Vitenskapskomiteen for mattrygghet
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Final health and environmental risk assessment of genetically modified carnation Moonberry IFD-25958-3

Scientific opinion on genetically modified carnation Moonberry IFD-25958-3 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/09/01)

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

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Norwegian Scientific Committee for Food Safety (VKM)
Po 4404 Nydalen
N – 0403 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

www.vkm.no
www.english.vkm.no

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Authors preparing the draft opinion

Åshild Kristine Andreassen (chair), Nana Asare (VKM staff), Anne Marie Bakke (VKM staff), Merethe Aasmo Finne (VKM staff), Anne-Marthe Jevnaker (VKM staff), Olavi Junttila, Ville Erling Sipinen (VKM staff), and Rose Vikse

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 IFD-25958-3 with product name Moonberry™, expresses three introduced traits. The *dfr* gene from *Petunia x hybrida* and the *f3′5′h* gene from *Viola hortensis*, coding for dihydroflavonol 4-reductase (DFR) and flavonoid 3′,5′-hydroxylase (F3′5′H), respectively, lead to the biosynthesis of anthocyanin pigments, which confer the desired violet/blue 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. Of note, carnation Moonberry IFD-25958-3 contained a hairpin RNA interference (RNAi) gene, which down-regulates endogenous *dfr*. Bioinformatics analyses of the inserted DNA and flanking sequences in carnation Moonberry IFD-25958-3 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* genes, have been shown over several generations of carnation Moonberry IFD-25958-3. Data reported from several field trials show that carnation Moonberry IFD-25958-3 petals contain higher levels of the anthocyanins delphinidin and cyanidin, and lower levels of pelargonidin compared to the non-GM (conventional) carnation counterpart Cerise Westpearl (CW). Other morphological traits were reported and along with differing petal colour, carnation Moonberry IFD-25958-3 differed significantly in nine traits compared to conventional carnation counterpart CW. Aqueous extracts from leaves or petals showed no mutagenic activity *in vitro*. ALS, DFR, and F3'5'H proteins do not show sequence resemblance to known toxins or IgE-dependent allergens, nor have they been reported to be toxic to animals or 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 Moonberry IFD-25958-3 represents an environmental risk in Norway.

Considering that carnation Moonberry IFD-25958-3 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 pelargonidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonberry IFD-25958-3 and its conventional carnation counterpart Cerise Westpearl (CW) 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 Moonberry IFD-25958-3.

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 Moonberry IFD-25958-3 is as safe as its conventional counterpart CW.

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 Moonberry IFD-25958-3 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.) IFD-25958-3 (Unique Identifier IFD-25958-3; trade name Moonberry™) is approved under Directive 2001/18/EC for import as cut flowers for ornamental use since 24 April 2015 (Application C/NL /09/01, Commission Decision 2015/692). The scope of the application is restricted to flowers produced by vegetative propagation, and do not cover progeny derived from sexual crosses with Moonberry IFD-25958-3 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”.

The current safety and environmental risk assessment of the carnation Moonberry IFD-25958-3 is based on information provided by the applicant in the application C/NL/09/01, relevant peer-reviewed scientific literature, and scientific opinions from EFSA (EFSA, 2014a). 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 violet/blue 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 Moonberry IFD-25958-3 contains one transgenic locus that contains the full length transfer-DNA-sequence (T-DNA) from the transformation vector pCFP3366. The T-DNA includes functional single copies of each of the four genes *dfr, f3′5′h, als*, and *Dcdfh*p. *Dcdfh*p is a hairpin-forming sequence which leads to downregulation of endogenous carnation DFR (DcDFR) by targeting its mRNA. Southern blot and PCR analyses indicate that no plasmid backbone sequences were integrated. Sequence analyses show no disruption of known endogenous genes. Presence of transcripts corresponding to *f3′5′h*, *dfr*, *Dcdfh*p and *als* genes in petals was examined by Northern blot analysis. The *Dcdfh*p probe hybridised to endogenous *dfr* sequences within the parental line CW as well as to the hairpin sequence in Moonberry IFD-25958-3. Although expression levels were not quantified, the results of the Northern blot suggest functional degradation of endogenous *dfr*-mRNA in Moonberry by the hairpin construct. The other probes did not hybridise with the parental line CW. Active F3′5′H and DFR enzymes, and the silencing function of the hairpin were further indicated by the altered flower colour, and metabolite analyses with both thin layer chromatography (TLC) and high-

pressure liquid chromatography (HPLC). The level of delphinidin based pigment in petals of Moonberry IFD-25958-3 was approximately 0.54 mg/g fresh weight. Tolerance to sulfonylurea herbicides indicated active ALS protein. Bioinformatic analyses of putative translation products from new open reading frames (ORFs) within the insert and junction sites returned no relevant similarities to known toxins. Partial identities were indicated for the ALS protein and two ORFs with known allergens, however these similarities are considered negligible. No relevant changes in the introduced flower colour have been reported during cultivation of carnation Moonberry IFD-25958-3, indicating genetic stability.

Based on current knowledge and information provided by the applicant, the VKM GMO panel concludes that the molecular characterisation of carnation Moonberry IFD-25958-3 does not indicate a safety concern.

Comparative assessment

Considering the intended use of carnation Moonberry IFD-25958-3, which excludes cultivation and use in food and feed, compositional studies were limited to the content of the three anthocyanin pigments delphinidin, cyanidin and pelargonidin. Compared to its non-GM parental cultivar Cerise Westpearl (CW), carnation Moonberry IFD-25958-3 petals contained higher levels of delphinidin and cyanidin and lower levels of pelargonidin, confirming the intended effects of the genetic modification. Other morphological traits were assessed and revealed that along with differing petal colour, carnation Moonberry IFD-25958-3 differed significantly in nine traits compared to carnation CW. 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 use of carnation Moonberry IFD-25958-3, which excludes 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 pelargonidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonberry IFD-25958-3 and the conventional carnation counterpart CW do not raise safety concerns.

Food and feed risk assessment

In vitro mutagenicity tests (Ames test) with four different strains of *Salmonella typhimurium* have been performed by the applicant on aqueous extracts from leaves and petals from carnation Moonberry IFD-25958-3. None of the tests 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 supplied by the applicant, and considering the intended use which excludes cultivation and use as food and feed, the VKM GMO Panel concludes that carnation Moonberry IFD-25958-3 is as safe as its conventional counterpart, carnation CW. 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 Moonberry IFD-25958-3.

Environmental assessment

Considering the intended use of Moonberry IFD-25958-3, 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, Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3. 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 Moonberry IFD-25958-3 is not intended for cultivation or use as food or feed, the VKM GMO Panel considers that the comparative analysis of the newly synthesised anthocyanins delphinidin, cyanidin and pelargonidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonberry IFD-25958-3 and the conventional carnation counterpart Cerise Westpearl (CW) 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 Moonberry IFD-25958-3 is as safe as its conventional counterpart, CW. 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 Moonberry IFD-25958-3.

Likewise, the VKM GMO Panel concludes that carnation Moonberry IFD-25958-3, based on current knowledge and intended use as cut flowers, does not represent an environmental risk in Norway.

Key words: GMO, carnation (*Dianthus caryophyllus* L.), Moonberry, 25958, 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.) linjen IFD-25958-3 (unik kode IFD-25958-3; produktnavn Moonberry™) fra Florigene ble godkjent til import og salg som avskårne prydblomster under EUs utsettingsdirektiv 2001/18/EC den 24.4.2015 (jfr. Kommisjonsbeslutning 2015/692). Søknad C/NL/09/01 omfatter nellikplanter som er produsert ved vegetativ formering, og omfatter ikke avledete sorter fra konvensjonelle kryssinger med Moonberry IFD-25958-3. 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 Moonberry IFD-25958-3.

Risikovurderingen av den genmodifiserte nelliklinjen er basert på søkers dokumentasjon og uavhengige vitenskapelige publikasjoner, samt vitenskapelige vurderinger og kommentarer fra EFSA (EFSA, 2014a) og andre medlemstater som er gjort tilgjengelig på EFSAs nettside EFSA GMO Extranet. Bortsett fra gjennomgang av nylig offentliggjort publikasjoner er resten av teksten i denne vurderingen en oppsummering av tidligere (EFSA, 2014a) 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 EU-direktiv 2001/18/EF (vedlegg 2, 3 og 3B) og veiledende notat til Annex II (2002/623/EF), samt prinsippene i EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler (EFSA, 2006a; EFSA, 2009a; EFSA, 2010a; EFSA, 2011a; EFSA, 2011b; EFSA, 2011c) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsmetoden og vektorkonstruksjonen, karakterisering og nedarving av genkonstruksjonen, komparativ analyse av antocyanin innhold i kronbladene og andre morfologiske egenskaper, kritiske toksiner, allergener og

nye proteiner. Videre er potensiale for utilsiktede effekter på fitness, genoverføring til målorganismer og ikke-målorganismer, og biogeokjemiske prosesser vurdert.

