Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

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# Final health and environmental risk assessment of genetically modified carnation Moonaqua 123.8.12

**Scientific opinion on genetically modified carnation Moonaqua 123.8.12 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/06/01)**

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

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# Final health and environmental risk assessment of genetically modified carnation Moonaqua 123.8.12. Scientific opinion on genetically modified carnation Moonaqua 123.8.12 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/06/01)

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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 123.8.12 with product name Moonaqua™, expresses three introduced traits. The *dfr* gene from *Petunia x hybrida* and the *f3′5′h* (*Hf1*) gene from *Viola* sp., 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 mauve colour to the flowers. A mutated *als* gene (*SuRB*) 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 123.8.12 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 123.8.12. Data reported from several field trials show that carnation 123.8.12 petals contain higher levels of the anthocyanins delphinidin and cyanidin compared to the non-GM (conventional) carnation counterpart FE123. Other morphological traits were reported and along with differing petal colour, carnation Moonaqua 123.8.12 differed significantly in one trait compared to conventional carnation counterpart FE123. An acute toxicity study in mice and an *in vitro* mutagenicity study 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 123.8.12 represents an environmental risk in Norway.

Considering that carnation Moonaqua 123.8.12 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 Moonaqua 123.8.12 and the conventional carnation counterpart FE123 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 Moonaqua 123.8.12.

Based on current knowledge and 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 Moonaqua 123.8.12 is as safe as its conventional counterpart FE123.

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 Moonaqua 123.8.12 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.) Moonaqua 123.8.12 (Unique Identifier FLO-40689-6) with modified flower colour is approved under Directive 2001/18/EC for import of cut flowers for ornamental use since 16 March 2009 (Application C/NL/06/01, Commission Decision 2009/244/EC). The scope of the application is restricted to flowers produced by vegetative propagation, and do not cover progeny derived from sexual crosses with Moonaqua 123.8.12 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”.

Moonaqua 123.8.12 has previously been assessed for import as cut ornamental flowers by EFSA (EFSA, 2008) upon application C/NL/06/01, but not by the VKM GMO Panel.

The current safety and environmental risk assessment of the carnation Moonaqua 123.8.12 is based on information provided by the applicant in the application C/NL/06/01, relevant peer-reviewed scientific literature, and scientific opinion from EFSA (EFSA, 2008). Except for a synopsis of more recent literature, this draft opinion is to a large extent a summary of the above-mentioned EFSA report, which is provided in Appendix I, and readers are referred to this for details.

The VKM GMO Panel has evaluated carnation Moonaqua 123.8.12 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 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 expresses three introduced traits: *dfr* gene from *Petunia x hybrida* coding for dihydroflavonol 4-reductase (DFR), *f3′5′h* gene from *Viola* sp. coding for flavonoid 3′,5′-hydroxylase (F3′5′H), both of which confer the mauve 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 Moonaqua 123.8.12 contains three transgenic loci. Locus 1 contains the full length transfer-DNA-sequence (T-DNA) which is comprised by single copies of each of the three genes *dfr, f3′5′h and als*, as well as other sequences necessary for their proper expression. The two other loci only contain incomplete copies of the *f3′5′h* gene and some adjacent sequences. Southern blot analyses indicate no integration of plasmid backbone sequences in carnation Moonaqua 123.8.12. No new unintended open reading frames (ORFs) were generated during the transformation process. Analyses performed by the applicant with bioinformatics tools, including general BLAST searches, did not return relevant sequence homologies between the transgene inserts in the carnation and known toxins and allergens. Northern blot analyses were used to confirm expression of the 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 a bulked petal sample were reported as 0.07 and 0.02 mg/g fresh weight, respectively. No relevant instability in the introduced trait, *i.e.* the particular flower colour, has been reported during the commercial cultivation of the carnation Moonaqua 123.8.12.

Based on current knowledge and the information provided by the applicant, the VKM GMO panel concludes that the molecular characterisation of carnation Moonaqua 123.8.12 does not indicate a safety concern.

### Comparative assessment

Considering the intended use of carnation Moonaqua 123.8.12, which excludes cultivation and use in 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 FE123, carnation Moonaqua 123.8.12 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 Moonaqua 123.8.12 differed significantly in six traits compared to carnation FE123. 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 Moonaqua 123.8.12, 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 Moonaqua 123.8.12 and the conventional carnation counterpart FE123 do not raise safety concerns.

### Food and feed risk assessment

A 14 day acute toxicity study with ICR mice and an *in vitro* test for mutagenicity (Ames test), both employing aqueous extracts from leaves or petals, have been performed by the applicant with carnation Moonaqua 123.8.12. Neither 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 Moonaqua 123.8.12 is as safe as its conventional counterpart, carnation FE123. 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 Moonaqua 123.8.

### Environmental assessment

Considering the intended use of Moonaqua 123.8.12, 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, Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12. 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 and its conventional carnation counterpart FE123 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12.

Likewise, the VKM GMO Panel concludes that carnation Moonaqua 123.8.12, 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.), Moonaqua, 123.8.12, 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.) Moonaqua 123.8.12 (unik kode FLO-40689-6) fra Florigene Ltd. ble godkjent til import og salg som avskårne prydblomster under EUs utsettingsdirektiv 2001/18 den 16. mars 2009 (jfr. Kommisjonsbeslutning 2009/244/EC). Søknad C/NL/06/01 omfatter nellikplanter som er produsert ved vegetativ formering, og omfatter ikke avledete sorter fra konvensjonelle kryssinger med Moonaqua 123.8.12. 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).

VKM har ikke tidligere uttalt seg om genmodifisert nelliklinje 123.8.12.

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

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 EUs 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, målorganismer, 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 Moonaqua 123.8.12 uttrykker tre nye egenskaper: *dfr* -genet fra *Petunia x hybrida* som koder for dihydroksyflavonol-reduktase (DFR) og *f3′5′h* -genet fra *Viola* sp. som koder for flavonol 3′,5′- hydroksylase (F3′5′H). Disse genene fører til endringen i produksjonen av antocyanin pigmenter i kronbladene, med fargeendring i blomsten som resultat. I tillegg, inneholder Moonaqua 123.8.12 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 nelliken Moonaqua 123.2.12 inneholder tre transgene loci. Locus 1 inneholder en fullstendig kopi av det transgene innskudds-DNAet (T-DNA), som består av én kopi for hver av de tre genene *dfr*, *f3'5'h* og *als*, og deres regulerende sekvenser. De to andre lociene inneholder kun ufullstendige kopier av *f3'5'h* genet, og enkelte andre tilstøtende sekvenser fra T-DNAet. Analyser med Southern blot indikerer at sekvenser som faller utenfor området til T-DNAet i plasmidet, ikke har blitt overført. Det har ikke blitt påvist utilsiktede nye åpne leserammer (ORFs) i nelliken som følge av genmodifiseringen. Søk etter homologier (BLAST-søk) mellom de nye innsatte gensekvensene og kjente toksiner og allergener, viste ingen relevante treff i aktuelle databaser. Northern blot ble brukt til å påvise faktisk uttrykk av de tre genene *dfr*, *f3'5'h* og *als*, mens væskekromatografi (HPLC) ble brukt til kvantifisering av nye metabolitter. I partier av kronblader ble nivået av plantepigmentene delphinidin og cyanidin målt til henholdsvis 0,07 og 0,02 mg/g ferskvekt. Ved kommersiell dyrking har det så langt ikke blitt rapportert om relevante avvik/ustabilitet ved de introduserte egenskapene, dvs. blomsterfargen til nellik Moonaqua 123.8.12.

