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Risk assessment of genetically modified carnation FLO-40685-2

Scientific opinion on genetically modified carnation FLO-40685-2 from Suntory Holdings Ltd with modified petal colour for import as cut flowers for ornamental use under Part C of Directive 2001/18/EC (Notification C/NL/13/02)

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016:35
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genetically modified carnation FLO-40685-2 from Suntory Holdings Ltd. with modified petal
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(Application C/NL/13/02).

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Scientific opinion on genetically modified carnation FLO-40685-2 from Suntory Holdings Ltd. with modified petal colour for import as cut flowers for ornamental use under Part C of Directive 2001/18/EC (Notification C/NL/13/02)

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Assessed and approved

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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

Carnation FLO-40685-2 is a genetically modified variety of *Dianthus caryophyllus* L. used as a decorative plant species. The purple colour of the flowers results from expression of the two introduced genes *dfr* and *f3'5'h*, encoding the enzymes dihydroflavonol 4-reductase (DFR) and flavonoid 3',5'-hydroxylase (F3'5'H). Together with endogenous enzymes involved in the anthocyanin biosynthesis pathway, DFR and F3'5'H enables the production of the anthocyanidins (plant pigments) delphinidin and cyanidin in the flower petals. Anthocyanidins and their sugar derivatives, anthocyanins, make up a large group of natural colours and are accepted food additives (E 163). The colours of most flowers, berries and fruits consist of a combination of anthocyanidins and anthocyanins.

Carnation line FLO-40685-2 also contains a mutated herbicide tolerance gene from *Nicotiana tabacum*, coding for an acetolactate synthase (ALS) variant protein, used to facilitate the selection of GM plantlets during the genetic transformation process. There are four transgenic inserts in the genome of FLO-40685-2, at four different loci. Only one of the loci includes the intended full length T-DNA sequence. Flanking sequences indicate no disruption of endogenous genes by the inserts. *In silico* analyses show no significant homologies between the DFR, F3'5'H and ALS proteins and known toxins or IgE-bound allergens. Expression of any new open reading frame(s) (ORF) with significant similarity to known toxin(s) or allergen(s) is highly unlikely. No observed changes in the introduced trait, *i.e.* the particular flower colour, indicative of instability, have been reported during numerous generations of vegetatively propagated plants since 1997.

Considering that carnation FLO-40685-2 is not intended for cultivation or use as food or feed, the VKM GMO Panel considers the comparative analysis of the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin in the flower petals sufficient for the risk assessment. The reported morphological differences between FLO-40685-2 and the conventional counterpart (parental variety) Cream Cinderella do not raise safety concerns.

Based on current knowledge and the scope of the application, the VKM GMO Panel concludes that the DFR, F3'5'H and ALS proteins and anthocyanidin pigments are unlikely to increase a potential health risk related to an accidental intake or other exposure routes to carnation FLO-40685-2 compared to the conventional counterpart or other non-GM carnations.

Likewise, the VKM GMO Panel concludes that carnation FLO-40685-2, based on current knowledge and the intended use as cut ornamental flowers, does not represent an environmental risk in Norway.

Summary

The Norwegian Scientific Committee for Food Safety (VKM) was asked by the Norwegian Environment Agency to deliver a scientific opinion on notification C/NL/13/02 from Suntory Holding Ltd submitted under Part C of EU Directive 2001/18. The scope of the notification C/NL/13/02 covers import, distribution and retailing of cut flowers of genetically modified carnation FLO-40685-2 for ornamental use in the EU/European Economic Area (EEA).

The flower petals of carnation FLO-40685-2 have a modified deep purple colour, whereas the parental variety (cream Cinderella) has white petals. The modified colour results from expression of the two introduced genes *dfi* and *f3'5'h* from *Petunia x hybrida* and *Viola hortensis*, respectively, encoding the enzymes dihydroflavonol 4-reductase (DFR) and flavonoid 3',5'-hydroxylase (F3'5'H). Together with endogenous enzymes involved in the anthocyanin biosynthesis pathway, DFR and F3'5'H enables the production of the anthocyanidins (plant pigments) delphinidin and cyanidin responsible for the purple colour. Anthocyanidins and their sugar derivatives, anthocyanins, make up a large group of natural colours and are accepted food additives (E 163). The colours of most flowers, berries and fruits consist of a combination of anthocyanidins and anthocyanins.

Carnation FLO-40685-2 also contains a mutated *als*- gene encoding a variant of the acetolactate synthase (ALS) enzyme that confers tolerance to ALS-inhibiting herbicides, such as chlorimuron, thifensulfuron and sulfonyleureas. A property used to facilitate the selection of GM plantlets during the genetic transformation process.

The current risk assessment of carnation FLO-40685-2 is based on information provided by the applicant in the notification C/NL/13/02, relevant peer-reviewed scientific literature, and scientific opinions from EFSA (EFSA 2016, Appendix I) and VKM (VKM, 2015 a,b,c).

The VKM GMO Panel has evaluated carnation FLO-40685-2 with reference to its intended use in the European Economic Area, and according to the principles described in the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. VKM has also decided to take account of the appropriate principles described in the EFSA guidelines on the risk assessment of GM plants used for non-food/feed purposes (EFSA, 2009a), the 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, 2011b).

The scientific risk assessment of carnation FLO-40685-2 considers the 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.

Molecular characterisation

The molecular characterisation shows that carnation FLO-40685-2 has four transgenic inserts in its genome at four different loci. Only one of the loci contains the intended full length T-DNA sequence with functional copies of each of the three genes *dfrr*, *f3'5'h* and *als*. The three other loci contain different partial versions of the T-DNA. Analyses of flanking sequences indicate no disruption of endogenous genes by the inserts. Bioinformatic analyses performed by the applicant show no significant homologies between the DFR, F3'5'H and ALS proteins and known toxins or allergens. Analyses of new open reading frames (ORFs) indicate that expression of any ORF showing significant similarity to known toxins or allergens is highly unlikely. Consistency of the intended new flower colour has been observed over multiple vegetative generations since 1997 indicating stability of the inserts in carnation FLO-40685-2.

Based on current knowledge and considering the intended use of carnation FLO-40685-2 as cut flowers the VKM GMO Panel finds the molecular characterisation of carnation FLO-40685-2 sufficient.

Comparative assessment

Considering the intended use of carnation FLO-40685-2, which excludes cultivation and use in food and feed, compositional studies were limited to analyses of the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin. The presence of delphinidin and cyanidin in carnation FLO-40685-2 and absence of all four anthocyanidins in the non-GM parental variety, measured by HPLC, confirmed the intended effects of the genetic modification. Other morphological traits were assessed in field trials and revealed that carnation FLO-40685-2 differed significantly in several traits compared to the parental variety. None of the reported differences in compositional or morphological traits are expected to influence the risk scenario upon accidental release to the environment or accidental intake or exposure to the GM carnation.

Based on current knowledge and considering the intended use of carnation FLO-40685-2, which excludes cultivation and use as food or feed, the VKM GMO Panel concludes that the comparative analysis is sufficient for the risk assessment. The reported morphological differences between FLO-40685-2 and its conventional counterpart do not raise safety concerns.

Food and feed risk assessment

In silico analyses performed by the applicant show no relevant sequence resemblance of the DFR, F3'5'H and ALS proteins to known toxins or IgE-bound allergens, and none of the proteins are known to cause allergic or toxic reactions. The anthocyanidin pigments produced in carnation FLO-40685-2 are natural constituents of numerous plant foods and are accepted as food additives.

Based on this and considering the scope of the application, the VKM GMO Panel concludes that the DFR, F3'5'H and ALS proteins and anthocyanidin pigments are unlikely to increase a potential health risk related to an accidental intake or other exposure routes to carnation FLO-40685-2 compared to the conventional counterpart or other non-GM carnations.

Environmental assessment

Considering the intended use of FLO-40685-2, 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, FLO-40685-2 has no altered survival, multiplication or dissemination characteristics compared to conventional carnation varieties, 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 FLO-40685-2 does not represent an environmental risk in Norway.

Post-market environmental monitoring

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

Based on current knowledge and considering its import, distribution and intended use as cut ornamental plants, the VKM GMO Panel concludes that the environmental risk assessment did not identify any potential adverse environmental effects of the transgenic line of carnation FLO-40685-2. 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 FLO-40685-2 is not intended for cultivation or use as food or feed, the VKM GMO Panel considers the comparative analysis of the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin in the flower petals sufficient for the risk assessment. The reported morphological differences between FLO-40685-2 and the parental variety do not raise safety concerns.

Based on current knowledge and the scope of the application, the VKM GMO Panel concludes that the DFR, F3'5'H and ALS proteins and anthocyanidin pigments are unlikely to increase a potential health risk related to an accidental intake or other exposure routes to carnation FLO-40685-2 compared to the conventional counterpart or other non-GM carnations.

Likewise, the VKM GMO Panel concludes that carnation FLO-40685-2, based on current knowledge and the intended use as cut ornamental flowers, does not represent an environmental risk in Norway.

Key words: GMO, carnation (*Dianthus caryophyllus* L.), FLO-40685-2, anthocyanin, anthocyanidin, petal colour, *dfr*, *f3'5'h*, *als*, *SuRB*, health safety, environmental risk evaluation, Directive 2001/18, VKM, risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Environment Agency

Sammendrag på norsk

Vitenskapskomiteen for mattrygghet (VKM) er bedt av Miljødirektoratet om å levere en vitenskapelig vurdering av den genmodifiserte nelliken FLO-40685-2 (unik kode FLO-40685-2, tidligere FLO-40685-1) fra Suntory Holdings Limited. Nelliklinjen er søkt godkjent til import og salg som avskårne prydblomster under EUs utsettingsdirektiv 2001/18/EC.