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

Nelliken Moonberry IFD-25958-3 uttrykker tre nye egenskaper: *dfr* -genet fra *Petunia x hybrida* som koder for dihydroksyflavonol-reduktase (DFR), *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 nellik Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 inneholder en fullstendig kopi av det transgene innskudds-DNAet (T-DNA) fra transformasjonsvektoren pCFP3366. T-DNAet består av én kopi for hver av de fire genene *dfr*, *f3′5′h*, *als* og *Dcdfr*hp. *Dcdfr*hp koder for en hårnålstruktur som fører til indirekte nedregulering av endogent DFR protein (DcDFR) i nelliken ved å degradere dets mRNA. Southern blot og PCR -analyser indikerer ingen overføring av vektorsekvenser utenfor området til T-DNA, i nelliken. Northern blot ble brukt til å undersøke uttrykk av de fire genene *dfr*, *f3′5′h, als*, og *Dcdfr*hp i kronblader. Proben til *Dcdfr*hp hybridiserte med både det endogene *dfr*-genet (*Dcdfr*) i foreldrelinjen CW, og med *Dcdfr*hp-sekvensen i Moonberry IFD-25958-3. Til tross for at genuttrykkene ikke ble kvantifisert, indikerer resultatene av analysen en aktiv degradering av endogent *Dcdfr*- mRNA i Moonberry av hårnålsstrukturen. Ingen av de andre probene hybridiserte med prøven fra foreldrelinjen CW. Den endrete blomsterfargen til Moonberry IFD-25958-3, og analyser med både tynnsjiktkromatografi (TLC) og væskekromatografi (HPLC) er videre indikasjoner på produksjon av fungerende F3'5'H og DFR -enzym, og aktiv nedregulering av endogent DcDFR. I kronblader ble delphinidin-basert pigment målt til 0,54 mg/g ferskvekt. Toleranse for sulfonylurea-herbicid viste tilstedeværelse av aktivt ALS-protein. Databasesøk utført av søker viste ingen relevante samsvar mellom antatte genprodukt fra de innsatte genene eller nye tilførte åpne leserammer (ORFs), og kjente toksiner. Søkene viste derimot en partiell likhet (~35%) mellom ALS proteinet og et kjent allergen. Liknende treff (~35% samsvar) ble også observert for eventuelt genprodukt fra to åpne leserammer. De observerte partielle likhetene til kjente allergener anses ikke som vesentlige.

Ved dyrking av Moonberry IFD-25958-3 har det så langt ikke blitt rapportert om relevante avvik ved den introduserte blomsterfargen, hvilket indikerer genetisk stabilitet.

Basert på dagens kunnskap og informasjonen fra søker, konkluderer VKMs faggruppe for GMO, at den molekylære karakteriseringen ikke tilsier noen økt risiko ved nellik Moonberry IFD-25958-3 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 til en stor grad lite kjent, ble kun innhold av de tre antocyanin pigmentene delfinidin, cyanidin and pelargonidin i kronblader fra Moonberry IFD-25958-3 rapportert av søker. Sammenlignet med den konvensjonelle motpart nellik Cerise Westpearl (CW) inneholder kronbladene fra nellik Moonberry IFD-25958-3 høyere nivåer av delfinidin og cyanidin, mens nivået av pelargonidin var lavere. 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 ni 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 Moonberry IFD-25958-3.

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 delphinidin, cyanidin og pelargonidin i kronbladene er tilstrekkelig for risikovurderingen. De rapporterte morfologiske forskjellene mellom Moonberry IFD-25958-3 og dens konvensjonelle motpart nellik CW medfører ikke en økt sikkerhetsrisiko.

Helserisiko

In vitro mutagenisitetsforsøk (Ames test) har blitt utført av søker, hvor ekstrakter av kronblad og blomsterblad fra Moonberry IFD-25958-3 ble testet på fire ulike typer av bakterien *Salmonella typhimurium.* Ingen av testene 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 Moonberry IFD-25958-3 er like trygg som dens konvensjonelle motpart, nellik CW. 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 Moonberry IFD-25958-3

Miljørisiko

Miljørisikovurderingen av nelliklinjen Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 pelargonidin i kronbladene til nellik Moonberry IFD-25958-3 er tilstrekkelig for risikovurderingen. De rapporterte morfologiske forskjellene mellom Moonberry IFD-25958-3 og dens konvensjonelle motpart nellik Cerise Westpearl (CW) medfører ikke en økt sikkerhetsrisiko.

Ut i fra dagens kunnskap og informasjon tilsendt av søker, og tatt i betraktning tiltenkt bruksområde som ekskluderer dyrking og bruk som mat og fôr, konkluderer VKM's GMO Panel at Moonberry IFD-25958-3 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 Moonberry IFD-25958-3.

Likeledes finner faggruppen, ut i fra dagens kunnskap, at den omsøkte bruken av Moonberry IFD-25958-3 som avskårne prydblomster ikke vil medføre en miljørisiko i Norge.

Abbreviations and glossary

ALS	Acetolactate synthase
CW	Cerise Westpearl
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 March 2009, an application (Reference C/NL/09/01) covering import of cut flowers of the genetically modified carnation IFD-25958-3 (Unique Identifier IFD-25958-3; trade name Moonberry™) for ornamental use was submitted by Florigene Ltd. to the competent authority of the Netherlands. The scope of the application was restricted to flowers produced by vegetative propagation, and did not cover progeny derived from sexual crosses with Moonberry IFD-25958-3 cultivar.

In July 2009, the European Commission received the full application and an assessment report from the Netherlands. In accordance with Directive 2001/18/EC (EC, 2001), the application was transmitted to the competent authorities of the other Member States for a 60-day public hearing. The applicant, Florigene Ltd., provided the MS with additional information after receiving comments and objections during this consultation period. Following an additional 45-day consultation period, some MS still maintained their objections 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/09/01 on 12 December 2014 (EFSA, 2014), and carnation Moonberry IFD-25958-3 was approved for import and ornamental use on 24 April 2015 (Commission Decision 2015/692/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".

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 Moonberry IFD-25958-3 for import of cut flowers for ornamental use (Application C/NL/09/01).

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 Moonberry IFD-25958-3 for import of cut flowers for ornamental use (Application C/NL/09/01).

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 Moonberry IFD-25958-3 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 by EFSA (EFSA, 2014a), reports/comments from other member states made available on the EFSA website GMO Extranet and relevant peer-reviewed scientific literature. The VKM GMO Panel has not previously published a risk assessment of carnation Moonberry IFD-25958-3. The above-mentioned EFSA report is provided in Appendix I, and readers are referred to this for details. The assessment was 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). Furthermore, the EFSA GMO Panel based its evaluation of carnation Moonberry IFD-25958-3 (EFSA, 2014a) on experience gained from previously assessing GM carnations with similar traits (EFSA, 2006b; EFSA, 2008) as well as considering the safety for humans in view of possible exposure routes through dermal contact, inhalation, and oral intake.

Carnation Moonberry IFD-25958-3 was developed for petal colour for decorative purposes. The expression of the newly introduced genes, *dfr* from *Petunia* × *hybrida* and *f3′5′h* from *Viola hortensis* coding for dihydroflavonol 4-reductase (DFR) and flavonoid 3′,5′-hydroxylase (F3′5′H), respectively, confers the violet/blue colour to the flowers. Biosynthesis of the anthocyanins, cyanidin and delphinidin in the petals is enabled via interplay between introduced and endogenous genes in the anthocyanin biosynthesis pathway. Of note, carnation Moonberry IFD-25958-3 contains a hairpin RNA interference (RNAi) gene, which down-regulates endogenous *dfr*. In addition, carnation Moonberry IFD-25958-3 expresses herbicide tolerance by the introduction of a mutated *als* gene from the *SuRB* locus of *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) and amounts 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 (reviewed by (Tranel and Wright, 2002). However, the introduction of the mutated *als* gene (*SuRB*) in carnation Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

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

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

2 Molecular characterisation

The EFSA GMO Panel (EFSA, 2014a in Appendix I) previously assessed the molecular characterisation of the event IFD-25958-3 (Moonberry; *dfr, f3'5'h, Dcdfr*hp, and *SuRB* [mutated version of *als*] inserts) with regards to the following:

1. The transformation system and vector constructs
2. Characterisation of the transgene insertions and constructs
3. 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 site and flanking sequences in the carnation Moonberry IFD-25958-3 genome.

Carnation variety Cerise Westpearl (CW) was transformed using a disarmed *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain AGL0, which carried the transformation vector pCGP3366. Vector pCGP3366 contains four expression cassettes, three that affect flower color and one that provides tolerance to sulfonylurea herbicides:

1) the dihydroflavonol 4-reductase (*dfr*) cassette, containing the *dfr* coding sequence and it's native promoter and terminator, from *Petunia* × *hybrida*

2) the flavonoid 3′,5′-hydroxylase (*f3′5′h*) cassette, containing the promoter sequence from *Antirrhinum majus* chalcone synthase gene (*chs*), the *f3′5′h* coding sequence from *Viola hortensis*), and the D8 terminator sequence from *Petunia* × *hybrida* putative phospholipid transfer protein homologue

3) the *Dcdfr*hp cassette, which contains a hairpin-forming construct targeted to specific, post-transcriptional down-regulation of endogenous carnation *dfr* (*dcdfr*) by RNAi. The hairpin-forming construct consisted of sense and antisense segments of the *dfr* coding sequence, separated by a petunia *dfr* intron. This sequence is regulated by the *Cauliflower mosaic virus* (*CaMV*) 35S promoter and terminator

4) the acetolactate synthase cassette (*als*) from tobacco, conferring tolerance to sulfonylurea herbicides. The cassette contains the *CaMV* 35S promoter, the mutated *als* coding region and terminator sequence from *Nicotiana tabacum*

Southern blot and PCR analyses performed by the applicant indicate that carnation Moonberry IFD-25958-3 contains insertion of the transfer DNA (T-DNA) region from the vector pCGP3366 at a single locus with a single copy of each integrated T-DNA component only. The analyses also indicated absence of plasmid backbone sequences in the GM flower.