Basert på dagens kunnskap og informasjonen fra søker, konkluderer VKMs faggruppe for GMO, at den molekylære karakteriseringen ikke tilsier noen økt risiko ved nellik Moonaqua 123.2.12 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 Moonaqua 123.8.12 rapportert av søker. Sammenlignet med den konvensjonelle motpart nellik FE123 inneholder kronbladene

fra nellik Moonaqua 123.8.12 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 seks egenskaper. Ingen av de rapporterte forskjellene i sammensetning eller morfologiske egenskaper er forventet å ha innvirkning på risikoscenarier ved utilsiktet miljøeksponering eller inntak av nellik Moonaqua 123.8.12.

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 Moonaqua 123.8.12. De observerte morfologiske forskjellene mellom Moonaqua 123.8.12 og dens konvensjonelle motpart nellik FE123 medfører ikke en økt sikkerhetsrisiko.

### Helserisiko

En 14 dagers akutt toksisitetsstudie med ICR mus og en *in vitro* mutagenisitetstest (Ames test), begge med ekstrakter fra frosne kronblad, har blitt utført av søker med Moonaqua 123.8.12. 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 Moonaqua 123.8.12 er like trygg som dens konvensjonelle motpart, nellik FE123. 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 Moonaqua 123.8.12.

### Miljørisiko

Miljørisikovurderingen av nelliklinjen Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 er tilstrekkelig for risikovurderingen. De rapporterte morfologiske forskjellene mellom Moonaqua 123.8.12 og dens konvensjonelle motpart nellik FE123 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 VKMs faggruppe for GMO at Moonaqua 123.8.12 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 Moonaqua 123.8.12.

Likeledes finner faggruppen, ut i fra dagens kunnskap, at den omsøkte bruken av Moonaqua 123.8.12 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 October 2006, an application (Reference C/NL/06/01) covering import of cut flowers of the genetically modified carnation Moonaqua 123.8.12 (Unique Identifier FLO-40689-6) for ornamental use was submitted by Florigene Ltd. to the competent authority of the Netherlands. The scope of the n was restricted to flowers produced by vegetative propagation, and did not cover progeny derived from sexual crosses with Moonaqua 123.8.12 cultivar.

On 1 March 2007, 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. Objections were raised by some Member States and according to EU legislation (Article 18[1] of Directive 2001/18/EC) EFSA's GMO Panel was therefore required to carry out a further assessment and provide an opinion.

The EFSA GMO Panel published its scientific opinion on application C/NL/06/01 (EFSA 2008) on 12 March 2008, and carnation Moonaqua 123.8.12 was approved for import and ornamental use 16 March 2009 (Commission Decision 2009/244/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 Moonaqua 123.8.12 has not been previously assessed by the VKM GMO Panel.

# 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 Moonaqua 123.8.12 for import of cut flowers for ornamental use (Application C/NL/06/01).

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 Moonaqua 123.8.12 for import of cut flowers for ornamental use (Application C/NL/06/01).

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 Moonaqua 123.8.12 (Unique Identifier FLO-40689-6) from Florigene Ltd. is a genetically modified (GM) 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 opinion from EFSA (EFSA, 2008), and relevant peer-reviewed scientific literature. The VKM GMO Panel has not previously published a risk assessment of carnation Moonaqua 123.8.12. The above-mentioned EFSA report is provided in Appendix I and readers are referred to this 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 Moonaqua 123.8.12 was developed for petal colour for decorative purposes. The expression of the newly introduced genes, *dfr* from petunia and *f3′5′h* from *Viola* sp. coding for dihydroflavonol 4-reductase (DFR) and flavonoid 3′,5′-hydroxylase (F3′5′H) respectively, confers the light mauve 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 Moonaqua 123.8.12 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 (review by Tranel and Wright, 2002). However, the introduction of the mutated *als* (*SuRB*) gene in carnation Moonaqua 123.8.12 with resulting tolerance to sulfonylurea herbicides was not primarily intended for plant protection purposes, but rather used as a marker trait for the selection of successfully transformed plants.

Carnation Moonaqua 123.8.12 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

Previously, the EFSA GMO Panel (EFSA, 2008 in Appendix I) assessed the molecular characterisation of the event FLO-40689-6 (Moonaqua 123.8.12; *dfr* and *f3'5'h* [from the *hf1* locus], and *SuRB* [*als*] inserts) with regards to the following:

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

The Panel concluded that the applicant had provided sufficient analyses to characterise the DNA insert, number of inserts, integration sites and flanking sequences in the carnation Moonaqua 123.8.12 genome.

Moonaqua 123.8.12 was developed by *Agrobacterium*-mediated transformation using disarmed *Agrobacterium tumefaciens* strain AGL0 carrying the transformation vector pCGP1991. *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 outside the T-DNA.

The vector pCGP1991 contained three expression cassettes between the left (LB) and right (RB) borders: 1) the promoter from a snapdragon (*Antirrhinum majus*) gene encoding chalcone synthase, cDNA encoding flavonoid 3'5' hydroxylase (F3'5'H) from *Viola* sp., the D8 terminator from the petunia gene encoding a phospholipid transfer protein homologue; 2) the entire petunia gene that encodes dihydroflavonol-4-reductase (DFR), including its promoter and terminator; and 3) a chimeric gene consisting of the cauliflower mosaic virus 35S promoter, 5' untranslated region (*ca*. 60 bp) from the cDNA corresponding to the petunia gene encoding chlorophyll a/b binding protein, and the *als* gene encoding the acetolactate synthase (ALS) variant protein derived from *Nicotiana tabacum* ,including its terminator.

Carnation Moonaqua 123.8.12 contains three transgenic loci. Analyses of sequences for all three inserts, including their flanking regions, have been provided by the applicant. Locus 1 contains an intact construct between the LB and RB. Locus 2 contains a fragment starting from the RB and extending to the D8 terminator, which is linked to another fragment containing an almost complete *f3'5'h* expression cassette (missing ca. 40 bp from the promoter). Locus 3 contains an incomplete *f3'5'h* expression cassette. Southern blot analysis of *Eco*R1-digested genomic DNA with seven probes covering the whole plasmid backbone outside the LB and RB indicated that none of these sequences had been integrated into carnation Moonaqua 123.8.12.

