3.8 mg/kg body weight per day. In 1982, JECFA (WHO/FAO Joint Expert Committee on Food Additives) established an ADI (acceptable daily intake) of 2.5 mg/kg body weight per day for anthocyanins from grapeskin (JECFA, 1982).

*Cyanidin*

In the petals of Moonlite 123.2.38, a cyanidin concentration of 0.03 mg/g was reported by the applicant. Cyanidin is also present in non-GM carnations that have red, pink and purple colours. The concentration of cyanidin in Moonlite 123.2.38 is 20-150 times lower than the non-GM carnation cultivars that Florigene has used in its comparison. Cyanidin concentration in e.g. blueberries is in the range of 0.3-0.7 mg/g fresh weight (Wu et al., 2006). The cyanidin level observed in the petals of Moonlite 123.2.38 is therefore not considered to pose a health risk compared to the cyanidin concentration found in petals of some non-GM carnation cultivars, blueberries, and estimated ADI.

*Delphinidin*

In the petals of Moonlite 123.2.38, a delphinidin concentration of 0.09 mg/g was reported by the applicant. Delphinidin is not a naturally occurring anthocyanidin in carnations. Delphinidin concentration in e.g. blueberries is in the range of 1.2-1.4 mg/g fresh weight (Wu et al., 2006). Thus, the delphinidin concentration in carnation Moonlite 123.2.38 petals is not considered to pose a health risk compared to the levels present in berries and estimated ADI.

## 4.5 Conclusion

The applicant has performed a 14 day acute toxicity study with ICR mice and two *in vitro* tests on cytotoxicity and mutagenicity (Ames test), respectively, with extracts from leaves or petals from carnation Moonlite 123.2.38. None of the experiments revealed adverse effects of the extracts. The DFR, F3'5'H and ALS proteins do not show relevant sequence resemblance to known toxins or IgE-dependent allergens, nor have they been reported to cause IgE-mediated allergic reactions. The anthocyanins delphinidin and cyanidin expressed as a result of the genetic modification are normally present in numerous plant foods and are authorised as food additives.

Based on current knowledge, information provided by the applicant, and considering the intended use which excludes cultivation and use as food and feed, the VKM GMO Panel concludes that Moonlite 123.2.38 is as safe as its conventional counterpart, carnation 123. It is unlikely that the DFR, F3'5'H or ALS proteins, or the delphinidin or cyanidin pigments, will introduce a toxic or allergenic potential in Moonlite 123.2.38.

# 5 Environmental risk assessment

## 5.1 Introduction

This assessment applies to carnation Moonlite 123.2.38 from Florigene Ltd, which has been transformed to modify the flower colour and possesses a herbicide resistance gene (*als*) for *in vitro* selection.

The application of this line covers only import, distribution and retailing of cut flowers, and does not include either cultivation or use of carnation as food or feed. The product is imported and sold as cut flowers, and exposure of the environment to living transgenic plants is therefore low.

The genus Carnation (*Dianthus* L.) contains approximately 300 annual, biannual and perennial species, native mainly to southern parts of Asia and Europe (OGTR, 2006). *Dianthus*-species are found in alpine regions of Europe and Asia, as well as coastal areas in Mediterranian and Europe. *Dianthus deltoides* L., *D. armeria* L., *D. barbatus* L. and *D. superbus* L. are native in Norway, and also isolated plants of non-native species (*D. carthusianorum* L., *D. chinesis* L. and *D. plumarius* L.) are reported from Norway (Lid and Lid, 2005). Carnations have been cultivated for more than 2000 years and extensive selection and breeding has resulted in thousands of commercial cultivars. They have been grown in Scandinavia as an ornamental species since the middle ages (http://www.plantearven.no). Wild populations of *D. caryophyllus* are only known from Greece, Italy, Sicily and Sardinia (Tutin and Walters, 1993). In this assessment, the term carnation is used for *D. caryophyllus*.

Carnations are grown in Norway as an annual ornamental plant for outdoor gardens. Cultivars used in Norway are frost sensitive and do not survive in regions with temperatures lower than -5°C. There is no greenhouse production of carnation for cut flowers in Norway. Thus, all the cut flowers of carnation are imported. According to Statistics Norway import of carnation in 2014 was about 427 metric tonnes (www.sbb.no).

Wild *D. caryophyllus* L. have simple, bisexual open flowers with five petals. Selection and breeding has increased flower size, number of petals, and stem length as well as disease resistance (OGTR, 2006). In the modern cultivars, most of the stamens have been converted to petals (between 30 and 100 petals) and the stamens and carpels are completely surrounded by the petals. Carnation cultivars are vegetatively propagated (Zuker et al., 2002).

The majority of *Dianthus* spp. are self-sterile because the stigma is not receptive to pollen until one week or more after anthers have shed pollen. Cultivated carnations normally produce very little pollen. As the pollen viability is also low, seed setting is very low or completely absent (Galbally and Galbally, 1997). The pollen is heavy and sticky and it is not

spread by wind. Insect pollination occurs in wild carnations, mainly by *Lepidoptera* species (OGTR, 2006). Insect pollination of *D. caryophyllus* is difficult due to the morphology of the flower, and there are no known reports on insect pollination of cultivated *D. caryophyllus* (OGTR, 2006). Hand pollination is needed for sufficient seed set (Bird, 1994). Inbreeding depression appears already in the third generation and production of F1-hybrids is not a useful approach (Sato et al., 2000). Seed development takes about five weeks from pollination. Vase life of carnation can be up to two weeks. Thus, even if the flowers were pollinated, cut flowers will not be able to produce ripe seed.

Commercially carnation is propagated either by cuttings or by various tissue culture methods *in vitro*. Carnation is perennial, but it does not produce stolons, rhizomes or other vegetative propagation units and it is not able to propagate spontaneously. Short side shoots are used as cuttings, which are rooted after a hormone treatment in greenhouse under proper temperature and high humidity. For propagation by tissue culture, appropriate laboratory facilities are needed.

## 5.2 Unintended effects on plant fitness due to the genetic modifications

Carnation is not a weed in Europe, and in spite of cultivation for several centuries, there are no reports of establishment of escaped populations of cultivated carnation in Europe. The transformed lines have modified flower colour. Genes responsible for those colours are taken from higher plants and they are common in many plant species. There are no reasons to expect, that changed flower colour has any effect on the fitness characters (seed production, growth potential, winter survival, etc.) under natural conditions, compared to non-transformed cultivars.

The transgenic line also contains the *SuRB* gene, a mutated acetolactate synthase (ALS) gene from tobacco. Due to ALS protein, the transgenic carnations have enhanced resistance to herbicides with sulfonylurea as an active component. This enzyme is important for production of amino acids leucine, isoleucine and valine. Resistance to sulfonylurea is used during in vitro cultivation to select the transformed cells from the untransformed ones. Herbicides with sulfonylurea are used in Norway to control annual dicotyledonous weeds in cereal fields (http://www.plantevernguiden.no). Resistance to this type of herbicides is rather common, mainly due to mutations in the *als* gene (Tranel and Wright, 2002). Sulfonylurea resistance in populations of common chickweed (*Stellaria media*) has been found in Norway (Fykse, 2004). Establishment of carnation populations in nature from cut flowers is highly unlikely, and presence of the *als* gene will not increase the probability of such establishment.

## 5.3 Potential for gene transfer

### 5.3.1 Plant to micro-organisms gene transfer

Experimental studies have shown that gene transfer from transgenic plants to bacteria rarely occurs under natural conditions and that such transfer depends on the presence of DNA sequence similarity between the DNA of the transgenic plant and the DNA of the bacterial recipient (Bensasson et al., 2004; de Vries and Wackernagel, 2002; EFSA, 2004; EFSA, 2009b; Nielsen et al., 2000; VKM, 2005).

In the case of carnation, possibility for horizontal gene transfer may occur when the transgenic plants are spilled or discarded. Unintended spill of the imported plants is negligible, and the used carnations are discarded as domestic and public waste. Based on established scientific knowledge of the barriers for gene transfer between unrelated species, likelihood of random transfer of the transgenes present in these carnation lines to microorganisms is highly unlikely. All of the genes used are already found in natural plant populations, and none of the used genes (*F3'5'H*, *dfr*, *als*) are expected to give any competition advantage to microorganisms. Thus, environmentally harmful horizontal gene transfer from the GM carnation lines to microorganisms is highly unlikely.

### 5.3.2 Plant to plant gene flow

Hybrids *D. caryophyllus* x *D. deltoids* and *D. caryophyllus* x *D. barbatus* have been made by hand pollination (Umiel et al., 1987), but no spontaneous hybrids between carnation and other *Dianthus*-species have been reported (OGTR, 2006). Due to the marginal pollen production and low vitality of pollen in cultivated carnation cultivars, gene transfer by pollination to other cultivars of carnation or to other species of *Dianthus* is highly unlikely. Even in the case of successful pollination, vase life of cut flowers (one to two weeks) is not long enough for production of viable seeds, which normally takes five to eight weeks (OGTR, 2006).

## 5.4 Interaction between the GM plant and target organisms

With the intended use as cut flowers, interaction between carnation Moonlite 123.2.38 and any target organisms is not an issue.

## 5.5 Interaction between the GM plant and non-target organisms

There are several herbivorous pests of the carnation and they could be affected by a change in delphinidin/cyanidin ratio. However, imported flowers will be used for decoration, mainly indoors, the local quantities are low, and the longevity of the flowers is short. Therefore, the exposure of herbivores to the transgenic carnations is very low. It is highly unlikely that non-target organisms will be affected as a result of import of transgenic carnations in question.

## 5.6 Potential interactions with the abiotic environment and biochemical cycles

The transgenic carnation lines are used as cut flowers and discarded in domestic or public waste. Dispersed quantities of organic mass are low, and all the genes used are already present in nature. It is highly unlikely that the intended use of carnation Moonlite 123.2.38 will have any adverse effect on abiotic environment or biochemical cycles.

## 5.7 Conclusion

Considering the intended use of Moonlite 123.2.38, which excludes cultivation and use as food or feed, the environmental risk assessment is concerned with accidental release into the environment of viable seeds/pollen and rooted plants during transportation and distribution.

With the exception of herbicide-tolerance, Moonlite 123.2.38 has no altered survival, multiplication or dissemination characteristics compared to conventional carnation cultivars, and there are no indications of an increased likelihood of spread and establishment of feral carnation plants in the case of accidental release into the environment. Carnations are cultivated in Norway but plant to plant gene flow is not considered to be an issue due to low pollen spread and viability and low likelihood of seed development from cut flowers.

Based on current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that carnation Moonlite 123.2.38 does not represent an environmental risk in Norway.

# 6 Post-market environmental monitoring

Directive 2001/18/EC introduces an obligation for applicants to implement monitoring plans, in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the Directive. According to Annex VII, the objectives of an environmental monitoring plan are 1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the environmental risk assessment (ERA) are correct, and (2) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment.

Post-market environmental monitoring is composed of case-specific monitoring and general surveillance (EFSA 2011c). Case-specific monitoring is not obligatory, but may be required to verify assumptions and conclusions of the ERA, whereas general surveillance is mandatory, in order to take account for general or unspecific scientific uncertainty and any unanticipated adverse effects associated with the release and management of a GM plant. Due to different objectives between case-specific monitoring and general surveillance, their underlying concepts differ. Case-specific monitoring should enable the determination of whether and to what extent adverse effects anticipated in the environmental risk assessment occur during the commercial use of a GM plant, and thus to relate observed changes to specific risks. It is triggered by scientific uncertainty that was identified in the ERA.

The objective of general surveillance is to identify unanticipated adverse effects of the GM plant or its use on human health and the environment that were not predicted or specifically identified during the ERA. In contrast to case-specific monitoring, the general status of the environment that is associated with the use of the GM plant is monitored without any preconceived hypothesis, in order to detect any possible effects that were not anticipated in the ERA, or that are long-term or cumulative.

The potential exposure to the environment of carnation Moonlite 123.2.38 would be mainly through (1) unintended release into the environment of GM carnations obtained by vegetative multiplication, (2) pollen dispersal from GM cut flowers to other carnations and wild relatives, (3) dispersal of seeds produced by GM cut flowers and possible progeny and (4) discarded GM carnation cut flowers resulting in possible exposure of environmental bacteria to recombinant DNA.

The PMEM plan proposed by the applicant includes (1) a questionnaire for the European importers and operators, including questions on unexpected adverse effects; (2) the consultation of a network of taxonomists and botanists to report on any wild populations or unusual *Dianthus* hybrids that might originate from the GM carnation; (3) European

consumers are invited to comment on Florigene products with all Florigene contact details. The names and locations of our importer customers will be listed on the website. The applicant proposes to submit a PMEM report on an annual basis.