Sequence analyses of flanking regions and the junction sites between the insert and plant DNA did not reveal disruption of endogenous genes.

The presence of transcripts corresponding to *f3′5′h*, *dfr*, *Dcdfh*p and *als* genes in petals of Moonberry IFD-25958-3 was examined by Northern blot analysis. The probes used in the analysis were based on the *als*, *f3′5′h*, *dfr* and *Dcdfh*p expression cassettes present in the transformation vector pCGP3366. No expression was detected in the control line CW for *als*, *f3′5′h*, and *dfr*. The *Dcdfh*p probe hybridised to endogenous *dfr* sequences within parental line CW as well as to the hairpin cassette in Moonberry IFD-25958-3. Expression levels were not quantified. Some additional small degradation products were observed in the autoradiograph for Moonberry IFD-25958-3 with the *Dcdfh*p probe. According to the applicant, the results suggest active post-transcriptional degradation of endogenous *dfr* mRNAs as a consequence of the gene silencing. Active F3'5'H and DFR enzymes, and siRNA were indicated by the flower colour, as well as from metabolite analyses with both thin layer chromatography (TLC) and high-pressure liquid chromatography (HPLC). The level of the anthocyanin (pigment) Delphinidin in flowers of Moonberry IFD-25958-3 was approximately 0.54 mg/g fresh weight. Tolerance to sulfonylurea herbicides indicated active ALS protein.

The putative translation products of open reading frames (ORFs) within the insert and spanning the junction sites were compared to known toxins and allergens in appropriate databases. No relevant similarities were found for toxins. A higher than 35 % sequence identity was however found within the ALS protein to an allergen from *Davidiella tassiana*, when employing an 80-amino-acid long sliding window, looking for a minimum of 35% contiguous identical amino acids. Likewise an identity of more than 35% was found with the allergen 'Amb a 4' from the common ragweed *Ambrosia artemisiifolia*. When searches were done for sequences of eight contiguous amino acids, a positive match was also found with a subtilisin protease allergen in *Bacillus licheniformis*. Due to lack of essential regulatory factors the likelihood that any of the above mentioned open reading frames would be transcribed and translated in carnation Moonberry IFD-25958-3, is deemed negligible.

Carnation Moonberry IFD-25958-3 was propagated vegetatively from April 2005 to September 2008, which represents multiple cycles of propagation. During 2007–2013, plants were cultivated in a field trial in Colombia and there were no incidents reported of flower colour change that would indicate genetic instability.

2.1 Conclusions

Based on current knowledge and information provided by the applicant, the VKM GMO panel concludes that the molecular characterisation of carnation Moonberry IFD-25958-3 does not indicate a safety concern.

3 Comparative assessments

Previously, EFSA (EFSA, 2014a in Appendix I) assessed compositional and morphological data of carnation Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 (EFSA, 2014a) or other GM carnations (EFSA, 2006b; EFSA, 2008; EFSA, 2014b). Thus the comparative compositional assessment as defined in EFSA guidance documents for GM plants and derived food and feed (EFSA, 2006a) was only partially applied and possible unintended effects of the genetic modification in carnation Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 pelargonidin – were analysed by HPLC in acetonitrile extracts of freeze-dried petals of carnation Moonberry IFD-25958-3 and its non-GM parental cultivar (conventional comparator; control) Cerise Westpearl (CW) according to the method described by Fukui et al. (2003). Carnation CW has cerise petals. The HPLC method included a hydrolysis step, which converted the pigments into their aglycones, allowing the determination of total delphinidin, total cyanidin and total pelargonidin, rather than as they occur *in planta* as glycosylated and/or acylated compounds.

For assessment of morphological traits, carnation Moonberry IFD-25958-3 and its conventional comparator CW were grown in a field trial in Australia during the 2007-2008 season.

3.2 Compositional analysis

HPLC data (Technical dossier; Fukui et al., 2003) indicated that compared to its non-GM parental cultivar Cerise Westpearl, carnation Moonberry IFD-25958-3 petals contained higher

levels of delphinidin and cyanidin and lower levels of pelargonidin (see Table 3.2-1). In other plant tissues, delphinidin-based pigments were not observed (stem, nodes, leaves and roots) or detected (leaves and roots) in carnation Moonberry IFD-25958-3.

Table 3.2-1. Mean levels of the various anthocyanins reported in petals from GM and respective non-GM comparator carnations. Values are mg pigment per g fresh weight (fw).

Cultivar	*Delphinidin*	*Cyanidin*	*Pelargonidin*
Cerise Westpearl (CW)	nd	0.01	1.06
Moonberry IFD-25958-3	0.54	0.10	0.02

EFSA (2014a) concluded that the altered levels and types of anthocyanins in carnation Moonberry IFD-25958-3 accounted for the intended morphological changes in petal colour. 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.

3.3 Morphological traits and GM phenotype

According to the applicant, carnation Moonberry IFD-25958-3 has been evaluated in field trials in Australia during the 2007-2008 growing season. In total, 18 morphological characteristics most relevant to potential gene dispersal were analysed in carnation Moonberry IFD-25958-3 and its conventional comparator (cultivar CW), 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 eight of these characteristics. Carnation Moonberry IFD-25958-3 had increased calyx diameter and length, and higher numbers of petals per flower, internodes per stem, and filaments, but reduced length to the fifth node, a thinner stem at the fifth node, and shorter filaments. The mean number of days to flowering was 138 days for Moonberry IFD-25958-3 compared to 146 days for carnation CW.

Additional data provided by the applicant from a field trial in Columbia did not confirm statistically significant differences between Moonberry IFD-25958-3 and its comparator in petal count per flower or days to flowering. The other parameters that had demonstrated statistically significant differences in the Australian study were not investigated in the Columbian study.

EFSA (EFSA, 2014a) concluded that the differences reported for 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 use of carnation Moonberry IFD-25958-3, which excludes 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 pelargonidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonberry IFD-25958-3 and its conventional carnation counterpart CW do not raise safety concerns.

4 Food and feed safety assessment

4.1 Previous evaluations by EFSA GMO Panel

Carnation Moonberry IFD-25958-3 was recently assessed by EFSA (EFSA, 2014a in Appendix I) and no adverse effects for the use of GM carnations in relation to non-GM cultivars, was identified.

4.2 Product description and intended uses

The EU Commission Decision 2015/692/EC stipulates that a condition for placing carnation Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3.

The scope of the application C/NL/09/01 is restricted to the import of cut carnations for ornamental use only. As is the case for 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 to be 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 Moonberry IFD-25958-3 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 carnation Moonberry IFD-25958-3 (Cacho et al., 1992). Notably, anthocyanins (E 163) are authorised food additives according to regulation 1333/2008 (Reference EC No. 1333/2008) on food additives. Previous evaluations of anthocyanins prepared by physical processes from natural foods identified no adverse effect or reason for concern (EFSA, 2013).

4.3.2.1 **In vitro** *studies*

The applicant performed studies on gene mutagenicity, Ames test, employing *Salmonella typhimurium* exposed to aqueous extracts from petals and leaves of GM carnation Moonberry IFD-25958-3 and non-GM parental CW carnations as control. No mutagenic activity was observed.

4.3.2.2 Acute toxicity study

Acute toxicity studies were not performed.

4.3.3 Toxicological assessment of the whole GM plant

Taking into account that carnation Moonberry IFD-25958-3 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 is 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 Moonberry IFD-25958-3 using the criterion of more than 35 % identity in a segment of 80 or more amino (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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 a 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 Moonberry IFD-25958-3 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 rather higher levels than in the petals of carnation Moonberry IFD-25958-3 (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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3, 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 Moonberry IFD-25958-3, a cyanidin concentration of 0.10 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 Moonberry IFD-25958-3 is considerably 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3, a delphinidin concentration of 0.54 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 Moonberry IFD-25958-3 petals is not considered to pose a health risk compared to the levels present in berries and estimated ADI.

4.5 Conclusion

In vitro mutagenicity tests (Ames test) with four different strains of *Salmonella typhimurium* have been performed by the applicant on aqueous extracts from leaves and petals from carnation Moonberry IFD-25958-3. None of the tests 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 supplied by the applicant, and considering the intended use which excludes cultivation and use as food and feed, the VKM GMO Panel concludes that carnation Moonberry IFD-25958-3 is as safe as its conventional counterpart, carnation CW. 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 Moonberry IFD-25958-3.

5 Environmental risk assessment

5.1 Introduction

This assessment applies to carnation line Moonberry IFD-25958-3 from Florigene Ltd, which has been transformed to modify the flower colour and possess a mutated herbicide resistance *als* gene (*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 species since the middle ages (http://www.plantearven.no). Wild populations of *D. caryophyllus* are only known from Greece, Italy, Sicily and Sardinia (Tutin and Walters, 1993). In this assessment, the term carnation is used for *D. caryophyllus*.

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

Wild *D. caryophyllus* L. 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 *als* 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 will have any adverse effect on abiotic environment or biochemical cycles.