Notifiseringen C/NL/13/02 omfatter nellikplanter som er produsert ved vegetativ formering, og omfatter ikke avledete sorter fra konvensjonelle kryssinger med FLO-40685-2.

FLO-40685-2 har ikke tidligere vært vurdert av VKMs faggruppe for GMO.

Risikovurderingen av den genmodifiserte nelliklinjen er basert på søkers dokumentasjon og uavhengige vitenskapelige publikasjoner, samt vitenskapelige vurderinger fra EFSA (EFSA 2016, Appendix I) og VKM (VKM 2015 a,b,c).

Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med 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 EFSA's retningslinjer for 1) risikovurdering av GM-planter for andre formål enn mat og fôr (EFSA 2009a), 2) miljørisikovurdering av GM-planter (EFSA 2010a), 3) valg av komparatorer for risikovurdering av GM-planter (EFSA 2011a) og 4) miljøovervåking av GM-planter etter markedsføring (EFSA 2011b) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsmetoden og vektorkonstruksjonen, karakterisering og nedarving av genkonstruksjonen, komparativ analyse av antocyanidininnhold i kronbladene og andre morfologiske egenskaper, toksiner, allergener og nye proteiner. Videre er potensiale for utilsiktede effekter på fitness, genoverføring, mulige effekter på målorganismer, ikke-målorganismer og biogeokjemiske prosesser vurdert.

Det presiseres at VKMs mandat ikke omfatter vurderinger av etikk, bærekraft og samfunnsnytte, i henhold til kravene i den norske genteknologiloven og dens konsekvensutredningsforskrift. Disse aspektene blir derfor ikke vurdert av VKMs faggruppe for genmodifiserte organismer.

Nellik FLO-40685-2 uttrykker tre nye egenskaper: *dfr*-genet fra *Petunia x hybrida*, som koder for enzymet dihydroksoflavonol-reduktase (DFR), og *f3'5'h*-genet fra *Viola hortensis*, som koder for enzymet flavonol 3',5'-hydroksylase (F3'5'H). Enzymene fører til produksjon av to antocyanidiner i kronbladene, hovedsakelig delfinidin (98%), men også noe cyanidin (2%). Antocyanidiner utgjør fargestoffkomponenter i en stor gruppe av naturlige pigmenter utelukkende i planteriket kalt antocyaniner (glykosider av antocyanidiner). Delfinidin og cyanidin finnes blant annet i mange bær, frukt og grønnsaker, og er godkjente tilsetningsstoffer i mat. Delfinidin er årsaken til den mørke lillafargen på blomstene til Nellik

FLO-40685-2. I tillegg inneholder FLO-40685-2 et mutert *als* (*SuRB*) gen fra tobakksplanten *Nicotiana tabacum*, som koder for en variant av acetolaktatsyntase (ALS)-enzymet. Enzymet gir økt toleranse for ALS-inhiberende ugressmidler som f.eks. sulfonylureaer, brukt til identifikasjon av transformerte planter under utvikling av nelliklinjen.

Molekylær karakterisering

Den molekylære karakteriseringen viser at nellik FLO-40685-2 inneholder fire transgene innskudd på fire ulike steder (loci) i genomet. Kun ett av innskuddene, ved ett locus, inneholder en fullstendig kopi av den ønskede T-DNA-sekvensen med fungerende kopier av hver av de tre nye genene *dfi*, *f3'5'h* og *als*. De tre andre innskuddene inneholder diverse ufullstendige versjoner av T-DNAet. Sekvensanalyser indikerer at ingen av de transgene innskuddene er satt inn i allerede uttrykte kodende områder av genomet. Databasesøk utført av søker viser ingen relevante likheter mellom de nye proteinene DFR, F3'5'H og ALS, og kjente toksiner og allergener. Uttrykk av nye åpne leserammer med potensiell likhet til kjente toksiner eller allergener er usannsynlig. Jevnt uttrykt blomsterfarge over atskillige vegetativt formerte generasjoner siden 1997 indikerer stabilitet av de innsatte genene i nellik FLO-40685-2.

Basert på dagens kunnskap, og tiltenkt bruk som avskårne blomster, konkluderer VKMs faggruppe for GMO at den molekylære karakteriseringen av nellik FLO-40685-2 er tilstrekkelig.

Komparative analyser

Med hensyn på tiltenkt bruksområde som ekskluderer dyrking og bruk i mat og fôr, ble kun innhold av antocyanidin-pigmentene delphinidin, cyanidin, petunidin og pelargonidin analysert av søker. Delphinidin og cyanidin ble begge påvist ved HPLC i nellik FLO-40685-2. Ingen av de undersøkte pigmentene kunne påvises i den umodifiserte foreldresorten (konvensjonell kontroll), noe som bekreftet den tilsiktede effekten av genmodifiseringen. Flere statistisk signifikante morfologiske forskjeller mellom den genmodifiserte nelliken og konvensjonell kontroll ble i tillegg vist i ulike feltstudier. Ingen av de rapporterte forskjellene i sammensetning eller morfologiske egenskaper er forventet å ha innvirkning på risikoscenarier ved utilsiktet miljøeksponering, håndtering eller utilsiktet inntak av nellik FLO-40685-2.

Ut i fra dagens kunnskap, og tatt i betraktning tiltenkt bruksområde som ekskluderer dyrking og bruk i mat og fôr, konkluderer VKMs faggruppe for GMO at de komparative analysene er tilstrekkelig for risikovurderingen. De rapporterte morfologiske forskjellene mellom FLO-40685-2 og dens konvensjonelle kontroll medfører ikke en økt sikkerhetsrisiko.

Helserisiko

Databasesøk (*In silico*-analyser) utført av søker viser ingen relevante sekvenslikheter mellom proteinene DFR, F3'5'H og ALS og kjente toksiner eller IgE-bundne allergener, og ingen av proteinene er kjent for å forårsake toksiske eller allergiske reaksjoner. Antocyanidin-pigmentene i FLO-40685-2 finnes naturlig i mange bær, frukt og grønnsaker og er godkjente tilsetningsstoffer i mat.

Basert på dette, og tatt i betraktning tiltenkt bruksområde, konkluderer VKMs faggruppe for GMO at det er usannsynlig at proteinene DFR, F3'5'H og ALS, og antocyanidin-pigmentene vil øke en potensiell helserisiko relatert til utilsiktet inntak, eller andre eksponeringsveier, av nellik FLO-40685-2 sammenliknet med konvensjonell kontroll eller annen konvensjonell nellik.

Miljørisiko

Miljøriskovurderingen av nelliklinjen FLO-40685-2 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 FLO-40685-2 ikke medført endringer i egenskaper knyttet til overlevelse og spredning sammenliknet med konvensjonell nellik. Det er ingen indikasjoner på økt sannsynlighet for etablering og spredning 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 eller viltvoksende arter i Norge.

Ut i fra dagens kunnskap og med bakgrunn i tiltenkt bruksområde som avskårne blomster/snittblomster, konkluderer VKMs faggruppe for GMO med at nelliklinjen FLO-40685-2 ikke vil medføre miljørisiko i Norge.

Miljøovervåkning

Med bakgrunn i tiltenkt bruksområde er det ikke behov for en spesifikk miljøovervåkningsplan for nellik FLO-40685-2

Samlet vurdering

Tatt i betraktning tiltenkt bruksområde som ekskluderer dyrking og bruk i mat og fôr, konkluderer VKMs faggruppe for GMO med at den komparative analysen begrenset til målinger av antocyanidin-pigmentene delphinidin, cyanidin, petunidin og pelargonidin er tilstrekkelig for risikovurderingen. De rapporterte morfologiske forskjellene mellom FLO-40685-2 og dens konvensjonelle kontroll medfører ikke en økt sikkerhetsrisiko.

Ut i fra dagens kunnskap og tatt i betraktning tiltenkt bruksområde, konkluderer VKMs faggruppe for GMO at det er usannsynlig at proteinene DFR, F3'5'H og ALS, og antocyanidin-pigmentene vil øke en potensiell helserisiko relatert til utilsiktet inntak, eller andre eksponeringsveier, av nellik FLO-40685-2 sammenliknet med konvensjonell kontroll eller annen konvensjonell nellik.

Likeledes finner faggruppen, ut i fra dagens kunnskap, at den omsøkte bruken av FLO-40685-2 som avskårne prydblomster ikke vil medføre en miljørisiko i Norge.

Abbreviations and glossary

ALS	Acetolactate synthase
Anthocyanidins	Common plant pigments. Sugar-free counterparts of anthocyanins
Anthocyanins	Common plant pigments. Anthocyanins are derived from anthocyanidins by adding sugars
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
OECD	Organisation for Economic Co-operation and Development
PCR	Polymerase chain reaction, a technique to amplify DNA by copying
PMEM	Post-market environmental monitoring
VKM	Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet)

Background

In October 2013, a notification (reference C/NL/13/02) covering import, distribution and retailing of the genetically modified carnation FLO-40685-2 (Unique Identifier FLO-40685-2, formerly FLO-40685-1) under Directive 2001/18/EC was submitted by Suntory Holdings Ltd. to the competent authority of the Netherlands. The scope of the notification C/NL/13/02 was restricted to cut flowers for ornamental uses from flowers produced by vegetative propagation. The scope did not cover progeny derived from sexual crosses with variety FLO-40685-2.

In April 2014, the European Commission (EC) received the full notification and the assessment report from the Netherlands. In accordance with Directive 2001/18/EC, the notification was then transmitted to the competent authorities of the other Member States for a 60-day public hearing. Some Member States raised objections and in February 2015, EC consulted EFSA for a scientific opinion addressing these objections. The EFSA GMO Panel published its scientific opinion on application C/NL/13/02 on 7th of April 2016 (EFSA, 2016).

