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Risk assessment of genetically modified carnation SHD-27531-4

Scientific opinion on genetically modified carnation SHD-27531 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/13/01)

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

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

Phone: +47 21 62 28 00

Email: vkm@vkm.no

www.vkm.no

www.english.vkm.no

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Authors preparing the draft opinion

Ville Erling Sipinen (VKM staff), Merethe Aasmo Finne (VKM staff)

Assessed and approved

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

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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Appendix I

Abstract

Carnation SHD-27531-4 is a genetically modified variety of *Dianthus caryophyllus* L. used as a decorative plant species. The red-purple colour of the flowers results from expression of the two newly introduced genes *dfr* and *f3'5'h*, encoding the enzymes dihydroflavonol 4-reductase (DFR) and flavonoid 3',5'-hydroxylase (F3'5'H). The two enzymes enable the production of the pigments delphinidin and cyanidin (anthocyanidins) 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 SHD-27531-4 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. Southern blot analysis and sequencing indicate only a single copy of the intended T-DNA insert in the SHD-27531-4 genome. Flanking sequences show no disruption of endogenous genes. *In silico* analyses show no significant homologies between the DFR, F3'5'H and ALS proteins and known toxins and IgE-bound allergens. No observed changes in the introduced trait, *i.e.* the particular flower colour, indicative of instability, have been reported during several generations of vegetatively propagated plants.

Considering that carnation SHD-27531-4 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 SHD-27531-4 and the parent cultivar 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 SHD-27531-4 compared to the conventional counterpart or other non-GM carnations.

Likewise, the VKM GMO Panel concludes that carnation SHD-27531-4, based on current knowledge and the intended use as cut ornamental flowers, does not represent an environmental risk in Norway.

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 SHD-27531-4 only has one transgenic insert in its genome, located at a single locus. Flanking sequences indicate no disruption of endogenous genes by the insert. The insert consists only of the intended T-DNA sequence comprised by single copies of each of the three genes *dfp*, *f3'5'h* and *als*, and sequences necessary for their proper expression. Bioinformatic analyses performed by the applicant show no significant homologies between the DFR, F3'5'H and ALS proteins and known toxins or allergens. Consistency of the intended new flower colour was observed over multiple vegetative generations indicating stability of the insert in carnation SHD-27531-4.

Based on current knowledge and considering the intended use of carnation SHD-27531-4 as cut flowers the VKM GMO Panel finds the molecular characterisation of carnation SHD-27531-4 sufficient.

Comparative assessment

Considering the intended use of carnation SHD-27531-4, which excludes cultivation and use in food and feed, compositional studies were limited to analyses of the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin. The altered anthocyanidin content between the GM and non-GM parent cultivar, measured by HPLC, confirmed the intended effects of the genetic modification. Other morphological traits were assessed by pot trials and revealed that carnation SHD-27531-4 differed significantly in several traits compared to the parent cultivar. 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 SHD-27531-4, 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 SHD-27531-4 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 SHD-27531-4 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 SHD-27531-4 compared to the conventional counterpart or other non-GM carnations.

Environmental risk assessment

Considering the intended use of SHD-27531-4, 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, SHD-27531-4 has no altered survival, multiplication or dissemination characteristics compared to conventional carnation cultivars, and there are no indications of an increased likelihood of spread and establishment of feral carnation plants in the case of accidental release into the environment. Carnations are cultivated in Norway but plant to plant gene flow is not considered to be an issue due to low pollen spread and viability and low likelihood of seed development from cut flowers.

Based on current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that carnation SHD-27531-4 does not represent an environmental risk in Norway.

Post-market environmental monitoring

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

Based on current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that the environmental risk assessment did not identify any potential adverse environmental effects of the transgenic line of carnation SHD-27531-4. 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 SHD-27531-4 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 SHD-27531-4 and the parent cultivar 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 SHD-27531-4 compared to the conventional counterpart or other non-GM carnations.

Likewise, the VKM GMO Panel concludes that carnation SHD-27531-4, 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.), SHD-27531-4, anthocyanidin, anthocyanin, petal colour, *dfr*, *f3'5'h*, *als*, *SuRB*, health safety, environmental risk assessment, Directive 2001/18/EC, VKM, risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Environment Agency

Molekylær karakterisering

Den molekylære karakteriseringen viser at nellik SHD-27531-4 kun inneholder et transgent locus, og at dette består av den ønskede T-DNA sekvensen med én fungerende kopi for hver av de tre nye genene *dfr*, *f3'5'h* og *als*. Sekvensanalyser indikerer at innskuddet (T-DNAet) ikke er satt inn i et uttrykt kodende område 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. Jevnt uttrykt blomsterfarge over flere vegetativt formerte generasjoner indikerer stabilitet av de innsatte genene i nellik SHD-27531-4.

Basert på dagens kunnskap, og tiltenkt bruk som avskårne blomster, konkluderer VKMs faggruppe for GMO at den molekylære karakteriseringen av nellik SHD-27531-4 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. Forskjellene i innhold av disse mellom nellik SHD-27531-4 og den umodifiserte foreldresorten (konvensjonell kontroll) ble målt ved HPLC, og bekreftet de tilsiktede effektene av genmodifiseringen. Flere statistisk signifikante morfologiske forskjeller mellom den genmodifiserte nelliken og konvensjonell kontroll ble i tillegg vist i pottforsøk. 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 SHD-27531-4.