The VKM GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the restricted intended uses of carnation Moonlite 123.2.38. No specific environmental impact of genetically modified carnation Moonlite 123.2.38 was indicated by the environmental risk assessment and thus no case specific monitoring is required.

## 6.1 Conclusion

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

The environmental risk assessment did not identify any potential adverse environmental effects of the transgenic line of carnation Moonlite 123.2.38. Thus, the general surveillance plan is sufficient and there is no need for a specific surveillance plan.

# 7 Conclusions

**Molecular characterisation**

The molecular characterisation provided by the applicant shows that Carnation Moonlite 123.2.38 contains two transgenic loci. Locus 1 contains one functional copy of each of the *dfr*, *f3'5'h* and *als* genes, as well as some plasmid backbone sequences. Locus 2 contains a truncated *dfr* gene, the *Mas* terminator, and a partial right border (RB) region. Nothern blot analyses were used to confirm expression of the three inserted genes *dfr, f3'5'h*, and *als*, and Liquid chromatography (HPLC) was used to quantify new metabolites. Levels of the anthocyanins (pigments) delphinidin and cyanidin measured in bulked petal samples were reported as 0.093 and 0.031 mg/g fresh weight, respectively. Two new open reading frames (ORFs) were created in Locus 1 during transformation of the Carnation. General BLAST searches performed by the applicant did not return relevant sequence homologies between the ORF sequences, the transgene insert, and known toxins and allergens. No instability in the introduced trait, *i.e.* the particular flower colour, has been reported during the commercial cultivation of carnation Moonlite 123.2.38.

Based on current knowledge and the information provided by the applicant, the VKM GMO panel concludes that the molecular characterisation of carnation Moonlite 123.2.38 does not indicate a safety concern.

**Comparative assessment**

The VKM GMO Panel considered the available information on compositional and morphological data. Considering the intended use of carnation Moonlite 123.2.38, which exclude cultivation and use as food and feed, compositional studies were limited to the content of the three anthocyanin pigments delphinidin, cyanidin and petunidin. Compared to its non-GM parental cultivar carnation 123, carnation Moonlite 123.2.38 petals contained higher levels of delphinidin and cyanidin, and neither cultivar contained petunidin, confirming the intended effects of the genetic modification. Other morphological traits were assessed following field trials and revealed that along with differing petal colour, carnation Moonlite 123.2.38 differed significantly in one trait compared to carnation 123. None of the reported differences in compositional or morphological traits were expected to influence the risk scenario upon accidental release to the environment or intake of the GM carnation.

Based on current knowledge and information provided by the applicant, and considering the intended uses of carnation Moonlite 123.2.38, which exclude cultivation and use as food or feed, the VKM GMO Panel concludes that the comparative analysis of the newly synthesised anthocyanin pigments delphinidin, cyanidin and petunidin in its petals is sufficient for the risk assessment. The reported morphological differences between carnation Moonlite 123.2.38 and its conventional carnation counterpart 123 do not raise safety concerns.

### Food and feed risk assessment

The applicant has performed a 14 day acute toxicity study with ICR mice and two *in vitro* tests on cytotoxicity and mutagenicity (Ames test), respectively, with extracts from leaves or petals from carnation Moonlite 123.2.38. None of the experiments revealed adverse effects of the extracts. The DFR, F3'5'H and ALS proteins do not show relevant sequence resemblance to known toxins or IgE-dependent allergens, nor have they been reported to cause IgE-mediated allergic reactions. The anthocyanins delphinidin and cyanidin expressed as a result of the genetic modification are normally present in numerous plant foods and are authorised as food additives.

Based on current knowledge, information provided by the applicant, and considering the intended use which excludes cultivation and use as food and feed, the VKM GMO Panel concludes that Moonlite 123.2.38 is as safe as its conventional counterpart, carnation 123. It is unlikely that the DFR, F3'5'H or ALS proteins, or the delphinidin or cyanidin pigments, will introduce a toxic or allergenic potential in Moonlite 123.2.38.

### Environmental assessment

Considering the intended use of Moonlite 123.2.38, which excludes cultivation and use as food or feed, the environmental risk assessment is concerned with accidental release into the environment of viable seeds/pollen and rooted plants during transportation and distribution.

With the exception of herbicide-tolerance, Moonlite 123.2.38 has no altered survival, multiplication or dissemination characteristics compared to conventional carnation cultivars, and there are no indications of an increased likelihood of spread and establishment of feral carnation plants in the case of accidental release into the environment. Carnations are cultivated in Norway but plant to plant gene flow is not considered to be an issue due to low pollen spread and viability and low likelihood of seed development from cut flowers.

Based on current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that carnation Moonlite 123.2.38 does not represent an environmental risk in Norway.

### Post-market environmental monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

Based on current knowledge and considering its import, distribution and intended use as cut ornamental plants, the VKM GMO Panel concludes that the environmental risk assessment did not identify any potential adverse environmental effects of the transgenic line of

carnation Moonlite 123.2.38. Thus, the general post-market surveillance plan is sufficient and there is no need for a specific post-market surveillance plan.

**Overall conclusion**

Considering that carnation Moonlite 123.2.38 is not intended for cultivation or use as food or feed, the VKM GMO Panel considers that the comparative analysis of the newly synthesised anthocyanin pigments delphinidin, cyanidin and petunidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonlite 123.2.38 and its conventional carnation counterpart 123 do not raise safety concerns.

Based on current knowledge, information supplied by the applicant, and considering the intended use which excludes cultivation and use as food and feed, the VKM GMO Panel concludes that Moonlite 123.2.38 is as safe as its conventional counterpart. It is unlikely that the DFR, F3'5'H or ALS proteins, or the delphinidin or cyanidin pigments, will introduce a toxic or allergenic potential in carnation Moonlite 123.2.38.

Likewise, the VKM GMO Panel concludes that carnation Moonlite 123.2.38, based on current knowledge and the intended use as cut ornamental flowers, does not represent an environmental risk in Norway.

# 8 Data gaps

Generally, carnations have no or very limited history of use in food and feed, and their content of nutrients, antinutritional factors and other components with biological activity is largely unknown. The import of carnation Moonlite 123.2.38 into the EU or Norway is not intended for food or feed use, nor for cultivation, and therefore components other than the anthocyanins delphinidin, cyanidin, petunidin and pelargonidin have not been analysed in carnation Moonlite 123.2.38 (EFSA, 2006b) or other GM carnations (EFSA, 2008; EFSA, 2014a; EFSA, 2014b). The comparative compositional assessment as defined in EFSA guidance documents for GM plants and derived food and feed (EFSA, 2006a) was therefore only partially applied and possible unintended effects of the genetic modification in carnation Moonlite 123.2.38 cannot be assessed.

Furthermore, ornamental plants may become popular as foodstuff species due to their intrinsic nutritional value, antioxidant capacity and attractive appearance (Mlcek and Rop, 2011). Flower species of *Dianthus*, *Chrysanthemum* and *Viola* have been found to possess high levels of mineral elements, with potassium being the most abundant element observed (Rop et al., 2012) and as such may be considered to have health benefits (Chandler et al., 2013). Thus, the possible use of carnation Moonlite 123.2.38 as food, dietary supplements or garnish (edible decoration) in food cannot be entirely ruled out. A need for a health risk assessment associated with such occasional consumption has therefore been suggested (Chandler et al., 2013).

Thus, more comprehensive compositional analysis and food safety assessments of Moonlite 123.2.38 are merited.

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# Appendix I

Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

**Uttalelse fra Faggruppe for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet**

**27.02.08**

# Helse- og miljørisikovurdering av genmodifisert hagenellik- linje Moonlite 123.2.38 fra Florigene Ltd. (C/NL/04/02)

## SAMMENDRAG

Helse- og miljørisikovurderingen av den genmodifiserte nelliklinjen Moonlite 123.2.38 fra Florigene Ltd. (C/NL/04/02) er utført av Faggruppe for genmodifiserte organismer under Vitenskapskomiteen for mattrygghet. I forbindelse med sluttføring av saksbehandling av søknad om godkjenning av nelliklinjen for import og salg som annen nellik (utelukkende avskårne blomster) i Norge, er Vitenskapskomiteen for mattrygghet blitt bedt av Direktoratet for naturforvalting (DN) og Mattilsynet om å foreta en vitenskapelig risikovurdering av Moonlite 123.2.38 med hensyn på eventuelle effekter på helse og miljø. Florigene Moonlite (C/NL/04/02) ble etter oppdrag fra DN vurdert av Faggruppe for genmodifiserte organismer i 2005.

Vurderingen av genmodifisert hagenellik, linje Moonlite 123.2.38 er basert på dokumentasjon som er gjort tilgjengelig fra DN, EFSA og Folkehelseinstituttet. I tillegg er det benyttet informasjon fra uavhengige vitenskapelige publikasjoner i vurderingen. Moonlite er vurdert i henhold til tiltenkt bruk, og i overensstemmelse med kravene i genteknologiloven og forskrift om konsekvensutredning etter genteknologiloven. Videre er kravene i EUs utsettingsdirektiv 2001/18/EF med annekser, og EFSAs retningslinjer for risikovurdering av genmodifiserte planter (EFSA 2006a) lagt til grunn for vurderingen. Den vitenskapelige vurderingen omfatter transformeringsprosess og vektorkonstruksjon, karakterisering og nedarving av det rekombinante DNA-fragmentet, toksiner, metabolitter, allergener, proteiner, morfologiske egenskaper, potensiale for genoverføring og ikke-intenderte effekter på fitness.

Moonlite er fremkommet ved at jordbakterien *Agrobacterium tumefaciens* (stamme AGL0), som inneholder plasmidvektoren pCGP1470, ble dyrket sammen med planteceller fra den umodifiserte nelliksorten 'White Unesco'. Det rekombinante DNA-fragmentet, som er satt inn

i vektoren pCGP1470, inneholder pigmentgenene *hf1* (syntetisk flavonol 3' 5' hydroksylasegen) og *dfr* (dihydroksyflavonol-reduktasegen), begge fra petunia (*Petunia* x *hybrida*). Transformasjonen har ført til endringer i produksjonen av antocyanin-pigmenter i kronbladene, med det resultat at blomsterfargen er endret fra hvit til lilla/blå. I tillegg inneholder Moonlite 123.2.38 *surβ*-genet, et acetolaktatsyntase gen, som uttrykker et mutert acetolactatsyntase (ALS)-enzym. Genet gir nelliklinjen økt toleranse mot herbicider med virkestoff sulfonylurea. Moonlite er genmodifisert med samme genetiske materiale som ble brukt for konstruksjon av nelliklinjen Florigene Moondust (C/NL/96/14). Florigene Moondust ble vurdert av Folkehelseinstituttet i september 1997, og godkjent for import og omsetning av norske myndigheter i 2000.

Utenfor EU/EØS-området er Moonlite godkjent for dyrking i Ecuador, Colombia, Japan og Australia, og for import og videresalg som snittblomst i Canada, USA og Japan.

Søknaden gjelder godkjenning av Moonlite for import og salg av avskårne blomster til prydformål. Faggruppen har derfor ikke vurdert mulige miljøeffekter knyttet til dyrking av den transgene nelliklinjen. Potensialet for spredning av transgener fra hagenellik beregnet på snittproduksjon vurderes til å være marginalt. Vegetativ spredning skjer ikke spontant hos nellik, og snittplanter har begrenset levetid, liten pollenproduksjon, lav fertilitet og vanskelig tilgjengelig pollen. Risiko for utkryssing med andre dyrkede nelliksorter vurderes derfor til å være ubetydelig. Det er ikke rapportert om spontan hybridisering mellom hagenellik og andre viltvoksende *Dianthus*-arter

Faggruppen finner det lite trolig at avskårne blomster fra nelliken Moonlite vil medføre endret risiko for helse og miljø i forhold til avskårne blomster fra umodifisert nellik.

**NØKKELORD**
Hagenellik, *Dianthus caryophyllus* L., genmodifisert linje Moonlite 123.2.38, herbicidtoleranse, *hf1*- og *dfr*-gen, *surβ*-gen, SuRB-protein (ALS protein), acetolactatsyntase (ALS), sulfonylureaherbicider, helsemessig trygghet, helse, miljørisiko

**INNHOLDSFORTEGNELSE**



## VURDERT AV

Faggruppe for genmodifiserte organismer:
Knut Berdal (leder), Jihong Liu Clarke, Sonja Klemsdal, Helge Klungland, Casper Linnestad, Anne I. Myhr, Audun Nerland, Ingolf Nes, Kåre M. Nielsen, Hilde-Gunn Opsahl Sorteberg, Odd E. Stabbetorp, Vibeke Thrane,

Koordinatorer fra sekretariatet: Arne Mikalsen, Merethe Aasmo Finne

## BAKGRUNN

Faggruppe for genmodifiserte organismer under Vitenskapskomiteen for mattrygghet er blitt bedt av Direktoratet for naturforvalting og Mattilsynet om å foreta en vitenskaplig vurdering av helse- og miljørisiko i forbindelse med nasjonal sluttbehandling av søknad om godkjenning av den genmodifiserte nelliklinjen Moonlite 123.2.38 fra Florigene Ltd., Melbourne, Australia (C/NL/04/02). Nelliklinjen er godkjent for omsetning i EU/EØS-området under direktiv 2001/18/EF, artikkel 13. Godkjenningen omfatter bruksområdene import og videresalg, og gjelder ikke utsetting/dyrking, eller bruk av nelliklinjen som mat og fôr. Produktet skal omsettes som snittblomst under handelsnavnet Florigene Moonlite™. Notifiseringen C/NL/04/02 omfatter nellikplanter som er produsert ved vegetativ formering (stiklinger), og omfatter ikke avledete sorter fra konvensjonelle kryssinger med Moonlite 123.2.38.