Carnation FLO-40685-2 has not previously been assessed by VKM.

Terms of reference as provided by the Norwegian Environment Agency

Miljødirektoratet viser til oppdragsbrev for 2016 og ber VKM om å foreta en vitenskapelig vurdering av helse- og miljørisiko ved *Dianthus caryophyllus* L., linje FLO-40685-2 til import, salg og distribusjon som avskårne prydblomster. Vurderingen skal tilfredsstillende kravene for miljørisikovurdering og helserisikovurdering i genteknologiloven. Miljørisikovurderingen skal peke på eventuelle effekter på norsk natur og norske forhold. Helserisikovurderingen skal foruten en vurdering av helserisiko ved håndtering og kontakt med den genmodifiserte nelliken, som følge av omsøkt bruksområde, også inkludere en vurdering av utilsiktet inntak som mat og fôr.

EFSA's risikovurdering kan legges til grunn for risikovurderingen, men dersom det finnes forhold som er spesielle for Norge som forhold i norsk natur, må dette utredes i risikovurderingen.

Assessment

1 Introduction

Carnation FLO-40685-2 (Unique Identifier FLO-40685-2, formerly FLO-40685-1) from Suntory Holdings 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 assessment by the VKM GMO Panel is based on documentation from the applicant, the scientific opinion from EFSA (EFSA 2016), and relevant peer-reviewed scientific literature. The VKM GMO Panel has not previously published a risk assessment of carnation FLO-40685-2. The above-mentioned EFSA report is provided in Appendix I and readers are referred to this for details.

Carnation FLO-40685-2 was developed for petal colour for decorative purposes. The expression of the introduced genes, *dfp* from petunia (*Petunia x hybrida*) and *f3'5'h* from *Viola hortensis* encoding dihydroflavonol 4-reductase (DFR) and flavonoid 3',5'-hydroxylase (F3'5'H) respectively, confers the purple colour to the flowers. Biosynthesis of the anthocyanidin pigment delphinidin (and a considerably lesser amount of cyanidin) in the petals is enabled via interplay between the introduced and endogenous genes in the anthocyanin biosynthesis pathway. In addition, carnation FLO-40685-2 expresses herbicide tolerance by the introduction of a mutated *als* gene (SuRB) from *Nicotiana tabacum* coding for an acetolactate synthase (ALS) variant protein, used to facilitate the selection of successfully modified shoots during the genetic transformation process.

Anthocyanidins and their sugar derivatives, anthocyanins, are widely distributed in nature and are accepted food additives (E 163). The colours of most flowers, berries and fruits consist of a combination of anthocyanidins and anthocyanins. Delphinidin and cyanidin based anthocyanins are among the most common of a class of about 100 water soluble pigments with common biosynthetic origins. They are stably localised in plant organs, such as petals, and are red, purple, blue, and black (Zhao and Tao, 2015). Delphinidin and cyanidin are naturally present in foods like aubergines, blueberries and blackcurrants at relatively high levels. Studies have shown that colour differences are related to the type(s) of anthocyanin present. Pink flowers contain cyanidin aglycone and pelargonidin aglycone as the core anthocyanins, and purple flowers contain mainly delphinidin aglycone and cyanidin aglycone as the core anthocyanins (Zhao and Tao, 2015).

The acetolactate synthase (ALS) enzyme is present in all plant species and catalyses the biosynthesis of branched amino acids (reviewed in Chandler et al., 2013). ALS-inhibiting herbicides, such as chlorimuron, thifensulfuron and sulfonyleureas, cause growth retardation in seedlings by impairing branch chain amino acid synthesis in treated grasses and broadleaf weeds, but not in crops such as rice, wheat, barley, soybean, maize and others due to their

high endogenous ALS expression. The herbicides have potency at extremely low concentrations, but rapid resistance development in weeds has limited their application (review by Tranel and Wright, 2002). However, the introduction of the mutated *als* gene in carnation FLO-40685-2 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 FLO-40685-2 was 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 Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, and Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. VKM has also taken into account the appropriate principles described in the EFSA guideline on the risk assessment of GM plants used for non-food/feed purposes (EFSA, 2009a), the 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, 2011b).

Owing to the scope of this notification, the VKM GMO Panel did not assess the possible consequences of the intentional consumption of GM carnations by humans and animals, as carnation FLO-40685-2 is not expected to enter the food and feed chain. Nevertheless, VKM has evaluated the safety of carnation FLO-40685-2 for humans considering accidental oral intake and other exposure routes e.g. dermal contact and inhalation.

Moreover, 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

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 molecular characterisation is adopted from the published EFSA opinion on Carnation FLO-40685-2 (EFSA 2016, Appendix I) and submitted data from the applicant with regard to the following:

1. The transformation system and vector constructs
2. Characterisation of the transgene inserts
3. Information on the expression of the inserts including quantification of new metabolites, and new open reading frames - ORFs
4. Inheritance and stability of the inserted DNA

Carnation FLO-40685-2 was developed by transforming the conventional carnation *Dianthus caryophyllus* L. with disarmed *Agrobacterium tumefaciens* strain AGL0, which carried the transformation vector pCGP1991. Vector pCGP1991 contained the transfer DNA (T-DNA) with the following expression cassettes:

- i) the dihydroflavonol 4-reductase (*dfr*) cassette, consisting of the promoter, the *dfr* coding sequence and the terminator, cloned as a whole from *Petunia × hybrida*. The encoded protein (DFR) is a key enzyme in the anthocyanin biosynthesis pathway.
- ii) the flavonoid 3',5'-hydroxylase (*f3'5'h*) cassette, consisting of the promoter sequence from *Antirrhinum majus* (snapdragon) chalcone synthase gene (*chs*), the *f3'5'h* coding sequence from *Viola hortensis*, and the terminator sequence of the *D8* gene encoding a *Petunia × hybrida* putative phospholipid transfer protein. The encoded protein (F3'5'H) is a key enzyme in the anthocyanin biosynthesis pathway leading to production of delphinidin.
- iii) the acetolactate synthase cassette (*als*), consisting of the *CaMV* 35S promoter, the coding region and the terminator sequence from a mutated *als* from the *SuRB* locus of *Nicotiana tabacum*. Encodes Acetolactate Synthase (ALS) which mediates tolerance to sulphonylurea-type herbicides e.g. Chlorsulfuron used during selection of transformed cells.

Size and structure of transgene inserts was determined by Southern blot and PCR analyses, and show that carnation FLO-40685-2 contains the following inserts at four different loci:

- Locus 1: one copy of the T-DNA, containing the three expression cassettes and an incomplete copy of the T-DNA containing only the *f3'5'h* cassette with the right T-DNA border. The two T-DNA copies are separated by a carnation genomic DNA region
- Locus 2: insert containing the *D8* terminator and the right T-DNA border
- Locus 3: one complete and one incomplete copy of the *f3'5'h* cassette, containing both copies of *D8* terminator sequences and the right T-DNA borders in a tail-to-tail orientation

- Locus 4: an incomplete copy of the *a/s* cassette containing the complete *a/s* gene, the CaMV 35S promoter and the left T-DNA border.

No plasmid backbone sequences were detected in carnation FLO-40685-2.

Analyses of the 5' and 3' flanking regions indicated no disruption of endogenous genes by the inserts. Bioinformatic analyses showed no significant homologies between the DFR, F3'5'H and ALS proteins and known toxins or allergens. In addition, bioinformatic analyses of all newly created open reading frames (ORFs) within the four loci, and at their junction sites, indicated that expression of an ORF showing significant similarity to known toxins or allergens is highly unlikely. Confirmation of the expression of functional DFR and F3'5'H enzymes were obtained from metabolite analysis using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Flowers of FLO-40685-2 contained predominantly delphinidin, ~1.79 mg/g fresh weight (fw) and a small amount of cyanidin ~0.02 mg/g fw, determined by HPLC. Successful isolation of transgenic shoots on selection indicated that the introduced *a/s* gene, encoding herbicide-resistant ALS, was also active.

Carnations are vegetatively propagated and the genetic stability of carnation can be readily measured by the frequency of flower colour change. The primary factor affecting this appears to be the genetic background of the variety. FLO-40685-2 was regenerated in 1997 and has been vegetatively propagated continuously since that time. According to the applicant no observed changes in flower colour, indicative of instability, has been reported.

2.1 Conclusions

Based on current knowledge and considering the intended use of carnation FLO-40685-2 as cut flowers the VKM GMO Panel finds the molecular characterisation of carnation FLO-40685-2 sufficient.

3 Comparative assessments

The comparative assessment is adopted from the published EFSA opinion on Carnation FLO-40685-2 (EFSA 2016, Appendix I) and submitted data from the applicant.

Generally, carnations have no or a 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 proposed marketing of carnation FLO-40685-2 in the EU does not include food or feed use, nor cultivation, and therefore components other than the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin were not analysed by the applicant.

3.1 Production of material for comparative assessment

Carnation FLO-40685-2 and its conventional counterpart (parental variety) Cream Cinderella were grown in field trials performed in the Netherlands (2000), Japan (1999-2000) and Australia (2003, 2010). The objective of the studies was to identify any morphological differences that could affect the vegetative or reproductive fitness of the transgenic line FLO-40685-2. Details regarding experimental design and statistical analysis of the trials are not included in the technical dossier from the notifier. The VKM GMO Panel considers this a short-coming in the application. However, since carnation FLO-40685-2 is not intended for cultivation or for use in food or feed, the documentation provided is sufficient for the scope of the application.

3.2 Compositional analysis

The comparative analysis of the composition of carnation FLO-40685-2 was limited to the anthocyanidin content in order to identify the intended changes. The concentration of the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin was determined in flower samples with high-performance liquid chromatography (HPLC) in accordance with the method of Fukui et al. (2003). Roots and stems were not assayed.