5.7 Conclusion

Considering the intended use of Moonberry IFD-25958-3, 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, Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3. No specific environmental impact of genetically modified carnation Moonberry IFD-25958-3 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 Moonberry IFD-25958-3. 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 Moonberry IFD-25958-3 contains one transgenic locus that contains the full length transfer-DNA-sequence (T-DNA) from the transformation vector pCFP3366. The T-DNA includes functional single copies of each of the four genes *dfr, f3′5′h, als*, and *Dcdfh*p. *Dcdfh*p is a hairpin-forming sequence which leads to downregulation of endogenous carnation DFR (DcDFR) by targeting its mRNA. Southern blot and PCR analyses indicate that no plasmid backbone sequences were integrated. Sequence analyses show no disruption of known endogenous genes. Presence of transcripts corresponding to *f3′5′h*, *dfr*, *Dcdfh*p and *als* genes in petals was examined by Northern blot analysis. The *Dcdfh*p probe hybridised to endogenous *dfr* sequences within the parental line CW as well as to the hairpin sequence in Moonberry IFD-25958-3. Although expression levels were not quantified, the results of the Northern blot suggest functional degradation of endogenous *dfr*-mRNA in Moonberry by the hairpin construct. The other probes did not hybridise with the parental line CW. Active F3'5'H and DFR enzymes, and the silencing function of the hairpin were further indicated by the altered flower colour, and metabolite analyses with both thin layer chromatography (TLC) and high-pressure liquid chromatography (HPLC). The level of delphinidin based pigment in petals of Moonberry IFD-25958-3 was approximately 0.54 mg/g fresh weight. Tolerance to sulfonylurea herbicides indicated active ALS protein. Bioinformatic analyses of putative translation products from new open reading frames (ORFs) within the insert and junction sites returned no relevant similarities to known toxins. Partial identities were indicated for the ALS protein and two ORFs with known allergens, however these similarities are considered negligible. No relevant changes in the introduced flower colour have been reported during cultivation of carnation Moonberry IFD-25958-3, indicating genetic stability.

Based on current knowledge and information provided by the applicant, the VKM GMO panel concludes that the molecular characterisation of carnation Moonberry IFD-25958-3 does not indicate a safety concern.

Comparative assessment

Considering the intended use of carnation Moonberry IFD-25958-3, which excludes cultivation and use in food and feed, compositional studies were limited to the content of the three anthocyanin pigments delphinidin, cyanidin and pelargonidin. Compared to its non-GM parental cultivar Cerise Westpearl (CW), carnation Moonberry IFD-25958-3 petals contained higher levels of delphinidin and cyanidin and lower levels of pelargonidin, confirming the intended effects of the genetic modification. Other morphological traits were assessed and revealed that along with differing petal colour, carnation Moonberry IFD-25958-3 differed significantly in nine traits compared to carnation CW. 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 use of carnation Moonberry IFD-25958-3, which excludes 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 pelargonidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonberry IFD-25958-3 and the conventional carnation counterpart CW do not raise safety concerns.

Food and feed risk assessment

In vitro mutagenicity tests (Ames test) with four different strains of *Salmonella typhimurium* have been performed by the applicant on aqueous extracts from leaves and petals from carnation Moonberry IFD-25958-3. None of the tests 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 supplied by the applicant, and considering the intended use which excludes cultivation and use as food and feed, the VKM GMO Panel concludes that carnation Moonberry IFD-25958-3 is as safe as its conventional counterpart, carnation CW. 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 Moonberry IFD-25958-3.

Environmental assessment

Considering the intended use of Moonberry IFD-25958-3, 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, Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3. 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 Moonberry IFD-25958-3 is not intended for cultivation or use as food or feed, the VKM GMO Panel considers that the comparative analysis of the newly synthesised anthocyanins delphinidin, cyanidin and pelargonidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonberry IFD-25958-3 and the conventional carnation counterpart Cerise Westpearl (CW) 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 Moonberry IFD-25958-3 is as safe as its conventional counterpart, CW. 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 Moonberry IFD-25958-3.

Likewise, the VKM GMO Panel concludes that carnation Moonberry IFD-25958-3, based on current knowledge and intended use as cut 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 (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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 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 Moonberry IFD-25958-3 are merited.

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Appendix I

SCIENTIFIC OPINION

Scientific Opinion on a notification (reference C/NL/09/01) for the placing on the market of the genetically modified carnation IFD-25958-3 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Florigene[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Genetically modified (GM) carnation IFD-25958-3 was developed to express anthocyanins in the petals conferring a mauve colour to the flowers. The GM carnation is intended to be imported in the European Union as cut flower for ornamental use only. Based on the molecular characterisation data, the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) confirms the stability of the newly introduced trait and the absence of disruption of known endogenous genes. Since anthocyanins are common pigments in many food plants, it is not expected that accidental intake of petals of carnation IFD-25958-3 would contribute substantially to the overall intake of anthocyanins from foods. Considering the ornamental use of cut flowers, and the limited exposure scenarios expected, the EFSA GMO Panel identified no reasons for any food safety concerns relating to carnation IFD-25958-3. The EFSA GMO Panel is also of the opinion that accidental release of GM carnations into the environment would not give rise to environmental safety concerns. The EFSA GMO Panel agrees with the methodology, including reporting intervals, proposed for post-market environmental monitoring. In response to the European Commission, the EFSA GMO Panel concludes that, in the light of the ornamental use of carnation IFD-25958-3 cut flowers, there is no scientific reason to consider that the placing on the market of the GM carnation will cause any adverse effects on human health or the environment.

KEY WORDS

carnation, cut flower, delphinidin, *Dianthus caryophyllus*, Directive 2001/18/EC, import, petal colour

[1] On request from the European Commission, Question No EFSA-Q-2013-00328, adopted on 4 December 2014.

[2] Panel members: Salvatore Arpaia, Andrew Nicholas Edmund Birch, Andrew Chesson, Patrick du Jardin, Achim Gathmann, Jürgen Gropp, Lieve Herman, Hilde-Gunn Hoen-Sorteberg, Huw Jones, József Kiss, Gijs Kleter, Martinus Lovik, Antoine Messéan, Hanspeter Naegeli, Kaare Magne Nielsen, Jaroslava Ovesna, Joe Perry, Nils Rostoks and Christoph Tebbe. Correspondence: gmo@efsa.europa.eu

[3] Acknowledgement: The GMO Panel wishes to thank the members of its standing Working Groups on Molecular Characterisation (MC), Food/Feed (FF) and Environmental Risk Assessment (ERA) for the preparatory work on this scientific opinion and EFSA staff: Irina Olaru, Antonio Fernandez Dumont and Sylvie Mestdagh, for the support provided to this scientific opinion.

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SUMMARY

Following a request from the European Commission, the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on notification C/NL/09/01 from Florigene submitted under Part C of Directive 2001/18/EC[4]. The scope of notification C/NL/09/01 covers the import, distribution and retailing in the European Union (EU) of genetically modified (GM) carnation IFD-25958-3 cut flowers for ornamental use only.

In accordance with Directive 2001/18/EC, an assessment of the GM carnation was requested by the European Commission in order to address the outstanding objections raised by some Member States following the evaluation at the national level, and to assess the overall safety of the GM carnation. The EFSA GMO Panel was, therefore, asked to consider if there is any scientific reason to believe that the placing on the market of GM carnation IFD-25958-3 for import is likely to cause any adverse effects on human health or the environment.

In delivering the present scientific opinion, the EFSA GMO Panel considered the full notification C/NL/09/01, including additional information provided by the notifier, the assessment report of the Dutch competent authority, the concerns raised by Member States, relevant scientific publications and the experience gained in assessing GM carnations with similar traits (EFSA, 2006a, 2008; EFSA GMO Panel, 2014). The EFSA GMO Panel performed its risk assessment in accordance with the principles of its guidance documents on the risk assessment of GM plants for non-food or non-feed purposes (EFSA, 2009a) and on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010). It should be noted that the comparative compositional assessment as defined in the EFSA guidance documents (EFSA, 2006b; EFSA GMO Panel, 2011a) could not be applied to identify possible unintended effects of carnation IFD-25958-3.

The scientific evaluation by the EFSA GMO Panel included molecular characterisation of the inserted DNA and expression of the new proteins. A comparative evaluation of the morphological characteristics was undertaken, and the safety of the newly expressed proteins and of the whole GM plant was evaluated with respect to potential toxicity and allergenicity. The potential environmental impacts of accidental release of GM carnations into the environment and the post-market environmental monitoring (PMEM) plan proposed by the notifier were evaluated in the context of the scope of notification C/NL/09/01.

Carnation IFD-25958-3 has a modified flower colour, a shade of mauve, whereas the parental line has a cerise flower colour. The colour has been achieved by introducing into the parental carnation three expression cassettes which, together with other genes of the anthocyanin biosynthesis pathway that are already present in the non-GM carnation, give rise to the anthocyanins delphinidin and cyanidin, the same pigments that give colour to blueberry, blackcurrant and red grape. Carnation IFD-25958-3 is also tolerant to sulfonylurea herbicides, but this newly introduced trait was used only for the selection of transformed plants and not for plant protection purposes.