Søknad om markedsføring av den genmodifiserte nelliken fra Florigene Ltd. ble forelagt nederlandske myndigheter i september 2004, som kom med sin anbefaling i desember 2005. Etter en 60-dagers høringsperiode til EU/EØS-landene, leverte EUs vitenskapskomité (EFSA) sin uttalelse i mai 2006 (EFSA 2006b). Endelig godkjenning av søknaden ble gitt i form av Kommisjonsbeslutning 2007/364/EF 23.mai 2007.

Utenfor EU/EØS-området er Moonlite 123.2.38 godkjent for produksjon i Ecuador, Colombia, Japan og Australia, og for import og omsetning som snittblomst i Canada, USA og Japan (Agbios 2008).

Pr. i dag er det godkjent 3 transgene linjer av hagenellik for omsetning som snittblomst på det norske markedet. I tillegg til økt resistens mot sulfonylurea-herbicider, er linjene modifiserte med hensyn på endret blomsterfarge (C/NL/96/14 og C/NL/97/13) og forlenget holdbarhet (C/NL/97/12). Moonlite er genmodifisert med samme genetiske materiale som ble brukt for konstruksjon av nelliklinjen Florigene Moondust (C/NL/96/14). Florigene Moondust ble vurdert av Folkehelseinstituttet i september 1997 (deres ref.: 97/01659, MINT/JAL/AMI/607.1), og godkjent for import og omsetning på det norske markedet i 2000.

## OPPDRAG FRA DIREKTORATET FOR NATURFORVALTING OG MATTILSYNET

I forbindelse med sluttføring av saksbehandling av søknad C/NL/04/02, genmodifisert hagenellik - linje 123.2.38 Moonlite fra Florigene Ltd., har Direktoratet for naturforvalting og Mattilsynet i brev datert henholdsvis 22.11.2007 (ref. 2005/3295 ART-BM-EBI) og 4.2.2008 (ref. 2008/13804) bedt Vitenskapskomiteen for mattrygghet om å foreta en vitenskapelig risikovurdering av nelliklinjen med hensyn på eventuelle effekter på helse og miljø. Søknaden omfatter bruksområdene import og salg som annen nellik (avskårne blomster).
Florigene Moonlite (C/NL/04/02) ble etter oppdrag fra DN vurdert av Faggruppe for genmodifiserte organismer i 2005.

Faggruppe for genmodifiserte organismer skal vurdere søknaden om markedsføring av genmodifisert nellik til bruk som avskårne blomster under direktiv 2001/18/EF artikkel 13. Oppdraget omfatter forhold knyttet til miljørisiko som gjelder for alle land som omfattes av godkjenningen (EØS-området), og på miljørisiko som vil være spesielt viktige for Norge. Det

skal også gis en samlet konklusjon om miljørisiko i tråd med kravene i forskrift om konsekvensutredning etter genteknologiloven, vedlegg 2C.

*Produktet som ønskes vurdert*
Genmodifisert hagenellik, linje Moonlite123.2.38. fra Florigene Ltd., Australia
Unik kode: FLO-4Ø644-4.
Notifikasjonsnummer i EU: C/NL/04/02
Status i EU: Godkjent under direktiv 2001/18/EF i 2007.
Svarfrist til DN: 5. februar 2008.

**RISIKOVURDERING**

# 1. Innledning

Risikovurderingen av den transgene hagenelliken Moonlite 123.2.38 er i hovedsak basert på dokumentasjon som er gjort tilgjengelig fra EFSA, samt uavhengige vitenskapelige publikasjoner. Vurderingen er gjort i henhold til tiltenkt bruk, og i overensstemmelse med kravene i genteknologiloven og forskrift om konsekvensutredning etter genteknologiloven, samt kravene i direktiv 2001/18/EF med annekser.

Faggruppe for genmodifiserte organismer har på faggruppemøtet 02.02.05 vedtatt å bruke EFSAs retningslinjer som gruppens retningslinjer for vurdering av genmodifiserte planter. Prinsippene som er lagt til grunn for vurderingen, er derfor hentet fra EFSAs dokument "Guidance document of the scientific panel on genetically organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA 2006a).

I henhold til Vitenskapskomiteen for mattrygghets uttalelse på møtet 23. april 2004 har Faggruppe for genmodifiserte organismer vedtatt at i de sakene hvor EFSA har kommet med sine uttalelser før Faggruppe for genmodifiserte organismer får sakene til behandling, skal søknadene behandles på samme måte som i EU-landene, dvs. ved en noe forenklet risikovurdering. EFSA har 17. mai 2006 avgitt en vitenskapelig vurdering av nellik Moonlite 123.2.38 (EFSA 2006b).

Det er kun medlemmene i Faggruppen som har vurdert den genmodifiserte nelliken.

## 1.1. Beskrivelse av egenskaper og virkningsmekanismer

Moonlite er fremkommet ved at jordbakterien *Agrobacterium tumefaciens* (stamme AGL0), som inneholder plasmidvektoren pCGP1470, ble dyrket sammen med planteceller fra den umodifiserte nelliksorten 'White Unesco'. Et rekombinant DNA-fragment som er satt inn i vektoren pCGP1470 inneholder pigmentgenene *hf1* (syntetisk flavonol 3' 5' hydroksylase gen) og *dfr* (dihydroksyflavonolreduktase gen), begge fra petunia (*Petunia* x *hybrida*). Dette rekombinante DNA-fragmentet ble overført (transformert) til nellikens planteceller. Genene på DNA-fragmentet fører til endringer i produksjonen av antocyaninpigmenter i kronbladene, med det resultat at blomsterfargen endres fra hvit til blå/fiolett. I tillegg er linje 123.2.38 modifisert med genet *surβ* fra tobakk, et acetolaktat-syntase gen, som uttrykker et mutert acetolactatsyntase(ALS)-enzym. Genet gir nelliklinjen økt toleranse mot herbicider med virkestoff sulfonylurea. Moonlite er genmodifisert med samme genetiske materiale som ble brukt for konstruksjon av linjen 'Florigene Moondust' (C/NL/96/14).

# 2. Molekylær karakterisering

## 2.1. Transformasjonssystem og vektorkonstruksjon

Til transformasjon er brukt *Agrobacterium tumefaciens* stamme AGL0 som inneholder vektoren pCGP1470 til transformering av celler fra den konvensjonelle nelliksorten 'White

Unesco'. Det rekombinante DNA fragmentet inneholder tre ekspresjonskassetter. En ekspresjonskassett for *surβ* -, en for *dfr* - og en for *hf1* genet.

## 2.2. Karakterisering av geninnsettingen og det rekombinante DNA-fragmentet

*Transformasjonssystemet/konstruksjon*
Transformasjonssystemet som er benyttet er Ti-plasmidet pCGP1470 fra *Agrobacterium tumefaciens*. Tetracyclinresistensgenet (*tet(A)*gen) er benyttet til oppformering av plasmidet i *E. coli*. Det er vist ved analyse at fullengde *tet(A)* genet ikke er til stede i nelliken.

Southern blot og PCR har blitt brukt for å karakterisere det rekombinante DNA-fragmentet i planten. Molekylærbiologisk karakterisering viser at det er satt inn bare en fullengde kopi av rekombinant DNA-fragment i nellikens genom. Dette fragmentet inneholder:

| | |
|---|---|
| LB | venstre grense fra Ti-plasmid *A. tumefaciens*, overfører DNA til planten |
| 35S | blomkål mosaikk virus (CaMV) promoter, |
| *surβ* | mutert acetolactatsyntasegen (*als*-gen); sulfonyltoleransegen fra tobakk (*Nicotiana tabacum*) |
| *surβ*3' | terminatorområde for *surβ* genet |
| P-CHS-A | kronbladspesifikk promoter, dirigerer fargeuttrykket til kronblad fra løvemunn (*Antirrhinum majus*) |
| *hf1* | flavonoid-3'5'-hydroksylase fra petunia (*Petunia* x *hybrida*), danner delfinidin avledet pigmenter |
| TD8 3' | DNA terminator fra petunia (*Petunia* x *hybrida*) |
| P-Mac-1 | konstitutiv promoter dannet ved fusjon av sekvenser fra CaMV og Mas promotere |
| *dfr* | dihydroflavonol reduktase, nøkkelenzym i antocyanin biosynteseveien fra petunia (*Petunia* x *hybrida*) |
| Tmas | mannopinsyntetase genet, blir ikke translatert, men terminerer transkriptet fra *A. tumefaciens* |
| RB | høyre grense, overfører DNA til planten, fra *A. tumefaciens* |

## 2.3. Beskrivelse av innskutte gener i fargeendrede transformanter

*Dfr*- og *hf1*-genene stammer fra petunia (*Petunia* x *hybrida*), en vanlig dyrket ettårig plante av slekten *Petunia* fra søtvierfamilien (*Solanaceae*). *Dfr*- og *hf1*-genenes enzymer danner antocyaner. Morplanten, cv. 'White Unesco', til den fargeendrede nelliken har et mutert *drf*-gen og danner ikke pigmenter. *Hf1*-genet er under regulering av en stedbunden promoter, og genet danner delfinidinpigmenter fra forløpermolekyler som produseres fra antocyanidin biosynteseveien. Delfinidin- og cyanidinpigmenter er blå eller fiolette, og finnes i for eksempel blåbær, solbær og blå druer. Enkelte antocyaner benyttes til farge av næringsmidler, og står oppført i Forskrift om tilsetningsstoffer til næringsmidler under sekkebetegnelsen antocyaner (E163).

*Surβ* er et mutert *als*-gen som finnes i tobakk, sukkerbete og vårskrinneblom (*Arabidopsis thaliana*). *Surβ*-genet koder for et mutert acetolactatsyntase enzym som ikke er sensitiv for sulfonylureaherbicider. Acetolactatenzymet er et viktig enzym i dannelsen av aminosyrer som

leucin, isoleucin og valin. *Als*-genet er til stede i alle planter. Sulfonylurea-herbicider er ikke vanlig å bruke ved produksjon av snittnellik i veksthus, og *suRF*-genet er ifølge søker introdusert for *in vitro*-seleksjon av transformerte celler. Det muterte *als*-genet er i 1995 vurdert av Arbeidsgruppe for næringsmiddeltoksikologi og risikovurdering under Nordisk Ministerråds Embetsmanns-komite for livsmedel (TemaNord 1996). Konklusjonen fra denne rapporten er at det muterte genet, som har en basepar substitusjon, koder for proteiner som normalt er til stede i planter. Det muterte enzymet opprettholder normal fysiologisk funksjon i plantens aminosyresyntese, men har endret affinitet til herbicidet. Mutanten kan derfor anses som lik villtypegenet, og som sådan ikke er noen ny komponent i matplanter.

*Andre innskutte gener*
P-CHS-A-, Pmac-1- og 35S- promoterene er regulatoriske elementer for uttrykket av *hfl-, dfr-* og *surβ*-genene. Promotorer binder RNA polymerase, men uttrykkes ikke som RNA og heller ikke som protein. *Tmas*- og *TD8-3′* -genene er utranslaterte gener som terminerer de forskjellige gentranskriptene. De uttrykkes ikke som RNA og derfor ikke som protein.

*Molekylærbiologiske analyser*
Molekylærbiologiske analyser viser at nelliklinjen Moonlite 123.2.38 inneholder to transgene loki, et lokus (lokus 1) som inneholder det rekombinante DNAet som ligger mellom høyre og venstre grense til plasmidet, samt < 20 % av *tet(A)* genet. Denne *tet(A)*-sekvensen består av ca. 190 basepar fra genets 3'-ende. Det andre lokuset (lokus 2) inneholder sannsynligvis et trunkert *dfr* gen, *mas* terminatoren og to kopier av høyre grense. Det er også påvist plasmidsekvenser på 528 bp i 5' enden av lokus 1, og 425 bp i 3'-enden av lokus 2.