The cream-coloured flower petals of the parental variety Cream Cinderella contained no anthocyanidins, whereas the purple petals of the carnation FLO-40685-2 contained delphinidin (1.79 mg/g fresh weight (fw)) and cyanidin (0.02 mg/g fw). Delphinidin-based pigments were not observed in other plant tissues of the GM plants (stem, nodes, leaves and roots). The altered levels of anthocyanidins in carnation FLO-40685-2 explain the intended phenotypic change in flower colour.

3.3 Morphological traits and GM phenotype

In total, 18 different quantitative morphological characteristics were measured in carnation FLO-40685-2 and its comparator in a field trial performed in the Netherlands in 2000. The

measured characteristics were: plant height, number of internodes per stem, length of fifth node, thickness of fifth node, flower diameter, leaf length of third node from top, height of corolla, calyx diameter, calyx length, number of lobes per calyx, number of petals per flower, petal length, petal width, number of stamens, number of styles, number of anthers, style length and stamen length. Seven statistically significant differences between carnation FLO-40685-2 and the comparator were found, i.e. plant height, length of fifth node, thickness of fifth node, petal length, petal width, number of styles and number of anthers.

Data collected in a field trial planted during winter in Japan showed a similar average time to flowering for carnation FLO-40685-2 and the parental variety.

The number of intact anthers was measured in flowers grown in the Netherlands in 1999 and in Australia in 2003: no significant differences were found between FLO-40685-2 and its comparator.

Studies on pollen viability were performed on pollen collected from flowers grown in the Netherlands in 2000 and from flowers grown in Australia in 2010. Pollen viability was assessed after acetocarmine staining and by studying pollen germination. No significant differences were identified in pollen viability between carnation FLO-40685-2 and its comparator. Studies on pollen morphology were performed on pollen collected from flowers grown in Australia in 2010. No significant differences were identified.

The notifier has also published results from observations of 27 qualitative morphological characteristics of carnation FLO-40685-2. These observations were based on a set of character measurements developed by UPOV, the international agency coordinating registration of new plant varieties for the purpose of distinguishing carnation varieties. No differences were found between carnation FLO-40685-2 and the parental variety Cream Cinderella.

EFSA (EFSA 2016, Appendix I) concluded that reported differences in morphological traits were not expected to influence the risk scenario upon accidental release to the environment or intake of the GM carnation.

3.4 Conclusion

Based on current knowledge and considering the intended use of carnation FLO-40685-2, which excludes cultivation and use as food or feed, the VKM GMO Panel concludes that the comparative analysis is sufficient for the risk assessment. The reported morphological differences between FLO-40685-2 and its conventional counterpart do not raise safety concerns.

4 Food and feed safety assessment

4.1 Previous evaluations by the VKM GMO Panel and EFSA

The VKM GMO Panel has previously not performed a risk assessment of Carnation FLO-40685-2, however three other GM-carnations expressing the same inserted genes as FLO-40685-2 were finalised and published by the Panel in October 2015 (VKM 2015 a,b,c.). No safety concerns related to human health or the environment were identified with the intended use of these GM-carnations.

The EFSA GMO Panel, published their opinion on Carnation FLO-40685-2 in March 2016 (EFSA 2016, Appendix I). The Panel identified no safety concerns to human health or the environment with the intended use of Carnation FLO-40685-2.

4.2 Product description and intended uses

The scope of the application C/NL/13/02 is restricted to the import of cut flowers for ornamental use only. Accordingly, if approved, products of Carnation FLO-40685-2 will be marketed with an accompanying label or document that states that it is genetically modified and not for human or animal consumption, nor cultivation.

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 an issue.

4.3 Toxicological assessment

Carnation FLO-40685-2 is intended for ornamental use only, and not for human or animal consumption. Accidental intake can however not be excluded. Possible health effects related to the genetic modifications in carnation FLO-40685-2 is therefore considered according to the EFSA guidelines on the risk assessment of GM plants used for non-food/feed purposes (EFSA, 2009a)

No toxicological *in vitro* or *in vivo* studies have been performed by the notifier on the new proteins (ALS, DFR and F3'5'H) or anthocyanidin pigments in carnation FLO-40685-2, nor on flower extracts or on the whole GM plant. Instead, the notifier has provided comprehensive literature data regarding the safety of these proteins and pigments to human and animal health, and refers to studies performed with similar GM-carnations expressing the same proteins and pigments (e.g. FLORIGENE® Moonaqua™ and Moonlite™).

4.3.1 Toxicological assessment of newly expressed proteins

Bioinformatics analyses performed by the applicant of the amino acid sequences of the newly expressed proteins in Carnation FLO-40685-2 (ALS, DFR and F3'5'H) do not show sequence resemblance to known toxins or IgE-bound allergens.

The ALS, DFR and F3'5'H proteins have previously been evaluated by the EFSA and VKM GMO Panels in risk assessments of other GM-carnations (EFSA 2006, 2008b, 2014a,b, VKM 2015a,b,c). Neither the EFSA nor VKM Panel could identify reasons for concern related to these proteins in the context of the limited scope of the previous notifications.

4.3.2 Toxicological assessment of new constituents other than proteins

The anthocyanidins delphinidin and cyanidin are naturally present in foods like aubergines, blueberries and blackcurrants at rather higher levels than in the petals of carnation FLO-40685-2 (Wu et al., 2006). Notably, anthocyanidins (E 163) are authorised food additives according to regulation 1333/2008 (Reference EC No. 1333/2008), on food additives. Previous evaluations of anthocyanidins prepared by physical processes from natural foods identified no reason for concern or adverse effects (EFSA 2013).

4.3.2.1 In vitro studies

Not applicable.

4.3.2.2 Acute toxicity study

Not applicable.

4.3.3 Toxicological assessment of the whole GM plant

Not applicable.

4.4 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).

4.4.1 Assessment of allergenicity of the newly expressed proteins

No significant similarities to known allergens were identified via bioinformatics analyses performed by the applicant, of the amino acid sequence of the newly expressed proteins in carnation FLO-40685-2 with the criterion of more than 35% identity in a segment of 80 or more amino acids (Codex Alimentarius, 2003). Likewise, analyses searching for matches of eight contiguous identical amino acid sequences between the newly expressed proteins and known allergens indicated no similarities to known allergens. 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,b; VKM 2008, VKM 2015 a,b,c).

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.

4.4.2 Assessment of allergenicity of the whole GM plant

As stated earlier, carnation FLO-40685-2 is not intended for food or feed purposes. Although dermal and respiratory allergies to carnations in workers handling cut flowers/carnations have been described (Cistero-Bahima et al., 2000; Sanchez-Fernandez et al., 2004; Sanchez-Guerrero et al., 1999; Stefanaki and Pitsios, 2008), the causes appear 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.

4.5 Nutritional assessment of GM food and feed

As stated in the comparative assessment, carnations have no or very limited history of use as food or feed, and their content of nutrients, antinutritional factors and other components with biological activity is largely unknown. Since carnation FLO-40685-2 is only meant for ornamental use, other components than the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin have not been analysed by the notifier.

Anthocyanidins and anthocyanins are naturally present in foods like aubergines, blueberries and blackcurrants, as well as some non-GM carnation cultivars and other edible flower petals, at higher levels than in the petals of carnation FLO-40685-2 (Cacho et al., 1992). According to regulation 1333/2008 (Reference EC No. 1333/2008) on food additives, anthocyanidins and anthocyanins (E 163) are authorised food additives. Previous evaluations of anthocyanidins and anthocyanins prepared by physical processes from natural foods identified no adverse effects or reason for concern (EFSA, 2013). Moreover, an evaluation by Chandler and colleagues (Chandler et al., 2013) suggested that the release of genetically

modified carnation varieties that express the *f3'5'h* gene and thereby delphinidin-based anthocyanins do not pose an increased risk of harm to human or animal health.

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 FLO-40685-2, a cyanidin concentration of 0.02 mg/g fw was reported by the applicant. Cyanidin is also present in non-GM carnations that have red, pink and purple colours. 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 FLO-40685-2 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 FLO-40685-2, a delphinidin concentration of 1.79 mg/g fw 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 FLO-40685-2 petals is not considered to pose a health risk compared to the levels present in berries and estimated ADI.

4.6 Conclusion

In silico analyses performed by the applicant show no relevant sequence resemblance of the DFR, F3'5'H and ALS proteins to known toxins or IgE-bound allergens, and none of the proteins are known to cause allergic or toxic reactions. The anthocyanidin pigments produced in carnation FLO-40685-2 are natural constituents of numerous plant foods and are accepted as food additives.

Based on this and considering the scope of the application, the VKM GMO Panel concludes that the DFR, F3'5'H and ALS proteins and anthocyanidin pigments are unlikely to increase a potential health risk related to an accidental intake or other exposure routes to carnation FLO-40685-2 compared to the conventional counterpart or other non-GM carnations.

5 Environmental risk assessment

5.1 Introduction

This assessment applies to carnation FLO-40685-2 from Suntory Holdings Ltd., which has been transformed to modify the flower colour and possesses a herbicide resistance gene (*als*) for *in vitro* selection.

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

The genus Carnation (*Dianthus* L.) contains approximately 300 annual, biannual and perennial species, native mainly to southern parts of Asia and Europe (OGTR, 2006). *Dianthus*-species are found in alpine regions of Europe and Asia, as well as coastal areas in Mediterranean 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. chinensis* 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 varieties. 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. Varieties used in Norway are frost sensitive and do not survive in regions with temperatures lower than -5°C. There is no greenhouse production of carnation for cut flowers in Norway. Thus, all the cut flowers of carnation are imported. According to Statistics Norway import of carnation in 2014 was about 427 metric tonnes (www.sbb.no).