Analyses of the flanking regions at the three loci indicate that no new ORFs were generated during the transformation process at any of the six junctions between integrated and genomic DNA. On request from the EFSA GMO Panel the applicant performed sequence homology searches to known toxin or allergen coding genes with an 80-amino-acid long sliding window, looking for a minimum of 35% contiguous identical amino acids. No matches were found. The applicant also performed a similarity search for short identical stretches of six contiguous amino acids, which returned several positive matches for each inserted transgene. In their assessment the EFSA GMO Panel notes however, that the 6-amino-acid threshold is likely to give rise to many false positives.

Northern analysis was carried out to measure the level of expression of the three introduced genes. Confirmation of the expression of functional enzymes was obtained from metabolite analysis with liquid chromatography (HPLC analysis). A strong hybridisation signal indicated that the introduced *als* mRNA was present in petal tissue. The level of *dfr* transcript may vary dependent on which flower stage is selected for analysis. The weak *dfr* signal observed in Moonaqua flowers was consistent with the relatively low measured levels of delphinidin, and pale flowers observed. The levels of delphinidin and cyanidin in a single assay of bulked petal samples were 0.07 and 0.02 mg/g fresh weight, respectively. It was estimated that the concentration of delphinidin in the genetically modified carnation flowers was approximately one-fiftieth of that in blueberry. The *f3'5'h* mRNA transcript was also detected in Moonaqua flowers. The *f3'5'h* gene is under the control of an *Antirrhinum* CHS promoter which typically directs expression through most stages of flower development. Controls showed no detectable transcript for the probes used.

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 Moonaqua 123.8.12, which includes the production of millions of flowers. In 2003, two off-types with white streaks were found among 1000 flowers assessed. No off-types were found during flower assessment in 2005 and 2006.

## 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 Moonaqua 123.8.12 does not indicate a safety concern.

# 3 Comparative assessments

Previously, EFSA (EFSA, 2008 in Appendix I) 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 (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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 and its non-GM parental cultivar (conventional comparator; control) FE123. Carnation FE123 does not produce anthocyanins and therefore has white petals. Other plant tissues were not analysed.

For assessment of morphological traits, carnation Moonaqua 123.8.12 and its non-GM conventional comparator (control) cultivar FE123 were grown in field trials in The Netherlands in 2000 and in Australia in 2005.

## 3.2 Compositional analysis

HPLC data (Technical dossier; Fukui et al., 2003) indicated that petals of carnation Moonaqua 123.8.12 and parental cultivar FE123 did not contain detectable levels of petunidin. Delphinidin and cyanidin were detected in Moonaqua 123.8.12 petals at levels of 0.07 and 0.02 mg/g fresh weight, respectively, but were not detected in cultivar FE123.

EFSA (EFSA, 2008) considered that since the intended uses of carnation Moonaqua 123.8.12 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, carnation Moonaqua 123.8.12 has been evaluated in field trials in the Netherlands in 2000 and in Australia in 2005. In total, 13 morphological characteristics most relevant to potential gene dispersal were analysed in carnation Moonaqua 123.8.12 and its conventional comparator cultivar FE123, 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 significant differences in several of these characteristics. Carnation Moonaqua 123.8.12 had smaller flowers, reduced stem thickness at the $5^{th}$ node, and reduced numbers of stamens, styles and anthers, as well as shorter stamen length. The applicant attributed these differences to somaclonal variation and/or environmental effects.

EFSA (EFSA, 2008) concluded that the data from the field trials confirmed the introduced trait, but also revealed numerous other morphological differences between carnation Moonaqua 123.8.12 and its parental cultivar FE123. However, the differences were not considered relevant for the safety assessment of carnation Moonaqua 123.8.12. The 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 Moonaqua 123.8.12, 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 Moonaqua 123.8.12 and the conventional carnation counterpart FE123 do not raise safety concerns.

# 4 Food and feed safety assessment

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

Carnation Moonaqua 123.8.12 has been evaluated (EFSA, 2008 in Appendix I). EFSA identified no adverse effects for use of GM carnations in relation to non-GM cultivars. However, a need for further assessment of the allergenic potential was identified by a Member State of EFSA.

## 4.2 Product description and intended uses

The EU Commission Decision 2009/244/EC stipulates that a condition for placing carnation Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12.

The scope of the application C/NL/06/01 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 Moonaqua 123.8.12 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 rather higher levels than in the petals of carnations; Moonaqua 123.8.12 (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 reason for concern or adverse effects (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 Moonaqua 123.8.12 and non-GM parental cultivar FE123 as control. No mutagenic activity was observed.

#### *4.3.2.2 Acute toxicity study*

To evaluate the impact of accidental exposure to carnation Moonaqua 123.8.12 on human or animal health, a 14-day acute toxicity study was conducted by the applicant. ICR male mice were administered a single oral dose of aqueous extract of Moonaqua 123.8.12 at 4 g/kg bw. The extract from carnation Moonaqua 123.8.12 contains delphinidin and cyanidin since anthocyanins are water soluble. Control groups received either aqueous extracts from leaves or petals of the parental cultivar FE123 or water. Body weights were measured and clinical observations were conducted regularly after the administration. Animals were autopsied at the end of the experiment and observed macroscopically. No treatment-related changes, adverse effects or deaths were observed.

### 4.3.3 Toxicological assessment of the whole GM plant

Taking into account that carnations Moonaqua 123.8.12 is not intended for human or animal consumption as food or feed but are 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. 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 was identified via bioinformatics analyses of the amino acid sequence of the newly expressed proteins in carnation Moonaqua 123.8.12 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 eight contiguous identical amino acid sequences between these 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, F3'5'H proteins in other GM carnations have not identified reason for concern (EFSA, 2006b; EFSA, 2008; EFSA, 2014a; EFSA, 2014b; VKM, 2008).

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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 (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 Moonaqua 123.8.12 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 Moonaqua 123.8.12, 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 Moonaqua 123.8.12, a cyanidin concentration of 0.02 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12, a delphinidin concentration of 0.07 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 Moonaqua 123.8.12 petals is not considered to pose a health risk compared to the levels present in berries and estimated ADI.

## 4.5 Conclusion

A 14 day acute toxicity study with ICR mice and an *in vitro* test for mutagenicity (Ames test), both employing aqueous extracts from leaves or petals, have been performed by the applicant with carnation Moonaqua 123.8.12. Neither 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 Moonaqua 123.8.12 is as safe as its conventional counterpart, carnation FE123. 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 Moonaqua 123.8.

# 5 Environmental risk assessment

## 5.1 Introduction

This assessment applies to carnation Moonaqua line 123.8.12 from Florigene Ltd, which has been transformed to modify the flower colour and possesses a mutated herbicide resistance gene *als* (*SuRB*) 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 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. has simple, bisexual open flowers with five petals. Many of the carnation species are self-sterile. 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).