PCR-analyser av det rekombinante DNA fragmentet i Moonlite viser at flankesekvensene til fragmentet er genomisk DNA fra nellik. Flankerende sekvenser til dette rekombinante DNA-fragmentet er sekvensert, 150 bp oppstrøms (5'-flankesekvens) og 150 bp nedstrøms (3'-flankesekvens). Både 5'- og 3'-flankesekvenser ble undersøkt med BLAST analyse for å undersøke egenskapen(e) og eventuelle funksjoner til flankesekvensene. Det er påvist to åpne leserammer (ORF) i 3'-fankerende området ved lokus 1. Det ble ikke påvist ORF ved lokus 2.

## 2.4. Informasjon vedr. uttrykk av introduserte gener og åpne leserammer (ORF)

Teoretiske analyser av mulige polypeptider fra hver leseramme v.h.a. allergen (BLAST2.2.13)- og toksin (GenBank, SWISS-PROT)-databaser viser ingen biologisk relevante strukturelle likheter til allergener og toksiner. ORF 2 viser stor likhet til tet(A) protein fra forskjellige klonigsvektorer. Størrelsen på peptidet er 69 aminosyrer.

Resultatene fra disse teoretiske analysene viser at det er lite sannsynlig at det dersom noen av disse leserammene skulle bli transkribert vil resultere i polypeptider som medfører potensielle toksiske eller allergene konsekvenser.

## 2.5. Nedarving og stabilitet av innsatt DNA

Hagenellik formeres utelukkende vegetativt, og spaltingsdata er følgelig ikke tilgjengelig. Det er ikke rapportert om instabilitet i introduserte egenskaper i den kommersielle produksjonen av Florigene Moonlite™ siden 1999. Søker opplyser at data fra produsenter og egne inspeksjoner viser at frekvensen av avvikende fenotyper mhp blomsterfarge, som resultat av somatiske mutasjoner, er svært lav (ikke kvantifisert).

### 2.6. Delkonklusjon

Faggruppen har vurdert de fysiske, kjemiske og funksjonelle karakteriseringene av proteinene og finner at informasjonen er tilstrekkelig. Faggruppen konkluderer at karakteriseringen av det rekombinante innskuddet i Moonlite er tilfredsstillende.

## 3. Komparative analyser

*Valg av komparator og forsøksdesign*
Nellik Moonlite 123.2.38 er sammenlignet med den ikke-transgene nelliklinjen 123. Kontrollinjen produserer ikke antocyaniner og har hvite kronblad.

### 3.1. Analyser av komponenter

Det er foretatt analyser av delfinidin, cyanidin og petunidin. Petunidin ble ikke påvist i kronblad. Mengdene av delfinidin og cyanidin i kronblad er henholdsvis 0,093 mg/g og 0,031 mg/g ferskvekt. Andre analyser er ikke aktuelt da avskåren nellik ikke benyttes til føde for mennesker eller dyr.

### 3.2. Morfologiske karakterer

Nelliklinjen Moonlite 123.2.38 og kontrollsorten 123 ble dyrket i feltforsøk og sammenlignet med hensyn på morfologiske karakterer som stilklengde, bladlengde og – bredde, knoppform, blomsterdiameter, antall grifler, samt lengde av kron- og begerblad. Med unntak av de introduserte egenskapene og kronbladlengde, ble det ikke funnet signifikante forskjeller mellom linjene. Gjennomsnittlig lengde på kronblad av Moonlite 123.2.38 var 3,5 cm sammenlignet med 2,7 cm hos kontrollsorten.

### 3.3. Delkonklusjon

Resultatene fra undersøkelsene av morfologiske karakterer viser at med unntak av herbicidresistens, er det ingen eller små forskjeller mellom nellik Moonlite og kontrollsorten.

## 4. Dokumentasjon av toksisitet og allergenisitet

### 4.1. Toksisitet

Hensikten med akutt toksisitetsstudie er å klarlegge om tilfeldig eksponering av Moonlite kan påvirke helsen til dyr og mennesker.

*Akutt oral fôringsstudie på mus*
Florigene har utført 14-dagers oral fôringsstudier på mus med ekstrakter av frosne kronblad (2 gram kronblad/kg kroppsvekt) fra Moonlite 123.2.38, samt vannuttrekk fra den umodifiserte varieteten 123. Siden antocyaniner er vannløslige vil ekstrakter fra Moonlite 123.2.38 inneholde delfinidin og cyanidin. Studiene er utført i henhold til retningslinjene fra OECD (akutt toksisitetstest nr. 401). Etter 14 dagers observasjonsperiode ble alle dyrene avlivet. Det

er utført patologiske undersøkelser. Det er ikke påvist testrelaterte skader på dyrene. Det ble påvist ca. 4 % vektøkning hos de dyrene som ble fôret med vannuttrekk fra Moonlite.

*Ames test*
Et Ames testsystem ved bruk av fire forskjellige stammer fra *Salmonella typhimurium* ble benyttet for å evaluere det mutagene potensialet til bladekstrakt fra nelliken. Ingen signifikant mutagen effekt ble påvist, sammenlignet med ekstrakt fra umodifisert nellik.

*In vitro cytotoksisitets-test*
Florigene har laget egen prosedyre for denne testen. Bladekstrakter, fra enten Moonlite eller umodifisert foreldresort, ble testet på vekst av humane tarmceller i kultur. Det ble ikke påvist noen forskjeller på celleveksten i forhold til ekstrakt fra umodifisert nellik.

*Teoretisk studie av toksisitet av antocyaninene delfinidin og cyanidin*
Antocyaniner er naturlige pigmenter som finnes i bær, frukt, grønnsaker og i kronblad hos blomster. Mengde antocyaniner i blåbær er ca. 1,5 mg/g, solbær 3 mg/g og krekling ca. 7 mg/g ferskvekt.

De naturlige forekommende antocyaniner kan avledes av et lite antall antocyanidiner. De viktigste antocyaninene er delfinidin og cyanidin, som uttrykkes i denne nelliken, samt pelargonidin. En antocyanin inneholder en fargekomponent, f.eks delfinidin, og et eller to glykosider, dvs. sukkerrester. Florigene har utført teoretiske studier av potensiell toksisitet av delfinidin og cyanidin.

*Cyanidin*
Cyanidin finnes naturlig i umodifisert nellik som har røde, rosa og lilla farger. Mengde cyanidin i Moonlite er ca. 20-150 ganger lavere enn de umodifiserte nellikvarietetene Florigene har brukt til sammenligning. Cyanidinmengden som finnes i kronblad i nelliken Moonlite anses ikke å utgjøre endret risiko for helse i forhold til cyanidinmengden i kronblad fra umodifisert nellik.

*Delfinidin*
I kronblad er det påvist 0,09 mg delfinidin/g. Mengde delfinidin i for eksempel blåbær er ca. 0,3 mg/g ferskvekt. Den akutte toksisiteten til antocyaniner er lav i gnagere. Delfinidin er ikke kjent for å være toksisk. Delfinidin og en delfinidin/cyanidin polymer var inaktiv i en rekke gentoksiske screening tester, men ga kromosomskade i pattedyrceller i kultur. I andre studier hemmet delfinidin den mutagene aktiviteten til benz(α)pyren. Den er også vist å være et fremragende anti-inflammatorisk middel. Delfinidin og dets glykosider er vist å være sterke antioksidanter og å hemme lipidperoksidering av UVB lys. Det estimerte akseptable daglige inntaket for mennesker er av IPCS satt til 2,5 mg/kg kroppsvekt (IPCS 2003). Delfinidin-mengden som finnes i kronblad i nelliken Moonlite anses ikke å utgjøre endret risiko for helse i forhold til de mengdene som er påvist i bær.

## 4.2. Allergenisitet

For å undersøke om transformasjonsprosessen kan ha ført til økning av endogene allergener i Moonlite nellik i forholdt til umodifiserte nellik ble det utført søk i databaser. Det er ikke påvist allergener fra slike baser. I henhold til EFSA er det påvist at arbeidere som har arbeidet med nellik i flere år har blitt allergiske mot nellik (Sanchez-Guerrero et al 1999; Sanchez-

Fernandez et al 2004). Denne allergien kan skyldes enten nellik eller midd (*Tetranychus urticae*), eller begge samtidig.

### 4.3. Delkonklusjon

Nelliklinjen skal benyttes som avskårne prydblomster. I enkelte tilfeller benyttes kronblad som garnityr. Ingen av proteinene betraktes som potensielle toksiske proteiner da de er til stede i de fleste planter og er ikke vist å være helseskadelige. Ingen av proteinene er kjent for å være allergener. Delfinidin og dets pigmenter er ikke å betrakte som toksiner. Sulfonylureaherbicider utgjør, etter Faggruppens syn, ikke noe problem da denne planten kun benyttes som prydplante og det er ikke meningen at disse herbicidene skal benyttes på planten. Resultater fra toksisitetstester viser at bladekstrakter fra Moonlite linje 123.2.38 ikke er akutt toksisk eller inneholder mutagener.

Faggruppen finner det lite trolig at bruk av Moonlite linje 123.2.38 som avskårne blomster samt som garnityr, vil medføre endret risiko for helse i forhold til umodifisert nellik.

## 5. Miljørisikovurdering

Godkjenningen/notifiseringen av den transgene nelliklinjen Moonlite 123.2.38 fra Florigene Ltd. under direktiv 2001/18/EF omfatter bruksområdene import og videresalg, og gjelder ikke utsetting/dyrking, eller bruk av nelliklinjen som mat eller fôr. Siden produktet som skal importeres og omsettes er snittblomster, vil det være en svært begrenset eksponering av levende plantedeler til miljøet.

### 5.1. Innledning

Nellikslekten (*Dianthus* L.) er en svært heterogen planteslekt med om lag 300 ett-, to- og flerårige arter, med opprinnelse i sørlige deler av Russland og sørlige og sentrale deler av Europa (OGTR 2006). *Dianthus*-artene er adapterte til alpine regioner i Europa og Asia, samt kystområder rundt Middelhavet. Slekten inneholder flere svært gamle kulturplanter med røtter tilbake i antikken. Hagenellik (*D. caryophyllus* L.) har trolig har vært dyrket som prydplante i Skandinavia siden middelalderen (http://www.plantearven.no). Viltvoksende populasjoner av hagenellik er bare kjent fra Hellas, Italia, Sicilia og Sardinia (Tutin *et al* 1993).

I dag dyrkes hagenellik som en ettårig utplantingsplante i Norge. Tilgjengelig sortsmateriale har dårlig overvintringsevne, og planten kan ikke dyrkes som staude i områder med temperaturer under -5 ºC. Det er ingen dyrking av hagenellik beregnet på snittproduksjon her i landet. Det foreligger ingen samlet statistikk over import av snittnellik til Norge.

Villformer av *D. caryophyllus* L. har enkle, åpne blomster med 5 kronblad. Som et resultat av langvarig vegetativ formering og seleksjon for blomsterkarakterer, har det skjedd betydelige morfologiske endringer av nellikplanten (ref. OGTR 2006). Hos sorter som benyttes til snittproduksjon har det blitt selektert for karakterer knyttet til økt blomsterstørrelse og økt antall kronblad over mange generasjoner. De fleste pollenbærere er omdannet til kronblad, og hos dagens sortsmateriale varierer antall kronblad mellom 30 og 100. Dette medfører at plantenes reproduksjonsorganer er fullstendig omsluttet av kronblad.

Hagenellik, som nyttes til produksjon av snittblomster, blir utelukkende oppformert ved stiklingsformering eller ulike typer vevskultur. Plantene viser innavlsdepresjon allerede etter tre generasjoner med selvbestøvning, og produksjon av $F_1$-hybrider er ikke aktuelt (Sato et al 2000). Nellik danner ikke vegetative formeringsorganer som stoloner, rhizomer eller yngleknopper, og vegetativ spredning skjer ikke spontant. Under oppformering tas stiklinger fra spesielle morplanter, som beskjæres kontinuerlig for å danne maksimalt antall vegetative skudd fra sideknopper. Etter behandling med plantehormoner som auxin (indoleddiksyre (IAA)), blir stiklingene satt til roting under betingelser med høy fuktighet.

Majoriteten av artene i slekten *Dianthus* er selvsterile. Nellikplantene er protandriske, dvs. at pollenet utvikles og spres før de hunnlige gametene er modne. Arret er ikke mottagelig for pollen før en til to uker etter pollespredning, og dyrkede former av nellik krever handpollinering for å sette frø (Bird 1994).