Wild *D. caryophyllus* L. have simple, bisexual open flowers with five petals. Selection and breeding has increased flower size, number of petals, and stem length as well as disease resistance (OGTR, 2006). In the modern varieties, 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 varieties are vegetatively propagated (Zuker et al., 2002).

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

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

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

5.2 Unintended effects on plant fitness due to the genetic modifications

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

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

Based on the nature of the introduced traits and the morphological data reported in 3.3, there are no indications of an altered fitness of carnation FLO-40685-2 that would suggest a selective advantage, or otherwise influence the risk scenario upon accidental release to the environment of the GM plant compared to non-GM varieties.

5.3 Potential for gene transfer

5.3.1 Plant to micro-organisms gene transfer

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

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

5.3.2 Plant to plant gene flow

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

5.4 Interaction between the GM plant and target organisms

With the intended use as cut flowers, interaction between carnation FLO-40685-2 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 FLO-40685-2 will have any adverse effect on abiotic environment or biochemical cycles.

5.7 Conclusion

Considering the intended use of FLO-40685-2, 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, FLO-40685-2 has no altered survival, multiplication or dissemination characteristics compared to conventional carnation varieties, 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 FLO-40685-2 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 FLO-40685-2 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 Suntory Holdings products with all 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 FLO-40685-2. No specific environmental impact of genetically modified carnation FLO-40685-2 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 FLO-40685-2. Thus, the general surveillance plan is sufficient and there is no need for a specific surveillance plan.

7 Conclusions

Molecular characterisation

The molecular characterisation shows that carnation FLO-40685-2 has four transgenic inserts in its genome at four different loci. Only one of the loci contains the intended full length T-DNA sequence with functional copies of each of the three genes *dfrr*, *f3'5'h* and *als*. The three other loci contain different partial versions of the T-DNA. Analyses of flanking sequences indicate no disruption of endogenous genes by the inserts. Bioinformatic analyses performed by the applicant show no significant homologies between the DFR, F3'5'H and ALS proteins and known toxins or allergens. Analyses of new open reading frames (ORFs) indicate that expression of any ORF showing significant similarity to known toxins or allergens is highly unlikely. Consistency of the intended new flower colour has been observed over multiple vegetative generations since 1997 indicating stability of the inserts in carnation FLO-40685-2.

Based on current knowledge and considering the intended use of carnation FLO-40685-2 as cut flowers the VKM GMO Panel finds the molecular characterisation of carnation FLO-40685-2 sufficient.

Comparative assessment

Considering the intended use of carnation FLO-40685-2, which excludes cultivation and use in food and feed, compositional studies were limited to analyses of the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin. The presence of delphinidin and cyanidin in carnation FLO-40685-2 and absence of all four anthocyanidins in the non-GM parental variety, measured by HPLC, confirmed the intended effects of the genetic modification. Other morphological traits were assessed in field trials and revealed that carnation FLO-40685-2 differed significantly in several traits compared to the parental variety. None of the reported differences in compositional or morphological traits are expected to influence the risk scenario upon accidental release to the environment or accidental intake or exposure to the GM carnation.

Based on current knowledge and considering the intended use of carnation FLO-40685-2, which excludes cultivation and use as food or feed, the VKM GMO Panel concludes that the comparative analysis is sufficient for the risk assessment. The reported morphological differences between FLO-40685-2 and its conventional counterpart do not raise safety concerns.

Food and feed risk assessment

In silico analyses performed by the applicant show no relevant sequence resemblance of the DFR, F3'5'H and ALS proteins to known toxins or IgE-bound allergens, and none of the proteins are known to cause allergic or toxic reactions. The anthocyanidin pigments produced in carnation FLO-40685-2 are natural constituents of numerous plant foods and are accepted as food additives.

Based on this and considering the scope of the application, the VKM GMO Panel concludes that the DFR, F3'5'H and ALS proteins and anthocyanidin pigments are unlikely to increase a potential health risk related to an accidental intake or other exposure routes to carnation FLO-40685-2 compared to the conventional counterpart or other non-GM carnations.

Environmental assessment

Considering the intended use of FLO-40685-2, 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, FLO-40685-2 has no altered survival, multiplication or dissemination characteristics compared to conventional carnation varieties, 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 FLO-40685-2 does not represent an environmental risk in Norway.

Post-market environmental monitoring

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

Based on current knowledge and considering its import, distribution and intended use as cut ornamental plants, the VKM GMO Panel concludes that the environmental risk assessment did not identify any potential adverse environmental effects of the transgenic line of carnation FLO-40685-2. 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 FLO-40685-2 is not intended for cultivation or use as food or feed, the VKM GMO Panel considers the comparative analysis of the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin in the flower petals sufficient for the risk assessment. The reported morphological differences between FLO-40685-2 and the parental variety do not raise safety concerns.

Based on current knowledge and the scope of the application, the VKM GMO Panel concludes that the DFR, F3'5'H and ALS proteins and anthocyanidin pigments are unlikely to increase a potential health risk related to an accidental intake or other exposure routes to carnation FLO-40685-2 compared to the conventional counterpart or other non-GM carnations.

Likewise, the VKM GMO Panel concludes that carnation FLO-40685-2, based on current knowledge and the intended use as cut ornamental flowers, does not represent an environmental risk in Norway.

8 Data gaps

The potential health risk evaluation is only based on *in silico* data due to the lack of compositional and toxicological data. The actual content of nutrients, antinutritional components and other factors of carnation FLO-40685-2 is lacking.

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Appendix I

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Part C notification (reference C/NL/13/02) from Suntory Holdings Limited for the import, distribution and retailing of carnation FLO-40685-2 cut flowers with modified petal colour for ornamental use

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

The Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) has evaluated the overall safety of genetically modified (GM) carnation FLO-40685-2 cut flowers to be imported into the EU for ornamental use. The genetic modification results in the flowers having purple petals. The stability of the newly introduced trait (purple flower colour) was observed over multiple vegetative generations. The purple colour of the petals comes from the altered expression levels of anthocyanins, common pigments found in edible fruits and vegetables. Considering the intended use of the GM carnation and the possible routes of exposure, the GMO Panel did not find indications that the genetic modification will increase the risk of allergy among those coming into contact with carnations. Overall there are no reasons for safety concerns of carnation FLO-40685-2 for humans. The GMO Panel also considered whether viable seed or pollen from GM carnation cut flowers could be dispersed into the environment and whether GM carnation can be propagated by rooting. Owing to the limited environmental exposure and the biology of the plant, the GMO Panel did not identify any environmental safety concerns and agrees with the scope of the post-market environmental monitoring (PMEM) plan. The GMO Panel concludes that the import, distribution and retailing of the GM carnation will not cause adverse effects on human health or the environment.

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

Requestor: European Commission

Question number: EFSA-Q-2015-00122

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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 (GMO Panel) was asked to deliver a scientific opinion on notification C/NL/13/02 from Suntory Holdings Limited submitted under Part C of Directive 2001/18/EC.¹ The scope of notification C/NL/13/02 covers the import, distribution and retailing in the European Union (EU) of genetically modified (GM) carnation FLO-40685-2 cut flowers for ornamental use only.

In accordance with Directive 2001/18/EC, a safety evaluation of the GM carnation was requested by the European Commission in order to assess the overall safety of the GM carnation. The GMO Panel was, therefore, asked to consider if there is any scientific reason to believe that the placing of carnation FLO-40685-2 on the market is likely to cause any adverse effects on human health and the environment.

In delivering the present scientific opinion, the GMO Panel considered the full notification C/NL/13/02, including additional information provided by the notifier, the assessment report of the Dutch competent authority, relevant scientific publications and the experience gained in assessing GM carnations with similar traits.

During its safety evaluation, the GMO Panel considered the molecular characterisation of the GM carnation, including the inserted DNA, the expression of new proteins and the stability of the modified flower colour trait. 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/13/02.

Carnation FLO-40685-2 has a modified flower colour, a shade of purple, whereas the parental line has a cream flower colour. The colour has been achieved by introducing into the parental carnation two 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 FLO-40685-2 is also tolerant to sulfonylurea herbicides, which was achieved by introducing an acetolactate synthase (*als*) expression cassette, but the herbicide tolerance trait was used only for the selection of transformed plants.

The GMO Panel concludes that the molecular characterisation data establish that carnation FLO-40685-2 contains inserts in four loci, consisting of three expression cassettes responsible for the intended trait (purple flower colour) conferred by the dihydroflavonol 4-reductase (*df*r) and flavonoid 3',5'-hydroxylase (*f3'5'h*) genes, and herbicide tolerance conferred by the mutated *als* gene. The stability of the newly introduced trait (purple flower colour) was observed over multiple vegetative generations.

Carnation flowers have a long history of use as ornamentals. Carnation FLO-40685-2 differs from its parental variety in that it synthesises different levels of anthocyanins in the petals, e.g. an increased content of delphinidin and cyanidin (common pigments in many ornamental flowers and food plants). The altered levels of anthocyanins in carnation FLO-40685-2 confer a purple colour to the flowers. It is not expected that the accidental intake of carnation FLO-40685-2 petals would contribute substantially to the overall intake of anthocyanins from foods.

From its assessment of the potential allergenicity and toxicity of the newly expressed proteins (DFR, F3'5'H and ALS), the GMO Panel concludes that there are no reasons for safety concern in the context of the limited scope of this notification. Given that the case reports of occupational allergies to carnations are rare and considering the assessment of the newly expressed proteins, 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/13/02 and the possible routes of exposure, the GMO Panel identified no reasons for any safety concerns of carnation FLO-40685-2 for humans related to the genetic modification.