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). Pollen develops before the pistils are receptive for pollination. 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 tissue various 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 D. 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 to 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 Moonaqua 123.8.12 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 Moonaqua line 123.8.12 will have any adverse effect on abiotic environment or biochemical cycles.

## 5.7 Conclusion

Considering the intended use of Moonaqua 123.8.12, 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, Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12. No specific environmental impact of genetically modified carnation Moonaqua 123.8.12 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 Moonaqua 123.8.12. 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 Moonaqua 123.8.12 contains three transgenic loci. Locus 1 contains the full length transfer-DNA-sequence (T-DNA) which is comprised by single copies of each of the three genes *dfr, f3'5'h and als*, as well as other sequences necessary for their proper expression. The two other loci only contain incomplete copies of the *f3'5'h* gene and some adjacent sequences. Southern blot analyses indicate no integration of plasmid backbone sequences in carnation Moonaqua 123.8.12. No new unintended open reading frames (ORFs) were generated during the transformation process. Analyses performed by the applicant with bioinformatics tools, including general BLAST searches, did not return relevant sequence homologies between the transgene inserts in the carnation and known toxins and allergens. Northern blot analyses were used to confirm expression of the 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 a bulked petal sample were reported as 0.07 and 0.02 mg/g fresh weight, respectively. No relevant instability in the introduced trait, *i.e.* the particular flower colour, has been reported during the commercial cultivation of the carnation Moonaqua 123.8.12.

Based on current knowledge and the information provided by the applicant, the VKM GMO panel concludes that the molecular characterisation of carnation Moonaqua 123.8.12 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 Moonaqua 123.8.12, which excludes cultivation and use in 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 FE123, carnation Moonaqua 123.8.12 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 Moonaqua 123.8.12 differed significantly in six traits compared to carnation FE123. 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 Moonaqua 123.8.12, 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 Moonaqua 123.8.12 and the conventional carnation counterpart FE123 do not raise safety concerns.

### Food and feed risk assessment

A 14 day acute toxicity study with ICR mice and an *in vitro* test for mutagenicity (Ames test), both employing aqueous extracts from leaves or petals, have been performed by the applicant with carnation Moonaqua 123.8.12. Neither 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 Moonaqua 123.8.12 is as safe as its conventional counterpart, carnation FE123. 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 Moonaqua 123.8.

### Environmental assessment

Considering the intended use of Moonaqua 123.8.12, 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, Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12. 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 and its conventional carnation counterpart FE123 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12.

Likewise, the VKM GMO Panel concludes that carnation Moonaqua 123.8.12, 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 (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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 are merited.

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# Appendix I

European Food Safety Authority

*The EFSA Journal* (2008) 662, 1-25

# Notification (Reference C/NL/06/01) for the placing on the market of the genetically modified carnation Moonaqua 123.8.12 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Florigene[1]

## Scientific Opinion of the Panel on Genetically Modified Organisms

**(Question No EFSA-Q-2007-177)**

## Adopted on 12 March 2008

**PANEL MEMBERS**

Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Lieve Herman, Marc De Loose, Niels Hendriksen, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Ilona Kryspin-Sørensen, Harry Kuiper, Ingolf Nes, Nickolas Panopoulos, Joe Perry, Annette Pöting, Joachim Schiemann, Willem Seinen, Jeremy Sweet and Jean-Michel Wal.

**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 Moonaqua 123.8.12, genetically modified (GM) for flower colour (Unique Identifier FLO-40689-6). The GM carnation also contains a gene conferring tolerance to sulfonylurea herbicides. Cut flowers of carnation Moonaqua 123.8.12 are intended to be imported in the European Union for ornamental use only.

The present opinion is based on a question raised by the European Commission related to a notification to place the GM carnation Moonaqua 123.8.12 on the market under Directive 2001/18/EC (Notification reference C/NL/06/01). 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

[1] For citation purposes: Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/NL/06/01) for the placing on the market of the genetically modified carnation Moonaqua 123.8.12 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* (2008) 662, 1-25

Moonaqua 123.8.12 was requested by the European Commission because of outstanding objections raised by some Member States following the evaluation 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 Moonaqua 123.8.12 for import only is likely to cause any adverse effects on human health and the environment.

In delivering its opinion, the GMO Panel considered the full notification, additional information provided by the notifier and the specific outstanding objections raised by the Member States. The carnation Moonaqua 123.8.12 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 compounds intended to be produced by the GM carnation. Furthermore, the potential environmental impact of carnation Moonaqua 123.8.12, including a monitoring plan, was assessed in the context of the restricted intended use of carnation Moonaqua 123.8.12.

Carnation Moonaqua 123.8.12 has a modified flower colour, a shade of light mauve, whereas the non-GM parent has cream-white flowers. The colour has been achieved by introducing into white carnation two genes of the anthocyanin biosynthesis pathway from *Petunia* and *Viola* sp. These genes, encoding dihydroflavonol 4-reductase (*dfr*) and flavonoid 3'5' hydroxylase (*f3'5'h*), together with other genes of the anthocyanin biosynthesis pathway already present in the non GM 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 Moonaqua 123.8.12 is also tolerant to sulfonylurea herbicides conferred by a mutated *SuRB (als)* gene used as marker gene for the selection of genetically modified plants but not for plant protection purposes. Other Florigene GM carnation varieties Moondust™, Moonshadow™ and Moonlite™ 123.2.38, which have also been genetically modified to express a specific blue-violet colour, were authorised to be placed on the market within the EU in 1997, 1998 and 2007, respectively.

The molecular analysis of the DNA inserts confirms that the three genes expressing the intended traits (light mauve flower colour encoded by *dfr* and *f3'5'h* genes and herbicide tolerance encoded by the mutated *SuRB (als)* gene) are present in carnation Moonaqua 123.8.12. Results of bioinformatic analyses of the three newly expressed proteins in carnation Moonaqua 123.8.12 did not indicate relevant homologies with known toxins or allergens. No new open reading frames were created in the flanking regions between the inserts and the carnation genome.

Given the intended use of carnation Moonaqua 123.8.12 (excluding human or animal consumption and cultivation), the GMO Panel considers that a compositional analysis limited

to the newly synthesised anthocyanins is sufficient for the risk assessment of the intended modification. The GMO Panel concludes that there is no indication of increased toxicity of the carnation Moonaqua 123.8.12 compared to the recipient variety.

The carnation Moonaqua 123.8.12 was assessed for imported cut flowers for ornamental use only. Scientific information on potential environmental effects associated with the cultivation of carnation Moonaqua 123.8.12 was therefore not required. Carnation Moonaqua 123.8.12 cut stems and flowers have marginal viability, negligible pollen production and little or no viable seed. However, in the very unlikely event of escape into the environment via seeds or rooted plants, the GMO Panel considers that the carnation Moonaqua 123.8.12 would not show enhanced fitness characteristics, except in the presence of sulfonylurea herbicides. The consequences of the potential transfer of the three genes into bacteria or plants would be negligible in terms of adverse effects on the environment. The GMO Panel concludes that there is no indication that GM carnation Moonaqua 123.8.12 will have adverse effects on the environment in the context of the intended use.