Carnation IFD-25958-3 was developed by *Agrobacterium tumefaciens*-mediated transformation of the conventional carnation Cerise Westpearl. The desired colour was obtained by introducing the flavonoid 3′,5′-hydroxylase (*f3′5′h*) coding sequence from *Viola* sp., the dihydroflavonol 4-reductase (*dfr*) coding sequence from *Petunia* sp. and a hairpin RNA interference (RNAi) construct which down-regulates endogenous *dfr*. Tolerance to sulfonylurea herbicides was conferred by the expression of a mutated *als* gene. The molecular characterisation data establish that carnation IFD-25958-3 contains a single insert consisting of the four expression cassettes. The stability of the newly introduced trait (mauve flower colour) was observed over multiple generations. Bioinformatic

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1–39.

analyses of the 5′ and 3′ flanking regions of the insert did not reveal disruption of known endogenous genes. Identities with allergens were found in putative translation products of open reading frames (ORFs) newly created by the genetic modification, but the likelihood that they are both transcribed and translated in carnation IFD-25958-3 was considered negligible.

Considering the scope of the notification and focusing on the limited information provided by the notifier, the EFSA GMO Panel is of the opinion that the altered flower colour and the differences observed for some morphological characteristics are not expected to influence the risk scenario of accidental intake of the GM carnation.

It is not expected that the accidental intake of petals of carnation IFD-25958-3 would contribute substantially to the overall intake of anthocyanins from foods. Considering the scope of notification C/NL/09/01 and given that case reports of occupational allergies to carnations are rare, there are no indications that the genetic modification will increase the risk of allergy among those coming into contact with carnations. Considering the scope of notification C/NL/09/01 and the limited exposure scenarios expected, the EFSA GMO Panel identified no reasons for any safety concerns of carnation IFD-25958-3.

Carnation IFD-25958-3 cut flowers have marginal viability, negligible pollen production and few or no viable seeds. However, in the very unlikely event of escape into the environment via pollen/seeds or rooted plants, the EFSA GMO Panel considers that carnation IFD-25958-3 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides. Considering the scope of notification C/NL/09/01 and the low level of exposure to the environment, interactions with the biotic and abiotic environment are not considered to be relevant issues by the EFSA GMO Panel. The EFSA GMO Panel also concludes that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation IFD-25958-3 to environmental bacteria does not give rise to environmental safety concerns. The scope of the PMEM plan provided by the notifier is in line with the intended use of carnation IFD-25958-3. The EFSA GMO Panel agrees with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

In the light of the scope of notification C/NL/09/01 (import, distribution and retailing in the EU of carnation IFD-25958-3 cut flowers for ornamental use only), the EFSA GMO Panel concludes that there is no scientific reason to consider that the placing on the market of the GM carnation IFD-25958-3 will cause any adverse effects on human health or the environment.

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1. Introduction

Carnation IFD-25958-3 is a genetically modified (GM) variety of *Dianthus caryophyllus* L. used as a decorative plant species. The mauve colour of the flowers results from the expression of two newly introduced genes encoding flavonoid 3′,5′-hydroxylase (*f3′5′h*), dihydroflavonol 4-reductase (*dfr*) and a hairpin RNA interference (RNAi) gene, which down-regulates endogenous *dfr*. These three constructs, together with endogenous genes involved in the anthocyanin biosynthesis pathway, enable the biosynthesis of delphinidin in the petals. Carnation IFD-25958-3 also contains a mutated herbicide tolerance *als* gene coding for an acetolactate synthase (ALS) variant protein, used to facilitate the selection of GM shoots during the genetic transformation process.

In the present scientific opinion, the GM carnation IFD-25958-3 is evaluated by the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) in the light of the scope of notification C/NL/09/01 (import, distribution and retailing in the European Union (EU) of GM carnation cut flowers for ornamental use only).

Therefore, the EFSA GMO Panel evaluated the safety of carnation IFD-25958-3 for humans considering three possible routes of exposure through: (1) dermal contact, (2) inhalation and (3) accidental oral intake[5]. Owing to the scope of this notification, the EFSA GMO Panel did not assess the possible consequences of the intentional consumption of GM carnations by humans[6]. In relation to animals, both intentional and accidental oral intake were excluded from this opinion, as carnation IFD-25958-3 is not expected to enter the feed chain or to be accidentally consumed in the field (cultivation being excluded from the scope) (EFSA, 2009a).

Moreover, considering the scope of this notification, a very limited environmental exposure with respect to viable plant parts of the GM carnation is expected. Hence, the environmental risk assessment (ERA) is mainly concerned with the consequences of exposure 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.

1.1. Background and Terms of Reference as provided by requestor

In July 2009, the European Commission received the full notification (reference C/NL/09/01), together with the positive assessment report from the competent authority of the lead Member State, The Netherlands.

In accordance with Directive 2001/18/EC[7], the notification was then transmitted to the competent authorities of other Member States. Some of them raised comments and objections during the statutory 60-day consultation period. The notifier, Florigene, provided the Member States with additional information in response to those comments and objections. However, some Member States maintained their reservations at the end of the additional 45-day consultation period, in which case, the European Commission is required to follow the procedure of Article 18(1) of Directive 2001/18/EC. In accordance with Article 18(1), the European Commission therefore consulted the EFSA GMO Panel for a scientific opinion.

Against this background, the EFSA GMO Panel identified mainly general comments rather than reasoned objections as in the sense of Directive 2001/18/EC. Moreover, concerns raised by Member

[5] Accidental oral intake should be considered as unintentional, infrequent and/or of relatively short duration.

[6] The EFSA GMO Panel is aware of a food habit in certain populations to intentionally consume carnation petals as garnish; however, this intentional use is outside the scope of this notification.

[7] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1–39.

States that relate, for example, to traceability, labelling, socio-economics, molecular detection methodologies and their validation fall outside the remit of EFSA or its GMO Panel.

EFSA is requested, in accordance with Article 28 of Directive 2001/18/EC, 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 line IFD-25958-3 for import is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC.

2. Data and methodologies

2.1. Data

The present evaluation of the safety of the GM carnation IFD-25958-3 by the EFSA GMO Panel is based on the information provided in notification C/NL/09/01, including additional information[8] provided by the notifier, the assessment report of the Dutch competent authority, the concerns raised by Member States, relevant scientific publications and the experience gained in assessing GM carnations with similar traits (EFSA, 2006a, 2008; EFSA GMO Panel, 2014).

2.2. Methodologies

The EFSA GMO Panel performed its risk assessment in accordance with the principles of its guidance documents on the risk assessment of GM plants for non-food or non-feed purposes (EFSA, 2009a) and on the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010).

3. Assessment

3.1. Molecular characterisation

3.1.1. Concerns raised by Member States

No objection raised by any Member State remained at the end of the 45-day Member States' consultation period. Therefore, notwithstanding its own risk analysis, the EFSA GMO Panel had no specific concerns to address from the Member States on the molecular characterisation of GM carnation IFD-25958-3.

3.1.2. Evaluation of relevant scientific data

3.1.2.1. Transformation process and vector constructs

To develop the IFD-25958-3 line, carnation variety Cerise Westpearl (CW) was transformed using disarmed *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain AGL0, which carried the transformation vector pCGP3366.

The transformation vector pCGP3366 contained the following expression cassettes, which are needed to obtain the desired flower colour:

1. the dihydroflavonol 4-reductase (*dfr*) cassette, encompassing the promoter, the *dfr* coding sequence and the terminator, cloned as a whole from the *Petunia* × *hybrida*;
2. the flavonoid 3′,5′-hydroxylase (*f3′5′h*) cassette, containing the promoter sequence from *Antirrhinum majus* chalcone synthase (CHS) gene, the *f3′5′h* coding sequence from *Viola hortensis* derived from a complementary DNA (cDNA) clone and the terminator sequence of a gene encoding a *Petunia* × *hybrida* putative phospholipid transfer protein homologue;
3. the *dcdfr*hp cassette, containing the *Cauliflower mosaic virus* (*CaMV*) 35S promoter, a hairpin-forming construct targeted to specific, post-transcriptional down-regulation of

[8] See section 'Documentation provided to EFSA' below.

endogenous carnation *dfr* and the *CaMV* 35S terminator sequence. The hairpin-forming construct consisted of sense and antisense segments of the *dfr* coding sequence, separated by a petunia *dfr* intron.

These three cassettes were inserted into the plant genome to obtain the desired flower colour. In addition, the vector pCGP3366 contained the acetolactate synthase cassette (*als*), consisting of the *CaMV* 35S promoter and the coding region and the terminator sequence from a mutated *als* from the *SuRB* locus of *Nicotiana tabacum*. Acetolactate synthase provided tolerance to sulfonylurea herbicides and was used as a marker trait in the selection of transformants.

3.1.2.2. Transgene constructs in the genetically modified plants

Carnation IFD-25958-3 contains one insert consisting of the transfer DNA (T-DNA) region of the transformation vector pCGP3366. Southern blot and polymerase chain reaction (PCR) analyses indicated that no plasmid backbone sequences had been integrated into carnation IFD-25958-3. The sequences of the insert and the flanking regions were provided.

Bioinformatic analysis of the 5′ and 3′ flanking regions did not reveal disruption of known endogenous genes.