Carnation FLO-40685-2 cut flowers have marginal viability and negligible pollen production, and no viable seeds have been reported. However, in the very unlikely event of escape into the environment via viable seeds, pollen or rooted plants, the GMO Panel considers that carnation FLO-40685-2 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides.

¹ 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.

Considering the scope of notification C/NL/13/02 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 GMO Panel. The GMO Panel also concludes that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation FLO-40685-2 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 FLO-40685-2. The GMO Panel agrees with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

The GMO Panel therefore concludes that there is no scientific reason to consider that the import, distribution and retailing in the EU of carnation FLO-40685-2 cut flowers for ornamental use will cause any adverse effects on human health or the environment.

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1. Introduction

Carnation FLO-40685-2 is a genetically modified (GM) variety of *Dianthus caryophyllus* L. used as a decorative plant species. The purple colour of the flowers results from the expression of two newly introduced genes encoding dihydroflavonol 4-reductase (*dfr*) and flavonoid 3',5'-hydroxylase (*f3'5'h*). This construct, together with endogenous genes involved in the anthocyanin biosynthesis pathway, enables the biosynthesis of delphinidin in the petals. Carnation FLO-40685-2 also contains a mutated herbicide tolerance gene coding for an acetolactate synthase (ALS) variant protein, used to facilitate the selection of GM plantlets during the genetic transformation process.

In the present scientific opinion, carnation FLO-40685-2 is evaluated by the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) in the light of the scope of notification C/NL/13/02, i.e. import, distribution and retailing of GM carnation FLO-40685-2 cut flowers in the European Union (EU) for ornamental use only.

Both intentional and accidental oral intake of GM carnation flowers by animals were excluded from this opinion, as carnation FLO-40685-2 is not expected to enter the feed chain or to be accidentally consumed in the field (cultivation being excluded from the scope) (EFSA, 2009a). Owing to the scope of this notification, the GMO Panel did not assess the possible consequences of the intentional consumption of GM carnations by humans.² Nevertheless, the GMO Panel evaluated the safety of carnation FLO-40685-2 for humans considering three possible routes of exposure: (1) dermal contact, (2) inhalation and (3) accidental oral intake.³

Moreover, 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 the requestor

In April 2014, the European Commission received the full notification (reference C/NL/13/02), together with the positive assessment report from the competent authority of the lead Member State, the Netherlands.

In accordance with Directive 2001/18/EC⁴, 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, Suntory Holdings Limited, provided the Member States with additional information in response to those comments and objections. However, one Member State maintained an objection which could not be solved during the statutory 105-day period, in which case the European Commission is required to follow the procedure of Article 18(1) of Directive 2001/18/EC.

In February 2015, the EFSA received the request from the European Commission to provide a scientific opinion as to *whether there is any scientific reason to believe that the placing on the market of carnation line FLO-40685-2 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 safety evaluation of GM carnation FLO-40685-2 by the GMO Panel is based on the information provided in notification C/NL/13/02, including additional information⁵ provided by the notifier, the assessment report of the Dutch competent authority, relevant scientific publications and

² The 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.

³ Accidental oral intake should be considered as unintentional, infrequent and/or of relatively short duration.

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.

⁵ See section 'Documentation provided to EFSA'.

the experience gained in assessing GM carnations with similar traits (EFSA, 2006, 2008; EFSA GMO Panel, 2014a,b,c, 2015).

2.2. Methodologies

The GMO Panel performed its safety evaluation of GM carnation FLO-40685-2 in accordance with the principles laid down in its guidance documents on the risk assessment of GM plants for non-food or non-feed purposes (EFSA, 2009a) and on the ERA of GM plants (EFSA GMO Panel, 2010).

3. Assessment

3.1. Molecular characterisation

3.1.1. Objections raised by Member States

No Member States' objection concerning the molecular characterisation of carnation FLO-40685-2 remained at the end of the 45-day Member States' consultation period.

3.1.2. Evaluation of relevant scientific data

3.1.2.1. Transformation process and vector constructs

To develop the FLO-40685-2 line, the conventional carnation *Dianthus caryophyllus* L. variety Cream Cinderella was transformed using disarmed *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain AGL0, which carried the transformation vector pCGP1991.

The transformation vector pCGP1991 contained within the transfer DNA (T-DNA) the following expression cassettes, which are needed to obtain the desired purple colour of the flowers:

- the dihydroflavonol 4-reductase (*dfr*) cassette, encompassing the promoter, the *dfr* coding sequence and the terminator, cloned as a whole from the *Petunia × hybrida*;
- 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 the *D8* gene encoding a *Petunia × hybrida* putative phospholipid transfer protein.

In addition, the T-DNA of vector pCGP1991 contained the acetolactate synthase (*als*) cassette, 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*. This acetolactate synthase provided tolerance to sulfonylurea herbicides and was used as a marker in the selection of transformants.

3.1.2.2. Transgene constructs in the genetically modified plants

Carnation FLO-40685-2 contains inserts in four loci, as described below:

- Locus 1: one copy of the T-DNA, containing the three expression cassettes and an incomplete copy of the T-DNA containing only the *f3'5'h* cassette with the right T-DNA border. The two T-DNA copies are separated by a carnation genomic DNA region;
- Locus 2: one insert containing the *D8* terminator and the right T-DNA border;
- Locus 3: one complete and one incomplete copy of the *f3'5'h* cassette, containing both copies of *D8* terminator sequences and the right T-DNA borders in a tail-to-tail orientation;
- Locus 4: an incomplete copy of the *als* cassette containing complete *als* gene, the *CaMV* 35S promoter and the left T-DNA border.

Southern blot and polymerase chain reaction (PCR) analyses indicated that no plasmid backbone sequences had been integrated into carnation FLO-40685-2. The sequences of the inserts and the flanking regions were provided.

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

Updated bioinformatic⁶ analyses of the amino acid sequences of the three newly expressed proteins (DFR, F3'5'H, ALS) revealed no significant similarities to known toxins. Using an 80-amino-acid sliding

⁶ Additional information: 7 May 2015.

window approach, no significant similarity over 35% identity with known allergens was found for DFR, F3'5'H and ALS proteins.

In addition, updated bioinformatic⁷ analyses of the newly created open reading frames (ORFs) within the inserts and at their junction sites indicate that the expression of an ORF showing significant similarity to known toxins or allergens is highly unlikely.

3.1.2.3. Information on the expression of the insert

The presence of transcripts corresponding to *dfr*, *f3'5'h* and *als* genes in the petals was demonstrated using northern blot analysis. The functionality of *dfr* and *f3'5'h* genes was confirmed by visual observation of the purple flower colour, as well as from delphinidin metabolite analysis using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Tolerance to sulfonylurea herbicides indicated the activity of the ALS protein.

3.1.2.4. Inheritance and stability of the inserted DNA

Genetic stability of carnation FLO-40685-2 was studied by visual observation of flower colour in vegetatively propagated plants grown since 2006. The stability of the newly introduced trait (purple flower colour) was observed over multiple vegetative generations. Although flowers of parental colour occurred at a low frequency (0.2–0.74%) during production, PCR analysis demonstrated that these plants (showing parental flower colour) were non-transgenic and therefore this occurrence was not an indication of genetic instability.⁸ As only the plants with intended phenotype will be imported to the EU, and no further issues with genetic stability of the transgenic carnation FLO-40685-2 were identified, the carnation FLO-40685-2 flowers that will be imported to the EU can be regarded as genetically stable.

3.1.3. Conclusion

The molecular characterisation data establish that carnation FLO-40685-2 contains inserts in four loci, consisting of three expression cassettes responsible for the intended trait, i.e. purple flower colour, conferred by the *dfr* and *f3'5'h* genes, and herbicide tolerance, conferred by the mutated *als* gene. The results of bioinformatic analyses of the newly expressed proteins in carnation FLO-40685-2 did not indicate relevant similarities with known toxins or allergens. The stability of the newly introduced trait (purple flower colour) was observed over multiple vegetative generations.

3.2. Comparative analysis

3.2.1. Objections raised by Member States

No Member States' objection concerning the comparative analysis of carnation FLO-40685-2 remained at the end of the 45-day Member States' consultation period.

3.2.2. Evaluation of relevant scientific data

The GMO Panel performed its comparative analysis in accordance with the principles of its guidance document on the risk assessment of GM plants for non-food or non-feed purposes (EFSA, 2009a).

3.2.2.1. Choice of comparator

Carnation FLO-40685-2, having purple-coloured petals, was compared with the parental non-GM carnation variety Cream Cinderella which is characterised by cream-coloured petals.

⁷ Additional information: 7 May 2015.

⁸ Occurrence of pink Cinderella type flowers is based on the chimeric nature of the non-transgenic carnation variety Cream Cinderella (white flowers), which itself resulted from a natural mutation in variety Cinderella (pink flowers). Cream Cinderella is considered to be a periclinal chimera, which after the transformation, resulting in FLO-40685-2 that gave rise to chimeric plants with its shoot apical meristem comprising transgenic Cream Cinderella L1 cell layer, while some or all cells of the L2 and/or L3 are of non-transgenic Cinderella genotype (pink). The notifier asserts that in the pink parental type plants from FLO-40685-2 culture, the L1 layer has been replaced during vegetative propagation by L1 cells of Cinderella genotype, resulting in appearance of the pink flower type. Additional information: 24 February 2016.

3.2.2.2. Compositional analysis

In order to identify the intended changes, the comparative analysis of the composition of carnation FLO-40685-2 was limited to the anthocyanin content. The content of anthocyanin colour pigments (delphinidin, cyanidin, petunidin and pelargonidin) was determined in acetonitrile extracts of freeze-dried petals using HPLC in accordance with the method of Fukui et al. (2003).