The GMO Panel is of the opinion 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 provided in the notification.

In conclusion, the GMO Panel considers that the information available for carnation Moonaqua 123.8.12 addresses the outstanding objections raised by the Member States and considers that, in the context of its intended use, carnation Moonaqua 123.8.12 is unlikely to have adverse effects on human and animal health or the environment.

**Key words:** acetolactate synthase (SuRB/ALS), anthocyanin, carnation, C/NL/06/01, cyanidin, 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-40689-6.

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## BACKGROUND

The Dutch Competent Authority forwarded the notification (Reference C/NL/06/01) to the European Commission on 1st of March 2007, together with a positive assessment report.

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 notifier, Florigene, provided the Member States with additional information in response to the objections raised during the 60-day period. The Member States had until 21 September 2007 to confirm or lift their objections. Where these objections are maintained, the Commission is required under Article 28 of Directive 2001/18/EC to consult the relevant Scientific Committee(s) for opinion, now 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 6 November 2007, after receipt of the complete background information (request from the Commission, full notification and final objections maintained by the Member States). During the 90-day period, EFSA requested further clarifications from the notifier. Therefore the deadline set for the delivery of this opinion was extended.

In delivering its opinion the GMO Panel considered the original notification, additional information provided by the notifier and the specific objections raised by three Member States.

The scope of notification C/NL/06/01 is restricted to the import of cut flowers of carnation Moonaqua 123.8.12 for ornamental use only. The progeny derived from sexual crosses with Moonaqua 123.8.12 variety are not covered under notification C/NL/06/01.

## 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 Moonaqua 123.8.12 for import is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC.

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 political 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.

## ACKNOWLEDGEMENTS

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**ASSESSMENT**

## 1. Introduction

The genetically modified (GM) carnation Moonaqua 123.8.12 (Unique Identifier FLO-40689-6) 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, 2006a). In its evaluation the GMO Panel focused in particular on the issues raised by the Member States during the initial assessment of the notification (Reference C/NL/06/01) introduced under Directive 2001/18/EC (EC, 2001). The evaluation presented here is based on the original notification, additional information provided by the notifier and the specific objections raised by three Member States and further scientific literature identified by the GMO Panel.

Carnation Moonaqua 123.8.12 is a new variety which contains a mutated herbicide tolerance *SuRB* (*als*) gene coding for an acetolactate synthase (ALS) variant protein, used to facilitate selection during the genetic transformation process. The light mauve 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 biosynthesis pathway, enable the biosynthesis of delphinidin and cyanidin in the petals.

The same transformation vector (pCGP1991) was used to produce the GM carnation variety Florigene Moonshadow™ (Notification reference C/NL/97/13), which was authorised within the EU for placing on the market in 1998 (http://europa.eu.int/comm/environment/biotechnology/authorised_prod_1.htm). This authorisation included cultivation and was issued by the Dutch Competent Authority. The new carnation Moonaqua 123.8.12 differs in the shade of flower colour and the morphology of the flower.

Another transformation vector with similar genes (pCGP1470) has been used in GM carnation varieties Florigene Moondust™ (Notification reference C/NL/96/14) and Florigene Moonlite™ (Notification reference C/NL/04/02) to modify the flower colour. Florigene Moondust™ was authorised for placing on the market in 1997. Following the opinion of the GMO Panel (EFSA, 2006b), Florigene Moonlite™ 123.2.38 was authorised by the European Commission for placing on the market in 2007 (EC, 2007). This authorisation did not include cultivation. The slight differences between the vectors pCGP1991 and pCGP1470 come from the source of the *f3'5'h* gene and some regulatory elements.

Upon request of the European Commission, EFSA is requested to make specific references to scientific objections from Member States. The objections as regards traceability, labelling and

validation of detection methods fall outside the remit of the GMO Panel. In addition, with respect to the objections related to post-market monitoring, the GMO Panel gave its opinion on the scientific quality of the monitoring plan provided by the notifier although a final adoption of the monitoring plan falls outside the mandate of the GMO Panel.

## 2. Molecular characterisation

### 2.1. Issues raised by Member States

No objection raised by a Member State remained at the end of the 45-day Member States consultation period. Therefore, notwithstanding its own risk analysis, the GMO Panel had no specific concerns to address from Member States on the molecular characterization of GM carnation Moonaqua 123.8.12.

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

### 2.2 Evaluation of relevant scientific data

#### 2.2.1. Transformation process and vector constructs

To develop carnation Moonaqua 123.8.12, new genetic material was introduced into carnation line FE123 (which is a DFR mutant and so does not contain the *f3'5'h* gene) by *Agrobacterium*-mediated transformation using disarmed *Agrobacterium tumefaciens* strain AGL0 carrying the transformation vector pCGP1991 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 outside the T-DNA.

The vector pCGP1991 contained the following three expression cassettes between the left (LB) and right (RB) borders that are commonly considered to define the region to be transferred to the plant: 1) the promoter from a snapdragon (*Antirrhinum majus*) gene encoding chalcone synthase, cDNA encoding flavonoid 3'5' hydroxylase (F3'5'H) from *Viola* sp., the D8 terminator from the petunia gene encoding a phospholipid transfer protein homologue; 2) the entire petunia gene that encodes dihydroflavonol-4-reductase (DFR), including its promoter and terminator. These two cassettes were needed to obtain the desired flower colour. The third cassette contained a chimeric gene consisting of the cauliflower mosaic virus 35S promoter, 5' untranslated region (*ca*. 60 bp) from the cDNA corresponding to the petunia gene encoding chlorophyll a/b binding protein, and the mutated *SuRB* (*als*) gene coding for a acetolactate synthase (ALS) variant protein derived from *Nicotiana*

*tabacum*, including its terminator. The *als* gene provided tolerance to sulfonylurea herbicides used as marker trait in the selection of genetically modified plants but not intended for plant protection purposes. In addition, small stretches (*ca*. 530 bp total) of *Escherichia coli* plasmid pBluescript/pUC were included in the region between the LB and RB.

The entire sequence of the transformation vector pCGP1991 and a description of the function of all genes present were provided.

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

Carnation Moonaqua 123.8.12 contains three transgenic loci. Integration Locus 1 (14433 bp) contains an intact construct between LB and RB. Integration Locus 2 (5140 bp) contains a fragment starting from RB and extending to D8 terminator, which is linked to another fragment containing an almost complete *f3'5'h* cassette (missing ca. 40 bp from the promoter). Integration Locus 3 (1741 bp) contains an incomplete *f3'5'h* cassette. Southern analysis of *Eco*R1-digested genomic DNA with seven probes covering the whole plasmid backbone outside the LB and RB indicated that none of these sequences had been integrated into carnation Moonaqua 123.8.12. Sequences have been provided for all three inserts including their flanking regions.