In order to assess if the open reading frames (ORFs) present within the insert and spanning the junction sites give rise to any safety issues, their putative translation products were compared to appropriate databases for similarities to known allergens and toxins by using suitable algorithms. There were no significant hits with known toxins. By using an 80-amino-acid sliding window approach, a 35 % sequence identity was found within the newly expressed protein ALS with a *Davidiella tassiana* protein (see section 3.3.2.2). By using the same approach, identity of over 35 % was found with allergen 'Amb a 4' from common ragweed *Ambrosia artemisiifolia* for ORF 5.242 within the insert. The putative translation product of ORF 5.242 is a 110-amino-acid sequence, generated from the reverse strand of the *als* gene cassette. Considering that ORF 5.242 is not in the codon frame intended to be expressed and does not include an ATG start codon, the likelihood that it is both transcribed and translated in carnation IFD-25958-3 is negligible. Furthermore, northern blot analysis did not reveal any other bands than the one expected for *als*. Searches of eight contiguous amino acids showed identity to a subtilisin protease allergen in *Bacillus licheniformis* for ORF 4.148 within the insert, putatively encoding a 25-amino-acid peptide. Considering that ORF 4.148 is not on the DNA strand of the insert intended to be transcribed and does not include an ATG, the likelihood that it is both transcribed and translated in carnation IFD-25958-3 is negligible.

3.1.2.3. Information on the expression of the insert

The presence of transcripts corresponding to *f3′5′h*, *dfr*, *dcdfr*hp and *als* genes in the petals was demonstrated using northern blot analysis. Confirmation of the functionality of *f3′5′h*, *dfr* and *dcdfr*hp was obtained visually from the flower colour, as well as from metabolite analysis using high-performance liquid chromatography (HPLC). Tolerance to sulfonylurea herbicides indicates the activity of the ALS protein.

3.1.2.4. Inheritance and stability of the inserted DNA

Carnation IFD-25958-3 was propagated vegetatively from April 2005 to September 2008, which represents multiple cycles of propagation. Furthermore, during 2007–2013, plants were cultivated in a field trial in Colombia and there were no incidents reported of flower colour change that would indicate genetic instability.

3.1.3. Conclusion

The molecular characterisation data establish that the carnation line IFD-25958-3 contains one insert, consisting of four expression cassettes responsible for the intended traits, i.e. mauve flower colour

conferred by the *dfr*, *dcdfr*hp and *f3′5′h* genes and herbicide tolerance conferred by the mutated *als* gene.

The results of bioinformatic analyses of the insert and flanking regions in carnation IFD-25958-3 did not indicate relevant similarities with known toxins. Identities with allergens were found in putative translation products of ORFs newly created by the genetic modification, but the likelihood that they are both transcribed and translated in carnation IFD-25958-3 is negligible. Sequence identity was found between the newly expressed protein ALS and an allergen from *Davidiella tassiana*. The safety relevance of this sequence identity is assessed in section 3.3.2.2.

3.2. Comparative analysis

3.2.1. Concerns raised by Member States

No objection remained among Member States concerning the comparative analysis of GM carnation IFD-25958-3 at the end of the 45-day Member States' consultation period.

3.2.2. Evaluation of relevant scientific data

Considering that there is no history of the use of carnations as food or feed to a significant degree, that the safety of carnations *per se* has never been assessed for food/feed uses and that the compositional profile of carnations is not known, the comparative compositional assessment as defined in the EFSA guidance documents (EFSA, 2006b; EFSA GMO Panel, 2011a) could not be fully applied to identify possible unintended effects in carnation IFD-25958-3 (see section 1 for further details).

3.2.2.1. Choice of comparator

Carnation IFD-25958-3 was compared with the non-GM carnation variety Cerise Westpearl (CW), which is the variety of carnation that was transformed to establish carnation IFD-25958-3. Carnation CW is characterised by cerise petals.

3.2.2.2. Compositional analysis

The contents of the anthocyanin colour pigments delphinidin, cyanidin and pelargonidin were determined in acetonitrile extracts of freeze-dried petals using HPLC in accordance with the method of Fukui et al. (2003). As these anthocyanins occur as glycosylated and/or acylated compounds in the plant, the analytical method used a hydrolysis step converting the pigments into their aglycones, allowing the determination of total delphinidin, total cyanidin and total pelargonidin and not the specific compounds in the plant.

The cerise flower petals of the comparator CW contained only cyanidin pigments (0.01 mg/g fresh weight (fw)) and pelargonidin pigments (1.06 mg/g fw), whereas those of the GM mauve-coloured carnation IFD-25958-3 contained delphinidin (0.54 mg/g fw), cyanidin (0.10 mg/g fw) and pelargonidin (0.02 mg/g fw). Delphinidin-based pigments were not observed in other plant tissues of the GM plants (stem, nodes, leaves and roots). This has been confirmed by HPLC studies of leaf and root material.

The altered levels and types of anthocyanins in carnation IFD-25958-3 account for the intended phenotypic changes in the flower colour. The altered flower colour is not expected to influence the risk scenario of accidental intake of the GM carnation.

3.2.2.3. Morphological traits and genetically modified phenotype

In total, 18 morphological characteristics were analysed in carnation IFD-25958-3 and its comparator (carnation CW) grown in a field trial in Australia, during the 2007–2008 season. An analysis of variance (ANOVA) identified eight significant differences between the GM carnation and its comparator. Thus, carnation IFD-25958-3 had a higher number of internodes per stem, a reduced length to the fifth node, a thinner stem at the fifth node, an increased calyx diameter and length, more

filaments, a reduced filament length and an increased number of petals per flower. In addition, the average number of days to flowering was shorter in carnation IFD-25958-3 than in carnation CW: 138 and 146 days, respectively. In response to a Member State comment, the notifier provided additional data from a field trial in Colombia. In that field trial, the average days to flowering and petal count per flower did not differ between carnation IFD-25958-3 and its comparator, whereas the other parameters that were statistically different in the Australian field trial were not investigated.

3.2.3. Conclusion

Considering the scope of the notification and focusing on the limited information provided by the notifier, the EFSA GMO Panel is of the opinion that the altered flower colour and the differences observed for some morphological characteristics are not expected to influence the risk scenario of accidental intake of the GM carnation. The relevance of the observed morphological differences for their potential environmental impacts is further assessed in section 3.4.3.1.

3.3. Food safety assessment

3.3.1. Concerns raised by Member States

No objection remained among Member States concerning the safety assessment of GM carnation IFD-25958-3 for humans at the end of the 45-day Member States' consultation period.

The possible need for an acute toxicity study with whole plant extracts to support the assessment of this GM carnation in relation to accidental intake by humans was suggested by a Member State and was also discussed by the EFSA GMO Panel. Such a study was not considered necessary, as the safety assessment is sufficiently supported by the available data (see sections 3.3.2.1(a) and (b)).

3.3.2. Evaluation of relevant scientific data

3.3.2.1. Toxicology

(a) Toxicological assessment of newly expressed proteins

Bioinformatic analyses of the amino acid sequences of the newly expressed proteins in carnation IFD-25958-3 reveal no significant similarities to known toxins to humans.

The EFSA GMO Panel has previously assessed the safety of the ALS, DFR and F3′5′H proteins and no reasons for concern were identified (EFSA, 2006a, 2008).

(b) Toxicological assessment of new constituents other than proteins

The anthocyanins delphinidin and cyanidin are present in carnation IFD-25958-3 at higher levels than in the non-GM carnation. These anthocyanins can also be found in many foods and, in some of them, at much higher concentrations than in the petals of carnation IFD-25958-3. Particularly high concentrations can be found, for example, in blackcurrants, aubergines, blueberries and red grapes (Cacho et al., 1992). According to Regulation 1333/2008[9] on food additives, anthocyanins (E 163) are authorised food additives in the EU. Anthocyanins have been evaluated by the 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. The SCF indicated that anthocyanins derived from natural sources are only acceptable as food additives if the quantities ingested do not differ substantially from the amounts that are likely to be ingested as a result of the normal consumption of the foods in which they occur naturally (SCF, 1975). In the re-evaluation of anthocyanins, the Scientific Panel on Food Additives and Nutrient Sources Added to Food of EFSA (EFSA ANS Panel, 2013) concluded that, provided that exposure from the use of food colours is

[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

comparable to that from the diet, the conclusion on safety in the 1975 opinion would still apply to anthocyanins extracted by aqueous processes from edible fruits and vegetables.

It is not expected that the accidental intake of petals of carnation IFD-25958-3 would contribute substantially to the overall intake of anthocyanins from foods. Therefore, the EFSA GMO Panel sees no reason for concern regarding the presence of delphinidin and cyanidin in petals of carnation IFD-25958-3.

In addition, the notifier also performed a study on gene mutations in bacteria using *Salmonella enterica* Typhimurium (Ames test) with water extracts of leaves or petals of carnation IFD-25958-3 and the control carnation CW. The water extracts did not show mutagenic activity under the conditions of the assay.

(c) Toxicological assessment of the whole genetically modified plant

Given that carnation IFD-25958-3 is not intended for human consumption as food but is intended for ornamental use only, the EFSA GMO Panel considered the possible effects of the genetic modification on human health in the case of accidental intake (EFSA, 2009a). Considering the assessment of the newly expressed proteins (section 3.3.2.1(a)) and of the new constituents other than proteins (section 3.3.2.1(b)), the EFSA GMO Panel identified no reasons for concern.

3.3.2.2. Allergenicity

(a) Allergenicity assessment of newly expressed proteins

Bioinformatic analyses of the amino acid sequence of the newly expressed proteins in carnation IFD-25958-3 using the criterion of more than 35 % identity in a segment of 80 or more amino acids (Codex Alimentarius, 2003) revealed no significant similarities to known allergens. In addition, the notifier performed analyses searching for matches of eight contiguous identical amino acid sequences between these newly expressed proteins and known allergens, which confirmed the outcome of the above-mentioned bioinformatic analyses showing no similarities to known allergens.