Dyrkede sorter av hagenellik produserer generelt lite pollen ofte med dårlig spireevne, og har følgelig dårlig eller manglende frøsetting (Galbally & Galbally 1997). Mengde og kvalitet av pollen kan imidlertid variere mellom sorter. Pollenkornene hos nellikplantene er tunge og klebrige og er ofte lite levedyktige. Vind spiller liten rolle i pollenspredningen, og under naturlige betingelser skjer krysspollineringen ved hjelp av insekter som vektorer. Blomsterformen til nellik, med lang avstand til nektarier ved basis av blomsten, gjør også at pollenet er vanskelig tilgjengelig for insektene.

Det er ikke kjent hvilke arter som primært pollinerer *D. caryophyllus*, men en antar at ulike *Lepidoptera*-arter, som er kjent fra andre *Dianthus-arter*, er involvert (OGTR 2006). I forbindelse med kommersiell snittproduksjon av hagenellik og videre handtering av avskårne blomster er det imidlertid ikke rapportert om insektpollinering.

### 5.2. Potensiale for ikke intenderte effekter på fitness relatert til genmodifiseringen

Nelliklinjen Moonlite 123.2.38 inneholder et rekombinant DNA-fragment med pigmentgenene *hf1* (syntetisk flavonol 3' 5' hydroksylase gen) og *dfr* (dihydroksyflavonol-reduktase gen), begge fra petunia (*Petunia* x *hybrida*). Transformasjonen har ført til endringer i produksjonen av antocyanin-pigmenter, med det resultat at fargen på kronbladene er endret fra hvit til blåfiolett. Antocyaniner er utbredt hos arter i planteslektene *Petunia, Rosa* og *Chrysantemum*. Det er ingen grunn til å anta at tilstedeværelse av pigmentene delfinidin og cyanidin vil medføre endret fitness utenfor dyrkingsmiljø sammenlignet med konvensjonelle nelliklinjer.

Nelliklinjen har også fått satt inn *surβ*-genet, et mutert *als*-gen fra tobakk. *Surβ*-genet koder for et mutert acetolactatsyntase-enzym, som gir nelliklinjen økt toleranse mot herbicider med virkestoff sulfonylurea. Acetolactatenzymet er et viktig enzym i dannelsen av aminosyrer som leucin, isoleucin og valin. I følge dokumentasjon fra søker benyttes ikke sulfonylurea herbicider ved produksjon av snittnellik, men *surβ*-genet er introdusert for *in vitro*-seleksjon av transformerte celler. I Norge brukes herbicider med virkestoff sulfonylurea i stor utstrekning mot frøugras i korn (http://www.planteverngguiden.no). Toleranse mot ALS-hemmende herbicider er utbredt i ugraspopulasjoner, hovedsakelig relatert til et mutert *surβ* (*als*)-gen (Tranel & Wright 2002). Det er også påvist resistens mot sulfonylurea i norske populasjoner av vassarve (Fykse 2004). Med bakgrunn i tiltenkt bruk av nelliklinjen er det ingen grunn til å anta at tilstedeværelse av *surβ*-genet vil ha noen økologisk betydning. Det

kreves optimale forhold for roting av stiklinger, og sannsynligheten for at kasserte planter eller avskårne blomster skal rote seg, og etablere nye planter og er derfor neglisjerbar. Til tross for omfattende dyrking av hagenellik i Europa over flere hundre år, er det ikke etablert naturaliserte populasjoner utenfor dyrkingsområder.

## 5.3. Potensiale for genoverføring

En forutsetning for genspredning er tilgjengelige veier for overføring av genetisk materiale, enten via horisontal genoverføring av DNA, eller vertikal genflyt i form av frøspredning og krysspollinering. Eksponering av mikroorganismer for rekombinant DNA kan skje under nedbryting av vegetativt plantemateriale og/eller pollen.

### 5.3.1. Horisontal genoverføring

Data fra tilgjengelige eksperimentelle studier viser at genoverføring fra transgene planter til bakterier etter all sannsynlighet inntreffer svært sjelden under naturlige forhold, og at denne overføringen forutsetter sekvenshomologi mellom overført DNA og bakterien (EFSA 2004; VKM 2005).

Ut fra dagens vitenskapelig innsikt mht barrierer for genoverføring mellom ubeslektede arter og flere års forskning for om mulig å framprovosere tilfeldig overføring av genetisk materiale fra planter til mikroorganismer, dyr eller mennesker gjennom inntak eller eksponering, er det ingenting som tyder på at transgenene i nelliklinjen skal kunne overføres til andre enn naturens kryssingspartnere, dvs. annen dyrket nellik. Nielsen et al. (2000) og De Vries og Wackernagel (2002) har undersøkt persistens av DNA og opptak av GM DNA i jord. I disse laboratorieforsøkene ble det detektert svært små mengder DNA som var overført fra planter til bakterier. Forutsetningen for at dette kunne skje var sekvenshomologi mellom plantetransgenet og mottagerbakterien.

Med bakgrunn i opprinnelse og karakter/egenskaper av de innsatte genene og mangel på seleksjonspress i miljøet, er sannsynligheten for at horisontal genoverføring vil gi selektive fordeler eller økt fitness på mikroorganismer svært liten (Nielsen 2003). Det er derfor usannsynlig at gener fra Moonlite 123.2.38 vil etableres stabilt i genomet til mikroorganismer i miljøet. Ut fra tilgjengelig kunnskap er det ikke grunn til å forvente at det vil skje horisontal genoverføring av DNA-materiale fra den transgene nelliklinjen.

### 5.3.2. Vertikal genoverføring

*Dianthus*-artenes reproduksjonsbiologi, inkludert marginal pollenproduksjon og dårlig fertilitet hos moderne sorter av hagenellik, indikerer at potensialet for genoverføring til viltvoksende populasjoner eller andre dyrkede nelliksorter via pollen er svært begrenset. I tillegg kommer at i forbindelse med produksjon av snittblomster blir nellikplantene høstet før de når pollenmodning. Frøutviklingen hos nellik tar fem til åtte uker (OGTR 2006), og ved en eventuell vellykket pollinering vil dette overstige forventet levetid som snittblomst.

Hagenellik er viltvoksende i kystområder rundt Middelhavet, nærmere bestemt i Hellas, Italia, Sicilia og Sardinia (Tutin *et al* 1993). Det er funnet forvillede planter av hagenellik på et fåtall lokaliteter i Norge, men arten etablerer ikke populasjoner utenfor dyrkingsområder (Lid og Lid 2005).

I Norge er *Dianthus*-artene engnellik (*D. deltoides* L.), saronnellik (*D. armeria* L.), busknellik (*D. barbatus* L.) og silkenellik (*D. superbus* L.) viltvoksende (Lid & Lid 2005). I tillegg er forvillede planter av kartunianernellik (*D. carthusianorum* L.), kinanellik (*D. chinesis* L.) og fjærnellik (*D. plumarius* L.) registrert, hovedsaklig rundt Oslofjorden. Det er laget hybrider mellom *D. caryophyllus* L. og henholdsvis busknellik og engnellik ved kontrollerte kryssinger (Umiel et al 1987). Det er imidlertid ikke rapportert om spontan hybridisering i felt mellom hagenellik og viltvoksende *Dianthus*-arter (OGTR 2006).

### 5.4. Delkonklusjon

Potensialet for spredning av transgener fra hagenellik beregnet på snittproduksjon vurderes til å være marginalt. Vegetativ spredning skjer ikke spontant hos nellik, og snittplanter har begrenset levetid, liten pollenproduksjon, lav fertilitet og vanskelig tilgjengelig pollen. Risiko for utkryssing med andre dyrkede nelliksorter vurderes derfor til å være ubetydelig. Det er ikke rapportert om spontan hybridisering mellom hagenellik og andre viltvoksende *Dianthus*-arter.

## KONKLUSJON

Søknaden gjelder godkjenning av nelliklinjen Moonlite 123.2.38 for import og omsetning som snittplanter til prydformål. Kronblader fra nellik har også vært benyttet som garnityr i matretter. Cyanidin og delfinidin er vanlige pigmenter i mange prydplanter og bær, som blåbær, krekling og solbær. Faggruppen vurderer at tilfeldig inntak av kronblad fra Moonlite er lavt, og at mengde delfinidin som inntas fra slike kronblad ubetydelig, sett i forhold til inntaket fra bær, frukt og vin. Ingen av proteinene betraktes som potensielle toksiske eller allergene. Faggruppen konkluderer med at avskårne blomster fra den genmodifiserte nelliken Moonlite ikke utgjør noen endret risiko for helse sammenlignet med umodifisert nellik.

Potensialet for spredning av transgener fra hagenellik beregnet på snittproduksjon vurderes til å være marginalt. Vegetativ spredning skjer ikke spontant hos nellik, og snittplanter har begrenset levetid, liten pollenproduksjon, lav fertilitet og vanskelig tilgjengelig pollen. Risiko for utkryssing med andre dyrkede nelliksorter vurderes derfor til å være ubetydelig. Det er ikke rapportert om spontan hybridisering mellom hagenellik og andre viltvoksende *Dianthus*-arter

Faggruppen finner det derfor lite trolig at bruk av nelliken Moonlite vil medføre endret risiko for helse og miljø i forhold til annen nellik.

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# Appendix II

# Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission 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[1]

## (Question No EFSA-Q-2005-282)

**Opinion adopted on 17 May 2006**

## SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on the notification to import carnation Moonlite 123.2.38 variety, genetically modified (GM) for flower colour (Unique Identifier FLO-40644-4). The GM carnation also contains a gene conferring tolerance to sulfonylurea herbicides. Cut flowers of carnation Moonlite 123.2.38 are intended to be imported within the European Union for ornamental use only.

The present opinion is based on a question raised by the Commission related to a notification to place carnation Moonlite 123.2.38 on the market under Directive 2001/18/EC (Reference C/NL/04/02). The question followed a scientific assessment that was initially made by the competent authority of the Netherlands and evaluated subsequently by all other Member States. An assessment of the GM carnation Moonlite 123.2.38 was requested by the Commission because of questions raised by several Member States following the evaluations at the national level. When this is the case, the EU legislation requires that EFSA carries out a further assessment and provides an opinion. The GMO Panel was, therefore, asked to consider whether there is any scientific reason to believe that the placing on the market of the GM carnation Moonlite 123.2.38 for import is likely to cause any adverse effects on human health and the environment.

In delivering its opinion, the GMO Panel considered the notification, additional information provided by the applicant and the specific questions and concerns raised by the Member States. The carnation Moonlite 123.2.38 was assessed with reference to its intended use and the appropriate principles described in the 'Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed'. The scientific assessment included examination of the DNA inserted into the GM carnation using *Agrobacterium*-mediated transformation and the nature and safety of the new products intended to be produced by the GM variety. Furthermore, the potential environmental impact of carnation Moonlite 123.2.38, including a monitoring plan, was assessed in the context of the restricted intended use of carnation Moonlite 123.2.38.

The carnation Moonlite 123.2.38 has a modified flower colour, a shade of violet. The colour has been achieved by introducing into white carnation two genes of the anthocyanin biosynthesis

[1] For citation purposes: Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission 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, *The EFSA Journal* (2006) 362, 1-19.

pathway from petunia. These genes, encoding dihydroflavonol 4-reductase (*dfr*) and flavonoid 3'5' hydroxylase (*f3'5'h*), in combination with other genes of the anthocyanin biosynthesis pathway already present in the carnation, give rise to the anthocyanins delphinidin and cyanidin, the same compounds that give colour to blueberry, blackcurrant and red grape. Both anthocyanins are present in the petals of the GM carnations. Carnation Moonlite is also tolerant to sulfonylurea herbicides conferred by a mutated *SuRB (als)* gene used as marker trait in the selection of genetically modified plants but not for plant protection purposes. Another GM carnation variety, Florigene Moondust™, which is genetically modified with the same transformation vector, received the consent for placing on the market, including cultivation, within the EU in 1997.

The molecular analysis of the DNA inserts confirms that the three genes expressing the intended traits (violet flower colour encoded by *dfr* and *f3'5'h* genes and herbicide tolerance encoded by the mutated *SuRB (als)* gene) are present into carnation Moonlite 123.2.38. The carnation Moonlite 123.2.38 does not contain a functional antibiotic resistance marker gene. Bioinformatic analysis shows that two new open reading frames (ORFs) were created but that neither shows homologies to any toxic or allergenic proteins. Results of bioinformatic studies of the three newly expressed proteins in carnation Moonlite 123.2.38 did not indicate relevant homology with known toxins or allergens.

Given the intended use of carnation Moonlite 123.2.38 (excluding human or animal consumption and cultivation), the GMO Panel considers that the comparative analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment. The GMO Panel concludes that there is no indication of increased toxicity of the carnation Moonlite 123.2.38 compared to the recipient variety.