Bioinformatic analysis of amino acid sequences encoded by the introduced genes indicated no homologies to known toxin or allergen coding genes. The analysis was carried out by comparing the translated sequences encoded by the three introduced genes with the GenBank and SwissProt databases by using the search program BLAST2.2.9 (FAO/WHO, 2001; Codex Alimentarius, 2003).

On the request from the GMO Panel the notifier performed sequence homology search using 80-amino-acid long sliding window, looking for a minimum of 35% non-contiguous identical amino acids. No matches were found. The notifier also performed a similarity search for short identical stretches of six contiguous amino acids. Several identities were found for each newly expressed protein. However, the GMO Panel notes that a number of reports in scientific literature indicate that the 6-amino-acid threshold is likely to give rise to many false positives. The GMO Panel therefore concludes that no relevant homologies exist between the newly expressed proteins in carnation Moonaqua 123.8.12 and known allergens.

Bioinformatic analysis of the flanking regions was carried out using the following criterion: the open reading frame (ORF) should be larger than 50 amino acids and start with methionine. No ORFs were found at the six junctions of the integrated DNA and genomic DNA of carnation.

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

The expression of the three genes, encoding F3'5'H, DFR and ALS enzymes in petals, was demonstrated by northern analysis. Confirmation of the expression of functional enzymes was obtained from metabolite analysis using liquid chromatography (HPLC analysis). The levels of delphinidin and cyanidin in a single assay of bulked petal samples were 0.07 and 0.02 mg/g fresh weight, respectively. It was estimated that the concentration of delphinidin in the genetically modified carnation flowers is approximately one-fiftieth of that in blueberry. Delphinidin is not produced in stems, nodes, leaves or roots of carnation Moonaqua 123.8.12. Cyanidin is not a novel metabolite in carnation.

### 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 Moonaqua 123.8.12, which includes the production of over seven million of flowers. In 2003, two off-types with white streaks were found among 1000 flowers assessed. No off-types were found during flower assessment in 2005 and 2006.

## 2.3. Conclusion

The molecular characterisation data establish that the carnation Moonaqua 123.8.12 contains, in one locus, the complete cassettes containing the genes responsible for the intended traits (light mauve flower colour encoded by *dfr* and *f3'5'h* genes and herbicide tolerance encoded by the mutated *SuRB (als)* gene). In addition, two other loci contain incomplete *f3'5'h* cassettes.

Results of bioinformatic analyses of the three newly expressed proteins in carnation Moonaqua 123.8.12 did not indicate relevant homologies with known toxins or allergens. No new open reading frames were created in the flanking regions covering the inserted DNA and the carnation genome. The GMO Panel concludes that the molecular characterisation of carnation Moonaqua 123.8.12 does not raise any safety concern for humans, animals or the environment.

## 3. Comparative analysis

### 3.1 Issues raised by Member States

No objection remained among Member States concerning the comparative analysis of carnation Moonaqua 123.8.12 to its non-GM parent at the end of the 45-day Member States consultation period.

### 3.2. Evaluation of relevant scientific data

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

Carnation Moonaqua 123.8.12 was compared with the parental variety FE123 which does not produce the anthocyanins, delphinidin and cyanidin, and has cream-white petals.

#### 3.2.2. Compositional analysis

Petals of carnation variety Moonaqua 123.8.12 and the parental variety FE123 were analyzed for three anthocyanins, namely delphinidin, cyanidin and petunidin. Roots, leaves and stems were not assayed. The GMO Panel reviewed the liquid chromatography (HPLC analysis) data provided on the concentrations of these three anthocyanins (Fukui *et al*., 2003). While petunidin was not detected in either the GM variety, or in the non-GM parent, delphinidin and cyanidin were detected in petals of carnation Moonaqua 123.8.12 at levels of 0.07 mg/g and 0.02 mg/g fresh weight, respectively (see Section 2.2.3). These anthocyanins were not present in petals of the white-flowered variety FE123.

The GMO Panel considers that the compositional analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment of the intended modification since the intended use of carnation Moonaqua 123.8.12 excludes cultivation and human or animal consumption.

#### 3.2.3. Agronomic traits and GM phenotype

Carnation Moonaqua 123.8.12 and the parental variety FE123 were grown in field trials in The Netherlands in 2000 and in Australia in 2005 and compared for several morphological characteristics. The comparison of data from these field trials identified significant differences between the GM carnation and the parental variety FE123. The GM carnation has smaller

flowers, reduced stem thickness at the 5th node, and reduced numbers of stamens, styles and anthers and stamen length. According to the notifier, the observed differences are most likely attributable to somaclonal variation and/or environmental effects.

### 3.3. Conclusion

On the basis of the data provided by the notifier and in consideration of the intended use of carnation Moonaqua 123.8.12 (excluding cultivation and human or animal consumption), the GMO Panel considers that a compositional analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment of the intended modification. In addition to confirming the introduced traits, the field trials identified significant morphological differences in some of the phenotypic characteristics observed between the GM carnation and the parental variety. The GMO Panel concludes that the GM carnation Moonaqua 123.8.12 is not agronomically equivalent to the parental variety FE123, as indicated by the morphological changes.

## 4. Safety assessment of GM carnation Moonaqua 123.8.12 for humans and animals

### 4.1. Issues raised by Member States

A need for further assessment of the allergenic potential of carnation Moonaqua 123.8.12 was identified by one Member State at the end of the 45-day Member States consultation period.

### 4.2. Evaluation of relevant scientific data

#### 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 grown in gardens and are available in the cut flower market as ornamental plants.

The scope of notification C/NL/06/01 is restricted to the import of cut carnations Moonaqua 123.8.12 for ornamental use only. Carnation Moonaqua 123.8.12 is a new variety with specific light mauve flower colour that results from the synthesis of delphinidin and cyanidin due to introduced *dfr* and *f3'5'h* genes. The GM carnation Moonaqua 123.8.12 also contains a mutated *SuRB* (*als*) gene conferring tolerance to sulfonylurea herbicides and used to facilitate selection during the transformation process *in vitro*.

### 4.2.2. Stability during processing

Since carnation Moonaqua 123.8.12 is intended to be imported as cut flower like other non-GM carnations, the petals of carnation Moonaqua 123.8.12 are not expected to be processed and used as food and feed. Consequently, the GMO Panel did not consider stability of the GM carnation during processing as an issue.

### 4.2.3. Toxicological assessment of expressed novel proteins

General BLAST searches were performed in order to compare the amino acid sequences of the proteins encoded by the three inserted genes with proteins from the GenBank and SwissProt databases. No homologies were observed with known toxic proteins using general BLAST searches (see Section 2.2.2).

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

Given that carnation Moonaqua 123.8.12 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.