The cream-coloured flower petals of Cream Cinderella contained no anthocyanidins, whereas the purple petals of the carnation FLO-40685-2 contained delphinidin (1.79 mg/g fresh weight (fw)) and cyanidin (0.02 mg/g fw). Delphinidin-based pigments were not observed in other plant tissues of the GM plants (stem, nodes, leaves and roots).

The altered levels of anthocyanins in carnation FLO-40685-2 explain the intended phenotypic change in the flower colour.

3.2.2.3. Morphological traits and genetically modified phenotype

Flower colour differed between the carnations FLO-40685-2 (purple) and the parental variety (cream). In the comparison of 27 qualitative morphological characteristics, no differences were found between carnation FLO-40685-2 and its comparator (i.e. the parental variety). In a trial performed in the Netherlands in 2000, 18 quantitative morphological characteristics were measured for carnation FLO-40685-2 and its comparator, and analysed statistically with a single-factor ANOVA.⁹ Seven statistically significant differences between carnation FLO-40685-2 and its comparator were found (for plant height, length of fifth node, thickness of fifth node, petal length, petal width, number of styles and number of anthers). Data collected in a trial in Japan (season 1999–2000) showed a similar average time to flowering for carnation FLO-40685-2 and the parental variety. The number of intact anthers was measured in flowers grown in the Netherlands in 1999 and in Australia in 2003: no significant differences were found between FLO-40685-2 and its comparator.

Studies on pollen viability were performed on pollen collected from flowers grown in the Netherlands in 2000 and from flowers grown in Australia in 2010. Pollen viability was assessed after acetocarmine staining and by studying pollen germination. No significant differences were identified in pollen viability between carnation FLO-40685-2 and its comparator. Studies on pollen morphology were performed on pollen collected from flowers grown in Australia in 2010. No significant differences in pollen diameter were identified.

3.2.3. Conclusion

The altered levels of anthocyanins in carnation FLO-40685-2 explain the intended phenotypic change in the flower colour. The relevance of the altered levels in anthocyanins in the GM carnation is further assessed for potential adverse effects on human health in Section 3.3.2. The relevance of the observed morphological differences is further assessed for potential environmental impact in Section 3.4.3.

3.3. Food safety assessment

3.3.1. Objections raised by Member States

No Member States' objection concerning the safety assessment of carnation FLO-40685-2 for humans remained at the end of the 45-day Member States' consultation period.

3.3.2. Evaluation of relevant scientific data

3.3.2.1. Toxicology

Toxicological assessment of newly expressed proteins

Bioinformatic analyses of the amino acid sequences of the three proteins newly expressed in carnation FLO-40685-2 (ALS, DFR and F3'5'H) reveal no significant similarities to known toxins to humans (see Section 3.1.3).

⁹ The characteristics measured were as follows: plant height, number of internodes per stem, length of fifth node, thickness of fifth node, flower diameter, leaf length of third node from top, height of corolla, calyx diameter, calyx length, number of lobes per calyx, number of petals per flower, petal length, petal width, number of stamens, number of styles, number of anthers, style length and stamen length.

These three new proteins have been previously assessed by the GMO Panel and no reasons for concern were identified in the context of the limited scope of previous notifications (EFSA, 2006, 2008; EFSA GMO Panel 2014b,c, 2015).

Toxicological assessment of new constituents other than proteins

As intended, the anthocyanin profile of carnation FLO-40685-2 differs from that of parental variety used as comparator (see Section 3.2.2). The anthocyanins, delphinidin and cyanidin are present in carnation FLO-40685-2 and not in its comparator. These anthocyanins can also be found in many foods and, in some of them, at much higher concentrations than in the petals of carnation FLO-40685-2. Particularly, high concentrations can be found, for example, in blueberries and blackcurrant (Wu et al., 2006). According to Regulation 1333/2008 on food additives, anthocyanins (E 163) are authorised food additives in the EU. Anthocyanins have been evaluated by the Scientific Committee on Food (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 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 carnation FLO-40685-2 petals would contribute substantially to the overall intake of anthocyanins from foods. Therefore, the GMO Panel sees no reason for concern regarding the anthocyanin profile in petals of carnation FLO-40685-2.

Toxicological assessment of the whole genetically modified plant

Given that carnation FLO-40685-2 is not intended for human consumption as food but is intended for ornamental use only, the 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 and of the new constituents other than proteins, the GMO Panel identified no reasons for food safety concern.

3.3.2.2. Allergenicity

Allergenicity assessment of newly expressed proteins

Bioinformatic analyses of the amino acid sequence of the newly expressed proteins in carnation FLO-40685-2 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 GMO Panel has previously assessed the potential allergenicity of the ALS, DFR and F3'5'H proteins, and no reasons for concern were identified in the context of the limited scope of previous notifications (EFSA, 2006, 2008; EFSA GMO Panel 2014b,c, 2015).

Allergenicity assessment of the whole genetically modified plant¹⁰

Occupational allergy (dermal and respiratory allergy) in workers handling carnation cut flowers 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). 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, but no occupational exposure was published (Brinia et al., 2013).

¹⁰ Additional information: 26 November 2015.

According to the notifier, no adverse reactions (including contact dermatitis) to carnation FLO-40685-2 cut flowers used for ornamental purpose have been reported in the populations handling the flowers (workers and users).

In the context of the scope of notification C/NL/13/02, given that the case reports of occupational allergies to carnations are rare and considering the assessment of the newly expressed proteins, 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 FLO-40685-2 differs from its parental variety in that it synthesises anthocyanins (delphinidin and cyanidin, common pigments in many ornamental flowers and food plants) in the petals. The altered levels of anthocyanins in carnation FLO-40685-2 confer a purple colour to the flowers. It is not expected that accidental intake of carnation FLO-40685-2 petals would contribute substantially to the overall intake of anthocyanins from foods.

Given that the case reports of occupational allergies to carnations are rare and considering the assessment of the newly expressed proteins, 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/13/02 and the possible routes of exposure, the GMO Panel identified no reasons for safety concerns for humans related to the genetic modification of carnation FLO-40685-2.

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

3.4.1. Objections raised by Member States

One Member State expressed concerns related to the possibility of crossing *D. caryophyllus* with other species of *Dianthus*, through spread of pollen by lepidopterans and cross-pollination. This issue is addressed in Section 3.4.3, under 'Plant-to-plant gene transfer'.

3.4.2. Evaluation of relevant scientific data

Considering the scope of notification C/NL/13/02, 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 assessment¹¹

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

Carnation is the common name of *D. 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 (e.g. in Italy and Spain), and is occasionally naturalised in some Mediterranean countries but appears to be restricted to the coastal Mediterranean regions of Greece, Italy, Sicily, Corsica and Sardinia (Tutin et al., 1993). In general, carnation varieties compete poorly outside their cultivated environment. In addition, carnation varieties do not show weedy characteristics.

The majority of *Dianthus* spp. is self-sterile because the stigma is not receptive to pollen until 1 week or more after anthers have shed pollen. Cultivated carnations require pollination by hand to set

¹¹ Notification C/NL/13/02, Section B.

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.

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 flowers 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 1–2 years. Flowers are produced in flushes, beginning from 3 to 5 months after rooted cuttings are planted. Plants can also be multiplied by tissue culture techniques.

Carnation FLO-40685-2 has a modified flower colour resulting from the expression of *dfr* and *f3'5'h* genes. This construct, together with endogenous genes involved in the anthocyanin biosynthesis pathway, enables 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 effects plant fitness of these species.

Carnation FLO-40685-2 also contains a mutated *als* gene conferring tolerance to sulfonylurea (or ALS-inhibiting) 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. They reported that little change in plant fitness of resistant weed types in the absence of the herbicide has been found. However, they reported that the seeds of some tolerant weed biotypes germinate more rapidly, especially in cool temperatures. No seeds have been found in cut flowers of carnation FLO-40685-2 and pollen production is reduced. However, in the very unlikely event of gene flow to *Dianthus* growing in the EU, this may result in a possible change in germination behaviour of the tolerant plants in the absence of the herbicide. Wild *Dianthus* populations exhibit a diversity of phenotypes exploiting niches in a wide geographical range in Europe (Tutin et al., 1993). In addition, seeds of *Dianthus* species are generally relatively short-lived (Mondoni et al., 2011) and so the consequences of changes in germination characteristics will vary with different populations and niches. The GMO Panel considered that small changes in seed germination characteristics induced by ALS tolerance are unlikely to be outside the current range of seed germination characteristics currently expressed by non-GM carnations and thus is unlikely to have an ecological impact.

In addition, fitness advantages and higher weediness of the GM plants in the presence of sulfonylurea herbicides and herbicides with similar mode of action are not considered significant as these herbicides are not known to be used on cultivated carnations. The notifier provided data on 18 quantitative morphological characteristics of carnation FLO-40685-2 compared with its parental variety from one trial in the Netherlands in 2000 (see Section 3.2.2 for more details). Statistically significant differences between the GM carnation and its parental variety were observed for seven out of the 18 characteristics studied (i.e. plant height, length of 5th node, thickness of 5th node, petal length, petal width, number of styles and number of anthers). None of the observed differences are considered to be related to characteristics associated with increased invasiveness or survival, except in the presence of sulfonylurea herbicides. Moreover, the notifier reported that there was no difference in time to flowering between the GM carnation and its parental line from a field trial in Japan. The notifier also measured number of viable anthers, pollen viability and germination for both GM carnation and its parental line, and did not report significant differences. Therefore, the GMO Panel is of the opinion that these characteristics for which differences were observed are unlikely to affect the survival, establishment and fitness of the GM carnation.

No evidence has been found that the flower colour and herbicide tolerant traits introduced by the genetic modification into carnation FLO-40685-2 would result in increased persistence and invasiveness of this or any other *Dianthus* species.