The carnation Moonlite 123.2.38 was assessed for imported cut flowers for ornamental use only. Scientific information on potential environmental effects associated with the cultivation of carnation Moonlite 123.2.38 was therefore not required. Carnation Moonlite 123.2.38 cut stems and flowers have very restricted viability, very low pollen emission and little or no viable seed. However, in the very unlikely event of accidental release into the environment, the GMO Panel considers that the carnation Moonlite 123.2.38 would not show enhanced fitness characteristics, except in the presence of sulfonylurea herbicides. The consequences of the potential transfer of the three genes would be negligible in terms of adverse effects on the environment. The GMO Panel concludes that there is no indication that GM carnation Moonlite 123.2.38 will have adverse effects on the environment in the context of the intended use.

The GMO Panel agrees with the applicant that the environmental risk assessment did not identify risks that require a case-specific monitoring plan. The GMO Panel also agrees with the general methods and approaches of the general surveillance plan.

In conclusion, the GMO Panel considers that the information available for carnation Moonlite 123.2.38 addresses the outstanding questions raised by the Member States and considers that, in the context of its intended use, carnation Moonlite 123.2.38 is unlikely to have adverse effects on human and animal health or the environment.

**Key words:** acetolactate synthase (SuRB/ALS), anthocyanin, carnation, C/NL/04/02, delphinidin, *Dianthus caryophyllus*, dihydroflavonol 4-reductase (DFR), Directive 2001/18/EC, environment, feed safety, flavonoid 3'5' hydroxylase (F3'5'H), Florigene, flower colour, GMO, health, herbicide tolerance, import, sulfonylurea, Unique Identifier FLO-40644-4.

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## BACKGROUND

The Commission received the notification (Reference C/NL/04/02) from Florigene, on 9 December 2005, together with a positive assessment report, from the lead Member State (The Netherlands).

In accordance with Directive 2001/18/EC (EC, 2001), the notification was then transmitted to the Competent Authorities of the other Member States, a number of which have raised objections during the statutory 60-day period. The applicant provided the Member States with additional information in response to the objections raised during the 60-day period. The Member States had until 6 November 2005 to confirm or lift their objections. Where these objections are maintained, the Commission is required to consult the relevant Scientific Committees for opinion, now represented by EFSA.

Article 18(1) of Directive 2001/18/EC states that the period of time during which the Commission is awaiting the opinion of the Scientific Committee shall not exceed 90 days. The evaluation by EFSA started on 4 January 2006, after receipt of the complete background information (request from the Commission, dossier of the applicant and final objections maintained by the Member States). During the 90-day period, EFSA requested further clarifications from the applicant. This procedure extended the final deadline set for the delivery of this opinion.

In delivering its opinion the GMO Panel considered the original notification, additional information provided by the applicant and the specific questions and concerns raised by the Member States.

The scope of notification C/NL/04/02 is restricted to the import of cut flowers of carnation Moonlite 123.2.38 for ornamental use only, produced by vegetative propagation. The progeny derived from sexual crosses with Moonlite 123.2.38 variety is not covered under notification C/NL/04/02.

## TERMS OF REFERENCE

EFSA was requested, under Article 29(1) and in accordance with Article 22(5)(c) of Regulation (EC) No 178/2002 (EC, 2002a), to provide a scientific opinion as to whether there is any scientific reason to believe that the placing on the market of the GM carnation Moonlite 123.2.38 for import is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC (EC, 2001).

In particular, EFSA was requested to take account of the scientific objections raised by the Competent Authorities of the Member States in this context, to highlight diverging scientific views, if any, and how these are resolved in the opinion.

EFSA was not requested to give an opinion on the non-scientific objections raised by the Competent Authorities in their replies, in the context of the entry into force of forthcoming legislation or requests for further legislative/implementing measures.

## ASSESSMENT

### 1. Introduction

The genetically modified (GM) carnation Moonlite 123.2.38 (Unique Identifier FLO-40644-4) was assessed with reference to its intended use, taking account of the appropriate principles described in the 'Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed' (EFSA, 2004) which was updated with a new version of chapter 11.4 on General Surveillance as part of the post market environmental monitoring (EFSA, 2006). In its evaluation the Panel focused in particular on the issues raised by the Member States during the initial assessment of the notification (Reference C/NL/04/02) introduced under Directive 2001/18/EC. The evaluation presented here is based on the information provided in the original notification related to carnation Moonlite 123.2.38 submitted to the Competent Authority of the Netherlands including additional information from the applicant in reply to the Member States questions. This information was provided to the Member States via EFSA-net.

The scope of notification C/NL/04/02 is restricted to the import of cut flowers of carnation Moonlite 123.2.38 for ornamental use only, produced by vegetative propagation. The progeny derived from sexual crosses with Moonlite 123.2.38 variety is not covered under notification C/NL/04/02.

Carnation Moonlite 123.2.38 is a new variety which contains the herbicide tolerance *SuRB* (*als*) gene coding for a mutant acetolactate synthase protein (ALS), used to facilitate selection during the genetic transformation process *in vitro*. The violet colour of the flowers results from the expression of two new genes encoding dihydroflavonol 4-reductase (DFR) and flavonoid 3'5' hydroxylase (F3'5'H) which, together with endogenous genes in the anthocyanin biosynthetis pathway, enable the biosynthesis of delphinidin in the petals.

The same transformation vector (pCGP1470) was used to produce the GM carnation variety Florigene Moondust™ (Notification reference C/NL/96/14) which was approved for placing on the market on December 1st 1997 (http://europa.eu.int/comm/environment/biotechnology/authorised_prod_1.htm). The consent for placing on the market in EU, including cultivation, was issued by the Dutch Competent Authority (see http://www.vrom.nl/ggo-vergunningverlening).

## 2. Molecular characterisation

### 2.1. Issues raised by Member States

Questions were raised regarding (1) the sequences of the inserts and flanking regions, (2) the presence/absence of an intact tetracycline (*tet*(A)) gene in carnation Moonlite 123.2.38, (3) the the expression levels of the three newly inserted genes and (4) the analysis of open reading frames (ORFs).

Comments raised by the Member States on specific molecular detection methodologies as well as on their validation are not within the scope of the GMO Panel remit.

Question (1) regarding the flanking sequences of the inserts is considered under section 2.2.2 of the present opinion. Question (2) regarding the presence/absence of an intact *tet*(A) gene is considered under section 2.2.2.1. Question (3) regarding the expression levels of the three newly inserted genes is considered under section 2.2.3. Question (4) regarding the analysis of ORFs is considered under section 2.2.2.

### 2.2 Evaluation of relevant scientific data

Having considered the information provided in the original notification and the Member States comments, the GMO Panel requested from the applicant further data on the nucleotide sequence(s) of the insert(s) and of the associated flanking sequences as well as on appropriate bioinformatic analysis.

#### 2.2.1. Transformation process and vector constructs

Genetic material was introduced into carnation Moonlite 123.2.38 by *Agrobacterium*-mediated transformation using disarmed *Agrobacterium tumefaciens* strain AGL0 carrying the transformation vector pCGP1470 described below. *Agrobacterium* was subsequently eliminated with ticarcillin and its absence was confirmed by PCR using *virG* gene primers; this gene is located in the Ti plasmid.

Details of the construction of the vector pCGP1470 used in the genetic modification of carnation Moonlite 123.2.38 are provided. The vector contains the following three expression cassettes ligated to the plasmid pWTT2132 backbone: 1) the promoter from a snapdragon gene encoding chalcone synthase, petunia flavonoid 3'5' hydroxylase (F3'5'H) cDNA, the terminator from the petunia gene encoding a phospholipid transfer protein homologue; 2) the constitutive promoter Mac, the petunia dihydroflavonol 4-reductase (DFR) cDNA, the terminator from the *Agrobacterium* gene encoding mannopine synthase (Mas); 3) the cauliflower mosaic virus 35S promoter, an untranslated region from the cDNA corresponding to the petunia gene encoding chlorophyll a/b binding protein 5, the *SuRB* (*als*) gene coding for a mutant acetolactate synthase protein (ALS) derived from *Nicotiana tabacum*, including its terminator. The first two cassettes were needed to obtain the desired flower colour.

The third cassette provided tolerance to sulfonylurea herbicides used as marker trait in the selection of genetically modified plants but not for plant protection purposes. Between the left (LB) and right (RB) borders that are commonly considered to define the region to be transferred, the vector also includes small stretches (*ca*. 400 bp total) of *Escherichia coli* plasmid pBluescript/pUC. Outside the LB and RB, the transformation vector pCGP1470 contained: 1) *ca*. 1.5 kb from *E. coli* for replication of the transformation vector in *E. coli*; 2) *ca*. 8 kb from

*Pseudomonas aeruginosa* for replication of the transformation vector in *A. tumefaciens*; 3) *ca*. 2 kb of a *tet* gene complex from *E. coli* for the selection of transformed bacterial cells based on tetracycline (tet) resistance. The complex includes *tet*(A) and *tet*(R) genes.

The entire sequence of the transformation vector pCGP1470 and a description of the function of all genes present were provided. The same transformation vector was used to produce the GM carnation variety Florigene Moondust™ (C/NL/96/14).

### 2.2.2. Transgenic constructs in the genetically modified plant

Carnation Moonlite 123.2.38 contains two transgenic loci:

- Locus 1: The genetic material located in the transformation vector between the partial LB and RB regions is stably integrated in the carnation Moonlite 123.2.38. In addition, Southern analysis of *Eco*RI-digested genomic DNA with 12 probes covering the entire *ca.* 25 kb transformation vector pCGP1470 indicated that some sequences outside the border regions have been integrated in the GM variety. The probe which partly overlapped the *tet* resistance gene complex showed weak hybridisation with plant DNA. Further studies using TAIL-PCR indicated that only a partial *tet*(A) gene is incorporated into the plant DNA. This sequence consists of *ca*. 190 nucleotides from the 3' end of the gene, representing less than 20% of the entire gene. No sequence corresponding to the *tet*(R) gene is incorporated into the carnation genome;

- Locus 2: Further Southern analysis was performed to understand the organization of the integrated sequences better. In contrast to all other probes used, the DFR and RB probes gave additional bands which would not be expected from a single copy of an intact T-DNA integrated in a single locus of the plant genome. The applicant concluded that, in addition to the sequence spanning from partial *tet*(A) gene through LB to RB, the carnation variety contains another integration site. Further sequence analysis indicated that the second integration site contained a truncated *dfr* gene and the *Mas* terminator as well as partial RB region.

Bioinformatic analysis showed that two new open reading frames (ORFs) were created at the junction region of locus 1. General BLAST searches were performed in order to compare the hypothetical protein sequences encoded by the three inserted genes and, the sequences of putative proteins that might be encoded by the two ORFs at the junction between the inserted DNA fragment and the plant DNA, with proteins from the GenBank and SwissProt databases. No relevant homologies were observed with known allergens and toxins using general BLAST searches. Additional searches for sequences homologies of at least six identical contiguous amino acids of the transgenic proteins with peptide sequences of identical length in known allergens were performed by the applicant (see sections 4.2.3 and 4.2.6).

#### 2.2.2.1 Absence of plasmid backbone sequences

Some plasmid backbone sequences were present in carnation Moonlite 123.2.38. These include the modified pACYC184 sequence necessary for replication of the transformation vector in *E. coli*, and part (ca. <20%) of the *tet(A)* resistance gene are integrated into locus 1 of the GM carnation. None of these sequences raise any concern (see section 2.2.3 regarding safety impact of *tet*(A) gene).

### 2.2.3. Information on the expression of the insert

The expression of the three genes, encoding F3'5'H, DFR and ALS enzymes, was demonstrated at the mRNA level by northern analysis. The expression analysis also included quantification of the resulting new metabolites by liquid chromatography. The levels of delphinidin and cyanidin in a single assay of bulked petal samples were 0.093 and 0.031 mg/g fresh weight, respectively. It was estimated that the amount of delphinidin in 200 genetically modified carnation flowers corresponds to that in 100 g blueberries.

The partial *tet*(A) gene incorporated into the carnation genome is unlikely to confer tetracycline resistance. Yamaguchi and co-workers (1993) found that a larger fragment of *tetA* corresponding to the C-terminal half of the TetA protein, which also comprises the smaller fragment encoded by the *tet(A)* fragment inserted into the topical GM carnation, was unable to convey antibiotic resistance to recipient bacteria. This was further confirmed by the applicant by cloning the *tet*(A) sequence present in the GM carnation into a bacterial vector which included a ribosome binding site necessary for transcription in *E. coli* and by adding an upstream ATG start codon in-frame with the *tet*(A) sequence and a terminal stop codon for translation. The correctness of the construct in the resulting plasmid pCGP3128 was confirmed by sequence analysis. In the tetracycline resistance assay appropriate positive and negative controls were used. Tetracycline resistance was studied by plating the bacteria on media containing tetracycline concentrations ranging from 0.5 to 12.5 mg/l. The cloned *TetA* fragment failed to confer resistance to tetracycline.