According to Directive 94/36/EC on colours for use in foodstuffs (EC, 1994), anthocyanins (E 163), including delphinidin and cyanidin, are authorised food additives in the EU. Anthocyanins have been evaluated by the previous Scientific Committee on Foods (SCF) which concluded that anthocyanins prepared by physical processes from natural foods are acceptable for use in food without further investigations (SCF, 1984). Therefore the GMO Panel sees no reason for concern regarding the presence of delphinidin and cyanidin in petals from carnation Moonaqua 123.8.12.

The anthocyanins delphinidin and cyanidin are present in many foods and in some of them at much higher concentrations than in the petals of carnation Moonaqua 123.8.12, particularly high concentrations being found, for example, in blackcurrants and red grapes (Cachio *et al*., 1992). Many other delphinidin-containing species (e.g. *Dampiera* spp., *Delphinium* spp., *Lisianthus* spp., *Wisteria* spp.) contain a higher concentration of delphinidin (as a percentage of total anthocyanins) than does carnation Moonaqua 123.8.12. 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) and *Vicia villosa* (Catalano *et al*., 1998).

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

The GMO Panel has considered the possible effects of the genetic modification on human and animal health of accidental consumption of carnation Moonaqua 123.8.12 petals.

4.2.5.1 Acute toxicity testing

The notifier conducted an acute oral toxicity study in mice for the purpose of assessing the impact of accidental consumption of carnation Moonaqua 123.8.12 on human or animal health.

Groups of five male mice received by gavage water extracts from leaves or petals (corresponding to a single dose of 4 g per kg body weight) of carnation Moonaqua 123.8.12. As anthocyanins are water soluble, the extracts from carnation Moonaqua 123.8.12 contained delphinidin and cyanidin. Control groups received either aqueous extracts from leaves or petals of the parental variety FE123 or water. There were no indications of adverse effects in mice administered aqueous extracts from carnation Moonaqua 123.8.12 compared with the non-GM controls at the end of the 14-day observation period.

4.2.5.2 Gene mutation assay

The notifier performed a study on gene mutations in bacteria using *Salmonella enterica Typhimurium* (Ames test) with water extracts of leaves or petals of carnation Moonaqua 123.8.12 and the parental variety FE123. The water extracts did not show mutagenic activity under the conditions of the assay.

### 4.2.6. Allergenicity

The notifier performed general BLAST searches comparing the amino acids sequences of proteins encoded by the three inserted genes with proteins found in the GenBank and SwissProt databases (FAO/WHO, 2001; Codex Alimentarius, 2003). No homologies were observed with known allergens.

The notifier performed a search for short identical stretches of at least six contiguous amino acids. Various positive outcomes consisting of solely six identical contiguous amino acids shared by the three transgenic proteins and allergens have thus been found. The GMO Panel, however, notes that a number of reports in the scientific literature indicates that stipulating only 6-amino-acid long stretches in the homology search is likely to give rise to many false positive outcomes. Therefore, for those proteins identified in the search, as well as for the transgenic proteins, hydrophilicity plots were drawn to predict the possible antigenic sites using a window of six amino acids. The prediction is based on the assumption that relatively hydrophilic residues are more exposed on the protein surface and thus likely to be bound by

antibodies. The scientific literature was also screened for data on IgE-binding epitopes in the identified allergens. No indication of potential allergenicity was found.

In response to a request from the GMO Panel, the notifier performed an additional sequence homology search this time between the three newly expressed proteins and known allergens using a 80 a.a. long sliding window looking for a minimum of 35% non-contiguous identical amino acids. No matches were found. The GMO Panel therefore concludes that no relevant homologies exist between the newly expressed proteins in carnation Moonaqua 123.8.12 and known allergens.

Sanchez (1999; 2004) has described occupational allergy (skin and respiratory allergy) to carnation in workers handling cut flowers/carnation over a long time. This allergy could be caused either by the flower, by mites such as *Tetranychus urticae* infesting carnations or by both simultaneously. According to the notifier, no adverse reaction to carnation Moonaqua 123.8.12 cut flowers used for ornamental purpose has been reported in the general populations where it is marketed. The notifier also reported to the GMO Panel that there have never been any reports of allergenicity or contact dermatitis from growers, distributors and purchasers in over 6 years due to production and processing in Ecuador and Colombia or from export of flowers to the United States.

Considering the scope of this notification and the limited exposure to carnation Moonaqua 123.8.12, 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.

Therefore the GMO Panel is satisfied with the data provided in the notification and is of the opinion that, in this specific case, no further tests are required with respect to allergenicity.

### 4.3. Conclusion

Carnation flowers have a long history of use as ornamentals. Carnation Moonaqua 123.8.12 differs from the parental variety FE123 by the synthesis of delphidinin and cyanidin in the petals, which confers a light mauve colour to the flowers. Delphinidin and cyanidin, which are common pigments in many ornamental flowers and food plants such as red grapes, blackcurrants, egg plants and blueberries, are produced as a result of the combined expression of the introduced *dfr* and *f3'5h* genes together with endogenous genes in the anthocyanin biosynthesis pathway.

The possibility of accidental consumption of carnation Moonaqua 123.8.12 petals cannot be ruled out. However the amount of delphinidin and cyanidin consumed will be negligible in comparison with the amount of delphinidin and cyanidin present in fruits containing high levels of delphinidin and cyanidin such as blackcurrant or red grapes.

No toxicity of water extracts of carnation Moonaqua 123.8.12 petals was observed in an acute oral toxicity study and no mutagenicity of aqueous extracts was indicated by a bacterial mutagenicity assay (Ames test). The amino acid sequences of the newly expressed proteins showed no similarity to known toxins or allergens.

Considering the intended use of carnation Moonaqua 123.8.12, 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

There was a question from a Member State on possible naturalization of carnation Moonaqua 123.8.12. Considering the scope of the notification, there will be a very limited environmental exposure with respect to viable plant parts of carnation Moonaqua 123.8.12.

A need for a more detailed post-market monitoring plan was identified by a Member State at the end of the 45-day Member States consultation period.

Monitoring is clearly related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of the GMO Panel. However, the GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the notifier under Section 5.2.4 of the present scientific opinion.

### 5.2. Evaluation of relevant scientific data

The GMO Panel considered the information provided in the original notification, the Member State objection and further scientific literature in the assessment of the potential for environmental risks and the requirement for a more detailed monitoring plan. As the notification concerns only import of cut flowers, no scientific information on potential environmental effects associated with the cultivation of carnation Moonaqua 123.8.12 was required. Considering the scope of the notification, there will be a very limited environmental exposure with respect to viable plant parts of carnation Moonaqua 123.8.12. 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 notifier, 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 to encourage root growth. 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 pollen. 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 only produces a negligible amount of 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. However there are no known reports of insect pollinators of *D. caryophyllus*, in particular. 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. *Dianthus* species are adapted to the cooler Alpine regions of Europe and Asia, and are also found in Mediterranean coastal regions. *D. caryophyllus* is a widely cultivated ornamental in Europe and occasionally naturalized in some Mediterranean countries but appears to be restricted to the coastal Mediterranean regions of Greece, Italy, Sicily, and Sardinia (Tutin *et al.,* 1993).