The EFSA GMO Panel has previously assessed the safety of the ALS, DFR and F3′5′H proteins and no reasons for concern were identified (EFSA, 2006a, 2008).

The EFSA GMO Panel noted that 35 % sequence identity over a window of 80 amino acids between the ALS protein and an allergen from *Davidiella tassiana* was reported. However, this hit was not considered relevant because the percentage of identity was not greater than 35 % and, in addition, a high E-value[10] was seen.

(b) Allergenicity assessment of the whole genetically modified plant

Occupational allergy (dermal and respiratory allergies) to carnations in workers handling cut flowers/carnations over a long time has been described (Sanchez-Guerrero et al., 1999; Cistero-Bahima et al., 2000; Sanchez-Fernandez et al., 2004; Stefanaki and Pitsios, 2008)[11]. This allergy could be caused by the flower, by mites such as *Tetranychus urticae* infesting carnations or by both simultaneously. Nevertheless, case reports of occupational allergies to carnations are rare.

More recently, a case report of an individual with a respiratory allergy to carnations with no occupational exposure was published (Brinia et al., 2013)[11].

[10] An alignment derived from a FASTA search of a database is accompanied with an E-value, which represents the number of times the corresponding alignment score is expected at chance.
[11] Additional information 28 August 2014.

According to the notifier, no adverse reactions (including allergenicity or contact dermatitis) to GM carnation IFD-25958-3 cut flowers used for ornamental purposes have been reported in the populations handling the flowers (growers, distributors and purchasers) where it is produced and/or marketed[11].

Considering the scope of notification C/NL/09/01 and given that case reports of occupational allergies to carnations are rare, there are no indications that the genetic modification will increase the risk of allergy among those coming into contact with carnations.

3.3.3. Conclusion

Carnation flowers have a long history of use as ornamentals. Carnation IFD-25958-3 differs from the non-GM carnation in that it synthesises anthocyanins, mainly delphinidin and cyanidin, in the petals, which confer a mauve colour to the flowers. Delphinidin and cyanidin are common pigments in many ornamental flowers and food plants such as red grapes, blackcurrants, aubergines and blueberries. It is not expected that accidental intake of petals of carnation IFD-25958-3 would contribute substantially to the overall intake of anthocyanins from foods.

Considering the scope of notification C/NL/09/01 and given that case reports of occupational allergies to carnations are rare, there are no indications that the genetic modification will increase the risk of allergy among those coming into contact with carnations.

Considering the scope of notification C/NL/09/01 and the limited exposure scenarios expected, the EFSA GMO Panel identified no reasons for any safety concerns of carnation IFD-25958-3.

3.4. Environmental risk assessment and post-market environmental monitoring plan

3.4.1. Concerns raised by Member States

Some Member States expressed concerns related to the possible illegal propagation of carnation IFD-25958-3 through vegetative multiplication by individuals. More details on the methods and implementation of the post-market environmental monitoring (PMEM) plan were requested by some Member States.

3.4.2. Evaluation of relevant scientific data

Considering the scope of notification C/NL/09/01, there will be very limited environmental exposure with respect to viable plant parts of the GM carnation. The ERA is mainly concerned with the consequences of exposure 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.

3.4.3. Environmental risk assessment[12]

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

Carnation is the common name of *Dianthus caryophyllus* (i.e. cultivated carnation). Members of the genus *Dianthus*, including wild and domesticated species, are fairly diverse, as their origins range from southern Russia to the Alpine region of Greece and the Auvergne mountains of France. *Dianthus* spp. 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 plant in Europe both in glasshouses and outdoors (i.e. in Italy and Spain) and is occasionally naturalised in some Mediterranean countries

[12] Notification C/NL/09/01, Section B.

but appears to be restricted to the coastal Mediterranean regions of Greece, Italy, Sicily, Corsica and Sardinia (Tutin et al., 1993).

Although *Dianthus* spp. do not spread vegetatively through organs such as bulbs, stolons or rhizomes, the cultivated carnations can be vegetatively propagated to produce plants for cut flower production. Cuttings are taken from 'mother plants/stems' which are continually pruned to produce a large 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 one to two years. Flowers are produced in flushes, beginning three to five months after rooted cuttings are planted.

The majority of *Dianthus* spp. is 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 produces only a negligible amount of pollen, and consequently seed set is low or absent (Galbally and Galbally, 1997). The quantity and quality of pollen varies with the cultivar (Kho and Baer, 1973; Galbally and Galbally, 1997). Carnation pollen is heavy and sticky and has low viability. Wind plays little role in pollen dispersal (OGTR, 2006). In the wild, cross-pollination of *Dianthus* spp. is by insect pollinators, in particular by Lepidoptera, which have probosces of sufficient length to reach the nectaries at the base of the flowers.

Carnation IFD-25958-3 has a modified flower colour resulting from the expression of two newly introduced genes encoding *f3′5′h*, *dfr* and a hairpin RNAi gene, which down-regulates endogenous *dfr*. These three constructs, together with endogenous genes involved in the anthocyanin biosynthesis pathway, enable the biosynthesis of delphinidin in the petals. These anthocyanins are also widely found, for example, 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 IFD-25958-3 contains a mutated *als* gene conferring tolerance to sulfonylurea herbicides. Given that the ALS enzyme is needed for the biosynthesis of some branched-chain amino acids such as isoleucine, ALS-inhibiting herbicides cause the death of the plant by interfering with this biosynthesis pathway. In relation to this, Tranel and Wright (2002) reported that tolerance to ALS-inhibiting herbicides was widespread among weeds and was mostly due to a mutated *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 in 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 exploiting niches in a wide geographical range in Europe (Tutin et al., 1993). The EFSA GMO Panel considered that a small change in seed germination characteristics induced by ALS tolerance is unlikely to be outside the current range of seed germination characteristics currently expressed by non-GM carnations and thus is unlikely to have an ecological impact.

In the very unlikely event of gene transfer to cultivated carnations, they may express the mutated *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[13] on cultivated carnations. Such herbicide-tolerant plants can be managed by a range of measures (Tranel and Wright, 2002). There is no evidence that the traits introduced by the genetic modification would result in increased persistence and invasiveness of a plant species.

[13] See additional information provided by the notifier to Member States after the 60-day consultation period.

In general, carnation varieties compete poorly outside their cultivated environment. In addition, carnation varieties do not show weedy characteristics. In notification C/NL/09/01, the notifier presented morphological or phenotypic data gathered from a field trial conducted in Australia during the 2007–2008 season (see section 3.2.2.3)[14]. A total[15] of 18 morphological characteristics were evaluated for the GM carnation, in comparison with the parental line CW. Statistically significant differences were observed between the GM carnation and its parental line for 8 out of the 18 characteristics studied. Carnation IFD-25958-3 had a higher number of internodes per stem, a reduced length to the fifth node, a thinner stem at the fifth node, an increased calyx diameter and length, more filaments, a reduced filament length and an increased number of petals per flower. The notifier attributed these variations in morphological characters to environmental factors. The notifier also reported from the 2007–2008 Australian field trial a lower average number of days to flowering for the GM carnation than its parental line. However, the notifier did not observe any difference in the number of petals per flower and mean days to flowering between the GM and non-GM carnation from a supplementary field trial carried out in Colombia. The EFSA GMO Panel is of the opinion that, owing to their morphological nature, these characteristics for which differences were observed are unlikely to affect the survival, establishment and fitness of the GM carnation.

Moreover, the EFSA GMO Panel is not aware of any scientific reports of increased spread and establishment of (GM) carnations or of any change in survival capacity, including overwintering (COGEM report[16]; EFSA, 2006a, 2008). In addition, *D. caryophyllus* has been imported into all EU countries as a garden ornamental plant and cut flower for many decades and EFSA is not aware of any reports of feral populations that have established outside of cultivation.

In order to assess the likelihood of unintended effects on the environment, the EFSA GMO Panel followed a weight-of-evidence approach in collating and assessing appropriate information from various data sources (e.g. molecular data, available phenotypic data from field trials performed by the notifier, literature and previous evaluations of similar transformation events (EFSA, 2006a, 2008; EFSA GMO Panel, 2014)). Therefore, considering the scope of notification C/NL/09/01 and the data available, the EFSA GMO Panel considered that there would be very little exposure of other *Dianthus* populations and no changes in plant characteristics of any ecological significance. Carnation IFD-25958-3 plants would show changed fitness characteristics only when exposed to sulfonylurea herbicides, but these herbicides are not used in carnation cultivation or in habitats where wild *Dianthus* spp. might occur. The EFSA GMO Panel also concludes that the propagation of the GM carnation (e.g. rooting) by individuals cannot be excluded. However, should this occur, carnation IFD-25958-3 would not show any potential for increased survival, fitness or weediness compared with its parental line.

3.4.3.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, through either horizontal gene transfer of DNA or vertical gene flow via seed dispersal and cross-pollination.

(a) Plant-to-bacteria gene transfer

Considering the scope of notification C/NL/09/01, the ERA is concerned with exposure through discarded GM carnation cut flowers resulting in possible exposure of environmental bacteria to

[14] Notification C/NL/09/01, Attachment A12.

[15] Plant height (mm), number of internodes per stem, length of fifth node (mm), thickness of fifth node (mm), leaf length of third node from the top (mm), height of corolla (mm), flower diameter (mm), calyx diameter (mm), calyx length (mm), number of lobes per calyx, number of petals per flower, petal length (mm), petal width (mm), number of styles, style length (mm), number of viable anthers, filament length (mm), number of filaments.