### 2.2.4. Inheritance and stability of inserted DNA

Carnations are propagated vegetatively. No instability in the introduced trait, *i.e.* the particular flower colour, has been reported during the commercial cultivation of the carnation Moonlite 123.2.38, which includes approximately seven generations and the production of millions of flowers.

## 2.3. Conclusion

The molecular characterisation data establish that the carnation Moonlite 123.2.38 contains in one locus the cassettes containing the genes responsible for the intended traits (violet flower colour encoded by *dfr* and *f3'5'h* genes and herbicide tolerance encoded by the mutated *SuRB (als)* gene).

Some vector backbone sequences were shown to be present at this locus. An additional locus was detected that does not express any functional protein. The carnation Moonlite 123.2.38 does not contain a functional antibiotic resistance marker gene. The bioinformatic analysis showed that two new ORFs were created at the first locus. These new ORFs do not share homology with any toxic or allergenic proteins. The GMO Panel concludes that, considering the intended use of the GM carnation, the molecular characterisation of carnation Moonlite 123.2.38 does not raise any safety concern for humans, animals or the environment.

## 3. Comparative analysis

### 3.1 Issues raised by Member States

A question was raised regarding the need for further information on sample preparation for the HPLC analysis of anthocyanins. This question is considered under section 3.2.2 of the present opinion.

### 3.2. Evaluation of relevant scientific data

Having considered the information provided in the original notification and the Member States comments, the GMO Panel considered the additional information provided by the applicant to the Member States with respect to the HPLC sample preparation.

#### 3.2.1. Choice of comparator and production of material

Carnation Moonlite 123.2.38 was compared with the parental variety 123 which does not produce anthocyanins and has white petals consequently.

#### 3.2.2. Compositional analysis

Freeze dried petals of carnation variety Moonlite 123.2.38 and the control variety 123 were analyzed for three anthocyanins, namely delphinidin, cyanidin and petunidin. Roots and stems were not assayed. The GMO Panel reviewed the HPLC data provided on the concentrations (mg/g fresh weight petal) of these three anthocyanins (Fukui *et al*., 2003). While petunidin was not detected in either the GM variety or the non-GM control, delphinidin and cyanidin were detected in carnation Moonlite 123.2.38 at levels of 0.093 mg/g and 0.031 mg/g fresh weight respectively. These anthocyanins were absent from the white-flowered variety 123.

The GMO Panel considers that the comparative analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment considering the intended use of carnation Moonlite 123.2.38 (excluding human or animal consumption and cultivation).

#### 3.2.3. Agronomic traits and GM phenotype

Carnation Moonlite 123.2.38 and the control variety 123 were grown in field trials and compared for several morphological characteristics including stem length, leaf length and width, bud shape, flower diameter and fragrance, number of petals, number of styles, and the height of the calyx and corolla. The two varieties showed no significant differences in any of these characteristics, except for the introduced traits and the mean height of the corolla of carnation Moonlite 123.2.38 (3,5 cm), which was higher than in the control variety (2,7 cm).

### 3.3. Conclusion

On the basis of the data provided by the applicant and in consideration of the intended use of carnation Moonlite 123.2.38 (excluding human or animal consumption and cultivation), the GMO Panel considers that the comparative analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment. The compositional data available in the application confirm the intended effects of the genetic modification (namely, the modified colour of flowers). The GMO Panel considers that the observed differences in the corolla height are not of significance with respect to the safety assessment of carnation Moonlite 123.2.38 for humans and animals in the unlikely event that carnation Moonlite 123.2.38 petals are consumed.

## 4. Safety assessment of carnation Moonlite 123.2.38 for humans and animals

### 4.1. Issues raised by Member States

Questions were raised regarding (1) the need for further explanations with respect to the outcomes of the acute toxicity study to analyze the anthocyanin content (in particular, the cyanidin content), (2) the limitation of the acute toxicity assay which administers petal extracts rather than feeding whole petals as part of the diet and (3) possible risk related to increasing use of GM carnation petals in food.

Questions (1) and (2) regarding the acute toxicity study are considered under section 4.2.4 of the present opinion and question (3) regarding the accidental consumption of carnation Moonlite 123.2.38 petals by humans under section 4.2.5.

### 4.2. Evaluation of relevant scientific data

Having considered the information provided in the original notification and the Member States comments, the GMO Panel requested from the applicant further clarifications and data with respect to the assessment for potential toxicity and allergenicity.

#### 4.2.1. Product description and intended use

The genus *Dianthus* comprises species that have been cultivated for ornamental uses for hundred of years (Office of the Gene Technology Regulator, 2005). Carnations are present in gardens and in the cut flower market as ornamental plants.

The scope of notification C/NL/04/02 is restricted to the import of cut carnations Moonlite 123.2.38 for ornamental use only. The progeny derived from sexual crosses with Moonlite 123.2.38 is not covered under notification C/NL/04/02. Carnation Moonlite 123.2.38 is a new variety with specific violet flower colour that results from the synthesis of delphinidin due to the introduction of the *dfr* and *f3'5'h* genes. The GM carnation variety also contains a *SuRB* (*als*) gene, coding for a mutant acetolactate synthase protein (ALS), which confers herbicide tolerance used to facilitate selection during the transformation process *in vitro*.

### 4.2.2. Stability during processing

Since carnation Moonlite 123.2.38 is intended to be imported for the cut flower market, as is the case for non GM carnations, the petals of carnation Moonlite 123.2.38 are highly unlikely to be processed and used as processed food and feed. Consequently, the GMO Panel did not consider stability of the GM carnation during processing as an issue.

### 4.2.3. Toxicology assessment of the newly expressed proteins

General BLAST searches were performed in order to compare the hypothetical protein sequences encoded by the three inserted genes and, the sequences of putative proteins that might be encoded by the two ORFs at the junction between the inserted DNA fragment and the plant DNA, with proteins from the GenBank and SwissProt databases. No homologies were observed with known toxins using general BLAST searches.

### 4.2.4. Toxicology assessment of new constituents other than proteins

#### (a) Acute toxicity testing

The purpose of an acute toxicity study is to determine the impact of accidental exposure to carnation Moonlite 123.2.38 on human or animal health.

A 14-day acute toxicity study was performed on four-week old mice fed with water extracts of frozen petals (2 g petals/kg body weight) from carnation Moonlite 123.2.38 and water extracts of the non-GM control variety 123, respectively. Acute toxicity studies on plant materials are commonly carried out with extracts made thereof (see section 3.2.2). As anthocyanins are water soluble, the extract from carnation Moonlite 123.2.38 contains delphinidin and cyanidin. Mice were split into two groups of five each for each exposure. No mortalities were observed. A slight body weight increase of 4% was observed in the group supplied with extracts from GM carnations compared to the group supplied with extracts from non-GM carnations.

#### (b) Additional in vitro studies

The applicant performed an Ames test and a cytotoxicity study on human embryonic intestinal cells *in vitro* with water extracts of leaves of carnation Moonlite 123.2.38 and control variety 123. The water extract showed neither mutagenicity nor toxicity.

### 4.2.5. Toxicological assessment of the whole GM plant

Carnation flowers have a long history of use as ornamentals. The genus *Dianthus* comprises species that have been cultivated for ornamental uses for hundred of years (Office of the Gene Technology Regulator, 2005).

Given that carnation Moonlite 123.2.38 is not intended for human or animal consumption as food or feed but for ornamental use only, the GMO Panel does not consider it necessary to perform a comprehensive food/feed safety assessment of the whole GM plant.

The GMO Panel has, nevertheless, considered the possible effects of the genetic modification on human and animal health of accidental consumption of carnation Moonlite 123.2.38 petals. The GMO Panel notes that the data on acute toxicity studies and on the two *in vitro* studies (see section 4.2.4) do not give any indication of increased toxicity of the carnation Moonlite 123.2.38

petals compared to the parental variety in the unlikely event of accidental consumption of GM petals.

In addition, delphinidin and cyanidin, belonging to the group of anthocyanins are present in many foods and at much higher concentrations than in the petals of carnation Moonlite 123.2.38, particularly high concentrations being found e.g. from blackcurrants. Many other delphinidin-containing species (e.g. *Dampiera* spp., *Delphinium* spp., *Lisianthus* spp., *Wisteria* spp.) show a higher concentration of delphinidin (as a percentage of total anthocyanins) than does carnation Moonlite 123.2.38. Cyanidin and its derivatives are commonly found in a number of plants including *Petunia* (Ando *et al*., 1999), carnation (Bloor, 1998), rose (Biolley and Jay, 1993), apple (Lancaster, 1992), sunflower seeds (Mazza and Gao, 1994), chrysanthemum (Schwinn *et al*., 1993; Andersen *et al*., 2000), *Vicia villosa* (Catalano *et al*., 1998) and *Vitis* spp. (Cachio *et al*., 1992).

### 4.2.6. Allergenicity

General BLAST searches comparing the hypothetical protein sequences encoded by the three inserted genes and, the sequences of putative proteins that might be encoded by the two ORFs at the junction between the inserted DNA fragment and the plant DNA, with proteins from the GenBank and SwissProt databases were performed. Additional searches for sequences homologies of at least six identical contiguous amino acids of the transgenic proteins with peptide sequences of identical length in known allergens were performed by the applicant. No homologies were observed with known allergens in using general BLAST searches. Various identical sequences of six amino acids were found in the three expressed proteins and known allergens, but there is no further indication of the allergenicity of these transgenic proteins.

Carnation Moonlite 123.2.38 is not intended to be used as food or feed. No adverse reaction to carnation Moonlite 123.2.38 cut flowers for ornamental purpose has been reported in the general populations. However Sanchez (1999; 2004) has described occupational allergy to carnation in workers handling cut flowers/carnation over a long time. This allergy could be caused either by the flower, by mites (*Tetranychus urticae* infesting carnations) or by both simultaneously.

Considering the limited exposure to carnation Moonlite 123.2.38 in the scope of this notification, the GMO Panel is of the opinion that, considering the rare reports of cases of occupational allergies, the issue of potential allergenicity is unlikely to be a safety concern.

## 4.3. Conclusion

Carnation flowers have a long history of use as ornamentals. Carnation Moonlite 123.2.38 differs from control variety 123 by the presence of delphidinin, which confers a violet colour to the flowers. Delphinidin, a common pigment in many ornamental flowers and food plants such as red grapes, black currants, egg plants, blueberries, is produced as a result of the combined expression of the introduced *dfr* and f*3'5h* genes together with endogenous genes in the anthocyanin biosynthesis pathway. Delphinidin is not known to be a toxic compound.

Furthermore no evidence for toxicity of the products of the three newly inserted genes (Petunia *dfr* gene ; Petunia *f3'5'h* gene and *SuRB* (*als*) gene) was reported based on a 14-day acute toxicity study, an Ames test and a cytotoxicity study on human embryonic intestinal cells *in vitro*. From BLAST searches using the GenBank and SwissProt databases, the GMO Panel concludes that no relevant homologies exist between the newly expressed proteins in carnation Moonlite 123.2.38 and known toxins or allergens.

The possibility of accidental consumption of carnation Moonlite 123.2.38 petals cannot be ruled out. However the amount of delphinidin consumed will be negligible in comparison with the amount of delphinidin present in fruits containing high levels of delphinidin such as blackcurrant or bilberry.

Considering the intended use of carnation Moonlite 123.2.38, the GMO Panel concludes that this carnation is unlikely to have adverse effects on human or animal health.

## 5. Environmental risk assessment and monitoring plan

### 5.1 Issues raised by the Member States

Questions were raised regarding (1) the possibility of gene transfer to wild carnations, (2) the need to consider more clearly the presence of cyanidin in the environmental risk assessment, (3) the need for a case specific monitoring plan focusing on hybridization of cut carnation flowers with wild *Dianthus* plants and (4) more details on general surveillance methods.

Question (1) regarding the possibility of gene transfer to wild carnations is considered under section 5.2.2 of the present opinion. Question (2) regarding the presence of cyanidin in the environmental risk assessment is considered under section 5.2.4 whereas questions (3) and (4) regarding the case specific monitoring plan and the general surveillance methods respectively, are considered under section 5.2.5.

### 5.2. Evaluation of relevant scientific data

The GMO Panel considered the information provided in the original notification, the Member State comments and further scientific literature in the assessment of the potential for environmental risks and the requirements of a monitoring plan. It was concluded that scientific information on potential environmental effects associated with the cultivation of carnation Moonlite 123.2.38 was not required. As the notification concerns import of cut flowers there will be a very limited environmental exposure with respect to viable plant parts of carnation Moonlite 123.2.38. The GMO Panel only considered this restricted exposure when evaluating the potential environmental impact of imported cut flowers and not issues associated with plant cultivation. In addition, the GMO Panel gave its opinion on the scientific quality of the environmental monitoring plan provided by the applicant, including the general surveillance (see section 5.2.4).