Carnation Moonaqua 123.8.12 are imported as cut flowers and thus have no roots and only occasional vegetative buds. The cut stems with vegetative shoots could be propagated by rooting or by micro-propagation.

### 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, to cover the very unlikely event of escape into the environment, the fitness of the GM plants was considered by the GMO Panel.

The carnation Moonaqua 123.8.12 has a modified flower colour achieved by introducing two genes of the anthocyanin biosynthesis pathway from *Petunia* and *Viola sp.*. These genes,

encoding dihydroflavonol 4-reductase and flavonoid 3'5' hydroxylase, give rise to the anthocyanins delphinidin and cyanidin. These anthocyanins are also widely found *e.g.* in flowers of the genus *Petunia* (Ando *et al*., 1999), *Rosa* (Biolley and Jay, 1993) or *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 Moonaqua 123.8.12 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. The GMO Panel took into account the phenotypic characteristics reported in Section 3.2.3. The GMO Panel considered that, because of the intended use of carnation Moonaqua 123.8.12 and therefore the very low exposure of recipient populations, there were no changes in plant characteristics of any ecological significance. The carnation Moonaqua 123.8.12 plant would not show changed fitness characteristics except in the presence of sulfonylurea herbicides and these herbicides are 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. However, these herbicides are not known to be used on cultivated carnations. Such herbicide tolerant plants can be managed by a range of measures (Tranel & Wright, 2002). The consequences of the potential transfer of the three genes would be negligible in terms of adverse effects on the environment.

The GMO Panel is of the opinion that the carnation Moonaqua 123.8.12 is unlikely to have adverse effects on the environment in comparison with non GM carnations.

### 5.2.2. Potential for gene transfer

5.2.2.1 Plant to bacteria gene transfer

The carnation Moonaqua 123.8.12 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 *Viola 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.

5.2.2.2 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 information provided by the notifier suggest that the proportion of flowers carrying pollen is low. The data indicate that pollen transfer is very unlikely to occur. In addition, viable seed set on cut flowers is very unlikely and has not been observed so far with carnation Moonaqua 123.8.12, most likely because of 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 cause an adverse environmental effect.

### 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 Moonaqua 123.8.12 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 notifier 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 of the GMO Panel on GM plants (EFSA, 2006a). 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 notifier, including the general surveillance.

The GMO Panel agrees with the notifier 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 which included a questionnaire to European importers. It was also noted that the notifier requested taxonomists and botanists to inform them of hybrids that might originate from the GM carnation. In addition the notifier will involve national botanic survey networks and plant protection services in his monitoring activities.

In the light of the very low environmental exposure of viable forms of GM carnation Moonaqua 123.8.12 due to the restricted intended use of the GM carnation, the GMO Panel concludes that the proposal of the notifier for general surveillance is in line with the Guidance Document of the GMO Panel on GM plants and in particular with its provisions on post-market environmental monitoring (EFSA, 2006a). The GMO Panel agrees with the proposal made by the notifier to report the monitoring activities on an annual basis as suggested in its Guidance Document (EFSA, 2006a).

## 5.3. Conclusion

The GMO Panel based its environmental risk assessment on cut flowers of carnation Moonaqua 123.8.12 to be imported for ornamental use only. From the information supplied by the notifier, 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 Moonaqua 123.8.12 was assessed for imported cut flowers for ornamental use only. Scientific information on potential environmental effects associated with the cultivation of carnation Moonaqua 123.8.12 was therefore not required. Carnation Moonaqua 123.8.12 cut stems and flowers have marginal viability, negligible pollen production 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 will have adverse effects on the environment in the context of the intended use.

The GMO Panel agrees with the notifier 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 provided in the notification.

## CONCLUSIONS AND RECOMMANDATIONS

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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 has a modified flower colour, a shade of light mauve, which is achieved by introducing into cream-white carnation two genes of the anthocyanin biosynthesis pathway, one from *Petunia* and the other from *Viola* sp. Carnation Moonaqua 123.8.12 also expresses sulfonylurea herbicide tolerance.

The GMO Panel has evaluated the molecular analysis of the genetically modified carnation Moonaqua 123.8.12 and concludes that the molecular characterisation of carnation Moonaqua 123.8.12 does not raise any safety concern for humans, animals or the environment.

Given the intended use of carnation Moonaqua 123.8.12 (excluding cultivation and human or animal consumption), the GMO Panel considers that a compositional analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment of the intended modification. In the case of accidental consumption of petals from carnation Moonaqua 123.8.12, the amount of delphinidin and cyanidin consumed will be negligible in comparison with the amount present in fruits containing high levels of delphinidin and cyanidin, such as blackcurrant or red grapes. An extract from petals did not induce adverse effects in an acute oral toxicity study and was not mutagenic in bacterial gene mutation tests. Furthermore, based on the results of bioinformatic studies, there is no evidence that any of the three proteins expressed is toxic or allergenic. The GMO Panel concludes that carnation Moonaqua 123.8.12 is unlikely to have adverse effects on human or animal health in the unlikely event that carnation Moonaqua 123.8.12 petals are consumed.

Considering the low environmental exposure due to the restricted scope of the notification, it is very unlikely that gene transfer and escape into the environment would occur. In the event that this did occur, the consequences of the escape of the three genes would be negligible with

regard to environmental impact. 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 Catherine Geslain-Lanéelle, Executive Director EFSA, and the annexes, dated 1st of October 2007 with ref. Directorate B D (2007) 17333, from Director Ladislav Miko – Notification C/NL/06/01 (Carnation Moonaqua 123.8.12), under Directive 2001/18/EC - request for EFSA opinion.
2. Letter from EFSA to the notifier with request for further copies of the notification (ref. SR/SM/shv (2007) 2430887, 9 October 2007).
3. Letter from notifier to EFSA, dated 18 October 2007, in response to EFSA request.
4. Letter from EFSA to the notifier – Acknowledgement of receipt (ref. CGL/SR/SM/shv (2007) 2460197, 6 November 2007).
5. Letter from EFSA to notifier, dated 19 December 2007, with request for clarifications/additional information (Ref. SR/SM/shv (2007) 2586837).
6. Letter from EFSA to notifier, dated 18 January 2008, with request for clarifications/additional information (Ref. SR/SM/shv (2008) 2630645).
7. Letter from notifier to EFSA, dated 24 January 2008 and received on 7 February 2008, providing additional information upon EFSA request.
8. Letter from EFSA to notifier, dated 18 February 2008, about additional data considered satisfactory (Ref. SR/SM/shv (2008) 2695632).

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