[16] Available online: http://www.cogem.net/index.cfm/en/publications/publicatie/advisory-report-import-distribution-and-retail-of-cut-flowers-with-modified-flower-colour-gm-carnation-shd-27531-4

recombinant DNA. If accidental intake of these GM carnations by humans occurs[17] (see section 3.3), it is likely to be at very low levels so that exposure of gastro-intestinal tract bacteria and microfloral decomposers of faecal material will be very low.

Current scientific knowledge of recombination processes in bacteria indicates that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as plants to microorganisms) is not likely to occur at detectable frequencies under natural conditions (see EFSA, 2009b, for further details).

Successful horizontal gene transfer would require the stable insertion of the transgene sequences into a bacterial genome and a selective advantage conferred on the transformed host. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules and, in addition to substitutive gene replacement, facilitates the insertion of non-homologous DNA sequences if their flanking regions have sequence similarity with bacterial sequences in the recipient.

Carnation IFD-25958-3 does not contain genetic elements with identity or high similarity to those of bacteria. The recombinant genetic elements used for the construction of carnation IFD-25958-3 originate from plants, i.e. *Petunia*, *Viola*, *Antirrhinum* and *Nicotiana tabacum* (tobacco) (for more details, see section 3.1.2.1). Owing to the absence of DNA with high similarity to that of bacteria, there is no indication of facilitated transfer of recombinant genes to bacteria when it is compared with the transfer of genes from non-GM carnations. Thus, based on the data provided by the notifier, no increased likelihood of horizontal gene transfer from carnation IFD-25958-3 to environmental bacteria is expected. The EFSA GMO Panel could not identify any selective advantage which would be provided to environmental bacteria when receiving the recombinant DNA of carnation IFD-25958-3.

Therefore, considering the scope of notification C/NL/09/01, the EFSA GMO Panel concluded that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation IFD-25958-3 to environmental bacteria does not give rise to environmental safety concerns.

(b) Plant-to-plant gene transfer

Considering the scope of notification C/NL/09/01, the ERA is mainly concerned with indirect exposure 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 and (3) dispersal of seeds produced by GM cut flowers and possible progeny.

Carnation IFD-25958-3 plants 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. The latter is a multiplication technique applied in the laboratory which requires particular expertise and adequate material for successful tissue culture. The EFSA GMO Panel is of the opinion that this technique is unlikely to be used by individuals (e.g. amateur gardeners) to propagate GM carnations. However, the GM carnation could be propagated by rooting and then released into the environment (e.g. gardens). The EFSA GMO Panel therefore considered the consequences of such potential releases (see section 3.4.3.1) and concluded that, should this occur, carnation IFD-25958-3 would not show any potential for increased survival, fitness or weediness compared with its parental line.

Wind plays little role in pollen dispersal of *Dianthus* spp. (OGTR, 2006). In the wild, cross-pollination of *Dianthus* spp. is by insect pollinators, in particular by Lepidoptera, which have probosces of sufficient length to reach the nectaries at the base of the flowers. However, the GM carnation has double flowers with a high density of petals. These obstruct insect pollinators from probing the

[17] Accidental oral intake should be considered as unintentional, infrequent and/or of relatively short duration.

flowers to reach the nectaries and therefore discourage insect pollinator activity and limit the amount of pollen they collect and transfer to other flowers. The EFSA GMO Panel is of the opinion that the potential spread of pollen of the GM carnation by Lepidoptera to wild *Dianthus* spp. cannot be eliminated but is highly unlikely to occur and, if it did occur, it is very unlikely that viable hybrids would be produced, survive and cause adverse environmental effects (see section 3.4.3.1).

Moreover, the reproductive biology of *Dianthus* (OGTR, 2006) and the information provided by the notifier suggest that pollen production by flowers and pollen viability are low. Moreover, in notification C/NL/09/01, the notifier pointed out that '*flowers must be harvested in tight bud (or closed bud for spray types) for distribution and marketing*', which rules out pollination during production, but suggests it could occur post-marketing. However, the data indicate that pollen transfer to other carnations is very unlikely to occur owing to very low fertility levels in most carnations. In addition, viable seed production of cut flowers is very unlikely and has not been observed to date with carnation IFD-25958-3, most probably because of its limited life time (i.e. three weeks) in comparison with the time needed for complete seed development (i.e. five weeks).

The EFSA GMO Panel also considered the possibility of natural exchange of genetic material with other carnation varieties, *Dianthus caryophyllus* L., and wild *Dianthus* species. Although hybridisation is mentioned in some floristic surveys, the EFSA GMO Panel is not aware of reports of gene flow between cultivated carnations and wild *Dianthus* spp. in the literature. The probability of spontaneous hybridisation between the GM carnation and other cultivated carnations or wild relatives, and then the establishment of viable hybrids, is considered to be very low.

Therefore, taking account of the very low potentials for hybridisation and/or seed production of (GM) carnations, the EFSA GMO Panel concludes that plant-to-plant gene transfer of the introduced genes is very unlikely and, if it did occur, it is unlikely to result in viable seed production leading to adverse environmental effects.

3.4.3.3. Potential interactions of the genetically modified plant with target organisms

Considering the scope of notification C/NL/09/01 and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered a relevant issue by the EFSA GMO Panel.

3.4.3.4. Potential interactions of the genetically modified plant with non-target organisms

Considering the scope of notification C/NL/09/01 and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered a relevant issue by the EFSA GMO Panel.

3.4.3.5. Potential interactions with the abiotic environment and biogeochemical cycles

Considering the scope of notification C/NL/09/01 and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the EFSA GMO Panel.

3.4.4. Post-market environmental monitoring[18]

According to Annex VII of Directive 2001/18/EC, the objectives of a post-market environmental monitoring (PMEM) 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 ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment that were not anticipated in the ERA.

[18] See notification C/NL/09/01, Section D.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the notifier (EFSA GMO Panel, 2011b). The potential exposure to the environment of carnation IFD-25958-3 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 scope of the PMEM plan provided by the notifier is in line with the restricted intended use of GM carnation cut flowers.

The PMEM plan proposed by the notifier includes (1) a questionnaire for the European importers and operators, including questions on unexpected adverse effects and '*illegal growing*'; (2) the involvement of existing networks (i.e. national plant protection services); and (3) the consultation of a network of European taxonomists, botanists and breeders to report on any wild populations or unusual *Dianthus* hybrids that might originate from the GM carnation. In addition, the notifier plans to survey the production sites in Colombia and Ecuador to report diverse observations, including adverse effects and the incidence of genetic off-types. The notifier proposes to submit a PMEM report on an annual basis. The report will include, for example, the number of imported GM cut flowers and a report of the identified hybrids and of feral carnation populations, if any.

The EFSA GMO Panel is of the opinion that the scope of the PMEM plan proposed by the notifier is in line with the restricted intended use of carnation IFD-25958-3, as the ERA did not cover cultivation and identified no potential adverse environmental effects. No case-specific monitoring is required.

3.4.5. Conclusion

Carnation IFD-25958-3 cut flowers have marginal viability, negligible pollen production and few or no viable seeds. However, in the very unlikely event of escape into the environment via pollen/seeds or rooted plants, the EFSA GMO Panel considers that carnation IFD-25958-3 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides. Considering the scope of notification C/NL/09/01 and the low level of exposure to the environment, interactions with the biotic and abiotic environment are not considered to be relevant issues by the EFSA GMO Panel. The EFSA GMO Panel also concluded that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation IFD-25958-3 to environmental bacteria does not give rise to environmental safety concerns. The scope of the PMEM plan provided by the notifier is in line with the intended use of carnation IFD-25958-3. The EFSA GMO Panel agreed with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

4. Conclusions

In the light of the scope of notification C/NL/09/01 (import, distribution and retailing in the EU of carnation IFD-25958-3 cut flowers for ornamental use only), the EFSA GMO Panel concludes that there is no scientific reason to consider that the placing on the market of the GM carnation IFD-25958-3 will cause any adverse effects on human health or the environment.

DOCUMENTATION PROVIDED TO EFSA

1. Notification C/NL/09/01 under Part C of Directive 2001/18/EC submitted by Florigene to the European Commission, and received from the European Commission on 4 April 2013.

2. Letter from the European Commission, dated 4 April 2013, to the EFSA Executive Director concerning a request for the placing on the market of genetically modified carnation IFD-25958-3 under Directive 2001/18/EC by Florigene.

3. Acknowledgement letter, dated 7 June 2013, from EFSA to the European Commission.

4. Letter from EFSA to the notifier, dated 24 July 2013, requesting additional information.

5. Letter from the notifier to EFSA, received on 8 November 2013, providing additional information.
6. Letter from EFSA to the notifier, dated 19 December 2013, requesting additional information.
7. Letter from the notifier to EFSA, received on 23 December 2013, providing additional information.
8. Letter from EFSA to the notifier, dated 3 April 2014, requesting additional information.
9. Letter from the notifier to EFSA, received on 25 April 2014, providing additional information.
10. Letter from EFSA to the notifier, dated 29 July 2014, requesting additional information.
11. Letter from the notifier to EFSA, received on 26 August 2014, providing additional information.
12. Letter from EFSA to the notifier, dated 2 October 2014, requesting additional information.
13. Letter from the notifier to EFSA, received on 29 October 2014, providing additional information.

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