Carnations are double-flowered cultivars and in the general trade and botanical and horticultural literature carnation cultivars are considered to belong to the species *Dianthus caryophyllus*. The cultivated carnation is vegetatively propagated to produce plants for cut flower production. Cuttings are taken from vegetative 'mother plants' which are continually pruned to produce a high number of vegetative cuttings from axillary buds. These cuttings are rooted in conditions of high humidity, after treatment with rooting powder. Rooted plants may be planted in soil or grown hydroponically, and are kept for 1-2 years. Flowers are produced in flushes, beginning 3-5 months after rooted cuttings are planted. Picking of all flowers is essential and flowers are harvested in tight bud (or closed bud for spray types) for distribution and marketing.

The majority of *Dianthus* species are self-sterile because the stigma is not receptive to pollen until one week or more after anthers have shed them. The cultivated carnations require pollination by hand to set seed (Bird, 1994). As a result of the long history of use of vegetative propagation and selection for flower characteristics, the carnation does not produce much

pollen, and consequently seed set is low or absent (Galbally & Galbally, 1997). The quantity and quality of pollen varies according to the cultivar (Kho & Baer, 1973; Galbally & Galbally, 1997). Carnation pollen is heavy and sticky and has low viability. Wind plays little role in pollen dispersal (Office of the Gene Technology Regulator, 2005).

In the wild, cross-pollination of carnation relies on insect pollinators. There are no known reports of insect pollinators of *D. caryophyllus*, in particular. However, pollination is likely to be affected by lepidopteran pollinators. Lepidopteran species of the genera *Aphantopus, Aporia, Cyaniris, Hesperia, Macroglossum, Melanargia, Mesoacidalia, Ochlodes, Pieris, Plusia, Polyommatus, Sartyrus,* and *Thymelicus* are documented pollinators of other *Dianthus* species in the EU (Office of the Gene Technology Regulator, 2005; Bloch *et al*., 2006).

Members of the genus *Dianthus* are fairly diverse, as their origins range from southern Russia to Alpine Greece and the Auvergne mountains of France. The *Dianthus* species are adapted to the cooler Alpine regions of Europe and Asia, and are also found in Mediterranean coastal regions. *D. caryophyllus* is widely cultivated for ornament in Europe and occasionally naturalized, but apparently not known in the wild, except perhaps in some Mediterranean countries, indicating that the distribution of naturalized *D. caryophyllus* carnation is restricted to the Mediterranean regions of Greece, Italy, Sicily, and Sardinia (Tutin *et al.,* 1993).

### 5.2.1. Potential unintended effects on plant fitness due to the genetic modification

Carnation varieties in general compete poorly outside their cultivated environment. However, in the very unlikely event of accidental release into the environment, the fitness of the GM plants was considered.

The carnation Moonlite 123.2.38 has a modified flower colour achieved by introducing two genes of the anthocyanin biosynthesis pathway from petunia. These genes, encoding dihydroflavonol 4-reductase and flavonoid 3'5' hydroxylase, give rise to the anthocyanins delphinidin and cyanidin. These anthocyanins are widely found in flowers like *Petunia* (Ando *et al*., 1999), rose (Biolley and Jay, 1993), chrysanthemum (Schwinn *et al*., 1993; Andersen *et al*., 2000). There is no evidence that the presence of delphinidin and cyanidin would lead to effects on plant fitness.

Carnation Moonlite 123.2.38 contains a mutated *SuRB* (*als)* gene conferring tolerance to sulfonylurea herbicides. Given that the ALS enzyme is needed for the biosynthesis of some branched-chain amino acids like isoleucine, ALS-inhibiting herbicides cause the death of the plant by interfering with this biosynthesis pathway. Against this background Tranel & Wright (2002) reported that tolerance to ALS-inhibiting herbicides was widespread among weeds and mostly due to a mutated *SuRB* (*als)* gene. In addition the ALS-tolerant biotype was shown to be less sensitive to feedback inhibition by branched-chain amino acids. This results in greater accumulation of branched-chain amino acids in tolerant biotypes, which may allow seeds from tolerant biotypes to germinate more rapidly, especially under cool temperatures. This may indicate a possible change in behaviour of the tolerant plants in the absence of herbicide selection, in the very unlikely event of escape into the environment. Wild *Dianthus* populations exhibit a diversity of phenotypes occupying niches in a wide geographical range in Europe (Tutin *et al.*, 1993). The GMO Panel considered that a small change in seed germination characteristics induced by ALS tolerance is unlikely to be outside the current range of seed germination characteristics currently expressed by non GM carnations and thus is unlikely to have an ecological impact. In addition, because of the intended use of carnation Moonlite 123.2.38 and therefore of the very low exposure of recipient populations, the GMO Panel considers this to be of no ecological significance. The carnation Moonlite 123.2.38 plant would not show changed fitness characteristics except in the presence of sulfonylurea herbicides and this herbicide is not used in habitats where wild carnation might occur.

In the very unlikely event of gene transfer to cultivated carnations, they may express the mutated *SuRB* (*als)* gene conferring tolerance to sulfonylurea herbicides. This could result in a possible fitness advantage and higher weediness of the tolerant plants in the presence of these herbicides and those with a similar mode of action. Such herbicide tolerant plants can be managed by a range of measures (Tranel & Wright, 2002).

The GMO Panel is of the opinion that the carnation Moonlite 123.2.38 is unlikely to have adverse effects on the environment in comparison with non GM carnations.

### 5.2.2. Potential for gene transfer

#### (a) Plant to bacteria gene transfer

The carnation Moonlite 123.2.38 contains a mutated acetolactate synthase (*SuRB/als*) gene conferring tolerance to sulfonylurea herbicides as well as a *dfr* gene, coding for dihydroflavonol 4-reductase (DFR), and the petunia *f3'5'h* gene, coding for flavonoid 3' 5' hydroxylase (F3'5'H) (see section 2.2.1 for further details on the molecular characterisation). Delphinidin is produced as a result of the combined expression of the introduced genes *dfr* and *f3'5'h* together with endogenous genes in the anthocyanin biosynthesis pathway. These genes are already present in other plant communities and thus in soil decomposition processes. Plant to bacteria gene transfer of the genes was not considered to pose an environmental risk by the Member States or the GMO Panel. In the very unlikely event that a plant to bacteria gene transfer would take place, no adverse effects on human and animal health or the environment are expected as no new genes from decomposing plants would be introduced into microbial communities.

#### (b) Plant to plant gene transfer

The reproductive biology of *Dianthus* (Office of the Gene Technology Regulator, 2005), including the low production and low viability of the pollen, and the limited information provided by the applicant suggesting that the proportion of flowers carrying pollen is low if at all, indicate that pollen transfer is very unlikely to occur. In addition, viable seed set on cut flowers is very unlikely given the limited life time in comparison to the time needed for complete seed development.

The GMO Panel considered the possibility of natural exchange of genetic material with other carnation varieties, *Dianthus caryophyllus* L., and some wild *Dianthus* species. Although hybridisation is mentioned in some floristic surveys, the GMO Panel is not aware of reports of gene flow between wild *Dianthus* spp. and cultivated carnations in the literature. The probability of spontaneous hybridisation between GM carnation and other cultivated carnations and establishment of a viable plant is considered to be very low. Therefore, the GMO Panel concludes that plant to plant gene transfer of the introduced genes is unlikely to be of environmental concern.

### 5.2.3. Potential interactions of the GM plant with non-target organisms

There are several herbivorous pests of the carnation and they could be affected by a change in delphinidin/cyanidin ratio. However, the scope of this notification does not include cultivation and therefore the exposure of herbivores to this GM carnation will be extremely limited and the exposure to detritivores would be localised (e.g. in waste processing). Thus the GMO Panel considered that carnation Moonlite 123.2.38 is unlikely to have adverse effects on non-target organisms in the context of the intended use.

### 5.2.4. Monitoring

The GMO Panel is of the opinion that the structure of the environmental monitoring plan provided by the applicant complies with the requirements defined in Directive 2001/18/EC, in Council Decision establishing guidance notes supplementing Annex VII (EC, 2002b) and in the Guidance document provided by EFSA (EFSA, 2004). The monitoring plan describes objectives, responsibilities and tasks, flow of information and monitoring methods. The GMO Panel gives its opinion on the scientific quality of the environmental monitoring plan provided by the applicant, including the general surveillance.

The GMO Panel agrees with the applicant that the environmental risk assessment did not identify risks that require case-specific monitoring.

The GMO Panel considered the general surveillance methods as provided in the notification (a.o. questionnaire to European importers). It was also noted that the applicant requested taxonomists and botanists to inform them of hybrids that might originate from their GM carnation. The GMO Panel additionally suggests that national botanic survey networks and plant protection services should also be considered.

In the light of the very low environmental exposure of viable forms of carnation line 123.2.38 due to the restricted intended use of the GM carnation, the GMO Panel concludes that the proposal of the applicant for general surveillance is in line with the EFSA Guidance on post-market environmental monitoring (EFSA, 2006). The GMO Panel recommends the adoption of the proposals for annual reporting made in the EFSA guidance document (EFSA, 2006).

### 5.3. Conclusion

The GMO Panel based its environmental risk assessment on cut flowers of carnation Moonlite 123.2.38 to be imported for ornamental use only. From the information supplied by the applicant, and from studies of relevant literature, there is no indication that this GM carnation will have adverse effects on the environment in the EU.

The carnation Moonlite 123.2.38 was assessed for imported cut flowers for ornamental use only. Scientific information on potential environmental effects associated with the cultivation of carnation Moonlite 123.2.38 was therefore not required. Carnation Moonlite 123.2.38 cut stems and flowers have very restricted viability, very low pollen emission and little or no viable seed. However, in the very unlikely event of accidental release into the environment, the GMO Panel considers that the carnation Moonlite 123.2.38 would not show enhanced fitness characteristics, except in the presence of sulfonylurea herbicides. The consequences of the potential transfer of the three genes would be negligible in terms of adverse effects on the environment. Exposure of non-target organisms to GM carnation would be very low and the GMO panel concludes that there is no indication that GM carnation Moonlite 123.2.38 will have adverse effects on the environment in the context of the intended use.

The GMO Panel agrees with the applicant that the environmental risk assessment indicates that there is no need for a case-specific monitoring plan. The GMO Panel also agrees with the general methods and approaches of the general surveillance plan.

## CONCLUSIONS

The GMO Panel was asked to consider whether there is any scientific reason to believe that the placing on the market of the GM carnation Moonlite 123.2.38 for import is likely to cause any

adverse effects on human health and the environment within the scope of Directive 2001/18/EC.

The carnation Moonlite 123.2.38 has a modified flower colour, a shade of violet, which is achieved by introducing into white carnation two genes of the anthocyanin biosynthesis pathway from petunia. Carnation Moonlite 123.2.38 also expresses sulfonylurea herbicide tolerance.

The GMO Panel has evaluated the molecular analysis of the genetically modified variety. The carnation Moonlite 123.2.38 does not contain a functional antibiotic resistance marker gene. From the bioinformatic analysis, there is no reason to assume that the DNA regions transferred code for toxic and/or allergenic products.

Given the intended use of carnation Moonlite 123.2.38 (excluding human or animal consumption and cultivation), the GMO Panel considers that the comparative analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment. Furthermore, based on the results of toxicity and allergenicity studies, there is no evidence that any of the three proteins expressed is toxic or allergenic. The GMO Panel concludes that carnation Moonlite 123.2.38 is unlikely to have adverse effects on human or animal health in the unlikely event that carnation Moonlite 123.2.38 petals are consumed.

Considering the low environmental exposure due to the restricted scope of the notification, this is very unlikely that gene transfer and escape into the environment would occur and, if any, the consequences of the three genes would be negligible for the environment in line with the intended use of Moonlite 123.2.38 cut flowers. The GMO Panel agrees with the general methods and approaches of the general surveillance plan provided in the notification.

## DOCUMENTATION PROVIDED TO EFSA

1. Note to Mr. Koëter and the annexes, dated 2 December 2005 with ref. DG ENV/B.4/KT D(05)25125, from Mr. Ladislav Miko – Notification C/NL/04/02 (Carnation Moonlite 123.2.38), under Directive 2001/18/EC - request for EFSA opinion.

2. Submission from Florigene (4 January 2006) to EFSA regarding the notification for the placing on the market of carnation Moonlite 123.2.38 in accordance with Directive 2001/18/EC: Ref C/NL/04/02, and the related annexes.

3. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/SM/jq (2006) 1412310, 8 March 2006).

4. Additional information submitted by Florigene on 31 March 2006 in response to EFSA request for further information.

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## ACKNOWLEDGEMENT

The GMO Panel wishes to thank Philippe Vain, Esther Kok and Henri Darmency for their contributions to the draft opinion.