Moreover, the 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 report¹²; EFSA, 2006, 2008; EFSA GMO Panel 2014a,b,c, 2015). In addition, *D. caryophyllus* with double flowers 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.

Considering the scope of notification C/NL/13/02 and the data available, the GMO Panel considered that there would be no changes in plant characteristics of any ecological significance. Carnation FLO-40685-2 plants would show changed fitness characteristics only when exposed to sulfonylurea herbicides, but these herbicides are not generally used in carnation cultivation or in habitats where wild *Dianthus* spp. might occur. The GMO Panel also concludes that the propagation of the GM carnation (e.g. by rooting) cannot be excluded. However, should this occur, carnation FLO-40685-2 would not show any potential for increased survival, fitness or weediness compared with its parental variety.

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.

Plant-to-bacteria gene transfer

Considering the scope of notification C/NL/13/02, the ERA is concerned with exposure through discarded GM carnation cut flowers resulting in possible exposure of environmental bacteria to recombinant DNA. Given that accidental oral intake of these GM carnations by humans is considered infrequent and/or of relatively short duration (see Section 3.3), it is likely to be at very low levels so that exposure of gastrointestinal tract bacteria and microbial decomposers of faecal material will be very low.

Current scientific knowledge of recombination processes in bacteria indicates that the 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 event FLO-40685-2 does not contain genetic elements with identity or high similarity to those of bacteria. The recombinant genetic elements used for the construction of carnation FLO-40685-2 originate from plants, i.e. *Petunia*, *Viola* and *Nicotiana tabacum* (tobacco) (for more details, see Section 3.1.2). 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 FLO-40685-2 to environmental bacteria is expected. The GMO Panel could not identify any selective advantage which would be provided to environmental bacteria when receiving the recombinant DNA of carnation FLO-40685-2.

Considering the scope of notification C/NL/13/02, the GMO Panel therefore concluded that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation FLO-40685-2 to environmental bacteria does not give rise to environmental safety concerns.

Plant-to-plant gene transfer

Considering the scope of notification C/NL/13/02, 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.

¹² 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>

Carnation FLO-40685-2 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 tissue culture. The latter is a multiplication technique applied in the laboratory which requires particular expertise and adequate material for successful tissue culture. The 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 GMO Panel therefore considered the consequences of such potential releases and concluded that, should this occur, carnation FLO-40685-2 would not show any potential for increased survival, fitness or weediness compared with its parental variety.

In the wild, cross-pollination of *Dianthus* spp. is mainly 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 flowers to reach the nectaries and therefore discourage insect pollinator activity and limit the amount of pollen they collect and transfer to other flowers.

Moreover, the reproductive biology of *Dianthus* (OGTR, 2006) and the information¹³ provided by the notifier suggest that the pollen production by flowers and the pollen viability are low. The data indicate that the pollen transfer to other carnations is very unlikely to occur owing to very low fertility levels in most carnations. Therefore, the GMO Panel is of the opinion that the potential spread of pollen of the GM carnation by Lepidoptera to wild *Dianthus* spp. 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.

In addition, viable seed production of cut flowers is very unlikely and has not been observed to date with carnation FLO-40685-2, most probably because of its limited life time (i.e. 3 weeks) in comparison with the time needed for complete seed development (i.e. 5 weeks).

The 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 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 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/13/02 and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered a relevant issue by the GMO Panel.

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

Considering the scope of notification C/NL/13/02 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 GMO Panel.

3.4.3.5. Potential interactions with the abiotic environment and biogeochemical cycles

Considering the scope of notification C/NL/13/02 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 GMO Panel.

3.4.4. Post-market environmental monitoring¹⁴

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

¹³ Notification C/NL/13/02, Attachment A12.

¹⁴ Notification C/NL/13/02, Section D.

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.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the notifier (EFSA GMO Panel, 2011). The potential exposure to the environment of carnation SHD-27531-4 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) a literature review; 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 GMO Panel is of the opinion that the scope of the PMEM plan proposed by the notifier is in line with the limited intended use of carnation FLO-40685-2. As no potential adverse environmental effects were identified during the ERA, no case-specific monitoring is required.

3.4.5. Conclusion

Carnation FLO-40685-2 cut flowers have marginal viability and negligible pollen production, and no viable seeds have been reported. However, in the very unlikely event of escape into the environment via viable seeds, pollen or rooted plants, the GMO Panel considers that carnation FLO-40685-2 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides. Considering the scope of notification C/NL/13/02 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 GMO Panel. The unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation FLO-40685-2 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 FLO-40685-2. The GMO Panel agreed with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

4. Conclusions

In response to the request from the European Commission to assess notification C/NL/13/02, the GMO Panel adopted the present scientific opinion on the import, distribution and retailing of carnation FLO-40685-2 cut flowers in the EU for ornamental use only.

The GMO Panel reports here its evaluation of (1) the molecular characterisation data, (2) the comparative analysis of morphological characteristics between the GM carnation and the parental non-GM variety, (3) the potential toxicity and allergenicity of the newly expressed proteins and of the whole GM carnation in the light of the possible routes of exposure to humans, (4) the potential environmental impacts of the GM carnation in case of escape into the environment via viable seeds, pollen or rooted plants, and (5) the scientific quality of the PMEM plan.

Based on a comprehensive information package (e.g. notification C/NL/13/02, additional datasets, initial assessment report by the Netherlands), the GMO Panel concludes that the molecular characterisation data establish that carnation FLO-40685-2 contains inserts in four loci, consisting of three expression cassettes responsible for the intended trait (purple flower colour), conferred by the *dfr* and *f3'5'h* genes, and the herbicide tolerance, conferred by the mutated *als* gene. The stability of the newly introduced trait was observed over multiple vegetative generations.

Carnation flowers have a long history of use as ornamentals. Carnation FLO-40685-2 differs from its parental variety in that it synthesises different levels of anthocyanins in the petals, e.g. an increased

content of delphinidin and cyanidin (common pigments in many ornamental flowers and food plants). The altered levels of anthocyanins in carnation FLO-40685-2 confer a purple colour to the flowers. It is not expected that accidental intake of carnation FLO-40685-2 petals would contribute substantially to the overall intake of anthocyanins from foods.

From its assessment of the potential allergenicity and toxicity of the newly expressed proteins (DFR, F3'5'H and ALS), the GMO Panel concludes that there are no reasons for safety concern in the context of the limited scope of this notification. Given that case reports of occupational allergies to carnations are rare and considering the assessment of the newly expressed proteins, 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/13/02 and the possible routes of exposure, the GMO Panel identified no reasons for any safety concerns of carnation FLO-40685-2 for humans related to the genetic modification.

Carnation FLO-40685-2 cut flowers have marginal viability and negligible pollen production, and no viable seeds have been reported. However, in the very unlikely event of escape into the environment via viable seeds, pollen or rooted plants, the GMO Panel considers that carnation FLO-40685-2 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides. Considering the scope of notification C/NL/13/02 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 GMO Panel. The GMO Panel also concludes that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation FLO-40685-2 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 FLO-40685-2. The GMO Panel agrees with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

The GMO Panel therefore concludes that there is no scientific reason to consider that the import, distribution and retailing in the EU of carnation FLO-40685-2 cut flowers for ornamental use will cause any adverse effects on human health or the environment.

Documentation provided to EFSA

- 1) Notification C/NL/13/02 under Part C of Directive 2001/18/EC submitted by Suntory Holdings Limited to the European Commission, and received from the European Commission on 10 February 2015.
- 2) Letter from the European Commission, dated 9 February 2015, to the EFSA Executive Director concerning a request for the placing on the market of genetically modified carnation FLO-40685-2 under Directive 2001/18/EC by Suntory Holdings Limited.
- 3) Acknowledgement letter, dated 24 February 2015, from EFSA to the European Commission.
- 4) Letter from EFSA to the notifier, dated 26 March 2015, requesting additional information.
- 5) Letter from the notifier to EFSA, received on 27 April 2015, providing additional information.
- 6) Letter from the notifier to EFSA, received on 8 May 2015, providing additional information.
- 7) Letter from EFSA to the notifier, dated 3 August 2015, requesting additional information.
- 8) Letter from the notifier to EFSA, received on 31 August 2015, providing additional information.
- 9) Letter from EFSA to the notifier, dated 12 October 2015, requesting additional information.
- 10) Letter from EFSA to the notifier, dated 16 November 2015, requesting additional information.
- 11) Letter from the notifier to EFSA, received on 30 November 2015, providing additional information.
- 12) Letter from the notifier to EFSA, received on 7 December 2015, seeking clarifications on question dated 16 November 2015.
- 13) Letter from the notifier, received on 4 January 2016, requesting a reply to the clarifications request submitted to EFSA on 24 December 2015.
- 14) Letter from EFSA to notifier, dated 20 January 2016, providing clarifications requested by the notifier on 7 December 2015.
- 15) Letter from the notifier, received on 24 February 2016, providing additional information requested.
- 16) Letter from EFSA to the notifier, dated 10 March 2016, re-starting the clock.

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Abbreviations

ALS	acetolactate synthase
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources Added to Food
CHS	chalcone synthase
cDNA	complementary DNA
DFR	dihydroflavonol 4-reductase
ERA	environmental risk assessment
F3'5'H	flavonoid 3',5'-hydroxylase
fw	fresh weight
GM	genetically modified
GMO Panel	EFSA Panel on Genetically Modified Organisms
HPLC	high-performance liquid chromatography
ORF	open reading frame
PMEM	post-market environmental monitoring
PCR	polymerase chain reaction
SCF	Scientific Committee on Food
T-DNA	transfer DNA
TLC	thin-layer chromatography