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 SHD-27531-4 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 SHD-27531-4 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 SHD-27531-4 sammenliknet med konvensjonell kontroll eller annen konvensjonell nellik.

Miljørisiko

Miljøriskovurderingen av nelliklinjen SHD-27531-4 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 SHD-27531-4 ikke medført endringer i egenskaper knyttet til overlevelse og spredning sammenlignet 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 SHD-27531-4 ikke vil medføre miljørerisiko i Norge.

Miljøovervåkning

Med bakgrunn i tiltenkt bruksområde er det ikke behov for en spesifikk miljøovervåkningsplan for nellik SHD-27531-4

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 delfinidin, cyanidin, petunidin og pelargonidin er tilstrekkelig for risikovurderingen. De rapporterte morfologiske forskjellene mellom SHD-27531-4 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 SHD-27531-4 sammenliknet med konvensjonell kontroll eller annen konvensjonell nellik.

Likeledes finner faggruppen, ut i fra dagens kunnskap, at den omsøkte bruken av SHD-27531-4 som avskårne prydblomster ikke vil medføre en miljørerisiko 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
DFR	Dihydroflavonol 4-reductase
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
ERA	Environmental risk assessment
EU	European Union
F3'5'H	Flavonoid 3',5'-hydroxylase
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
GM	Genetically modified
GMO	Genetically modified organisms
GMP	Genetically modified plants
mRNA	Messenger RNA
MS	Member states
OECD	Organisation for Economic Co-operation and Development
PCR	Polymerase chain reaction, a technique to amplify DNA by copying
PMEM	Post-market environmental monitoring
VKM	Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet)

Background

In March 2013, a notification (reference C/NL/13/01) covering import, distribution and retailing of the genetically modified carnation SHD-27531-4 (Unique Identifier SHD-27531-4) 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/13/01 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 cultivar SHD-27531-4.

In July 2013, 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 May 2014, EC consulted EFSA for a scientific opinion addressing these objections (ref EFSA, 2015).

In February 2015, the EFSA received an additional request from the European Commission to provide a consolidated scientific opinion as to *'whether there is any scientific reason to believe that the placing on the market of carnation line SHD-27531-4 is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC.'* The EFSA GMO Panel published its scientific opinion on application C/NL/13/01 on 15 December 2015 (EFSA, 2015).

Carnation SHD-27531-4 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 SHD-27531-4 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.

concentrations, but rapid resistance development in weeds has limited their application (review by Tranel and Wright, 2002). However, the introduction of the mutated *als* (SuRB) gene in carnation SHD-27531-4 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 SHD-27531-4 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 SHD-27531-4 is not expected to enter the food and feed chain. Nevertheless, VKM has evaluated the safety of carnation SHD-27531-4 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 SHD-27531-4 (EFSA 2015, 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 insertions and constructs
3. Information on the expression of the insert including quantification of new metabolites
4. Inheritance and stability of the inserted DNA

Carnation SHD-27531-4 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 the *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* chalcone synthase (CHS) gene, 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 which mediates tolerance to sulphonylurea-type herbicides e.g. Chlorsulfuron used during selection of transformed cells.

The size and structure of the inserted sequence in Carnation SHD-27531-4 was determined by Southern blot analysis and sequencing. According to the applicant, only a single copy of the intended T-DNA insert is present in the genome, located at a single locus. Southern and PCR analyses indicated no carrier or vector DNA other than the intended T-DNA insert.

The production of delphinidin-based pigments is confined to the petals of the transgenic flowers which turns them red-purple, compared to the pink flowers of the parental variety. Expression of the insert was determined by detecting delphinidin-type pigments using high pressure liquid chromatography (HPLC). Flowers of carnation SHD-27531-4 contain approximately 1.2 mg delphinidin per gram fresh weight (fw). Delphinidin-based pigments

have not been observed in other tissues e.g. stems, nodes, leaves and roots. Analyses of flanking sequences indicated no disruption of endogenous genes. Bioinformatic analyses showed no significant homologies between the DFR, F3'5'H and ALS proteins and known toxins or allergens.

Carnations are propagated vegetatively. According to the applicant no observed changes in the introduced trait, *i.e.* the particular flower colour, indicative of instability, has been reported during vegetatively propagated plants grown since 2007.

2.1 Conclusion

Based on current knowledge and considering the intended use of carnation SHD-27531-4 as cut flowers the VKM GMO Panel finds the molecular characterisation of carnation SHD-27531-4 sufficient.

3 Comparative assessments

The comparative assessment is adopted from the published EFSA opinion on Carnation SHD-27531-4 (EFSA 2015, Appendix I) and submitted data from the applicant.

Generally, carnations have no or very limited history of use in food and feed, and their content of nutrients, antinutritional factors and other components with biological activity is largely unknown. The proposed marketing of carnation SHD-27531-4 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

According to the applicant, carnation SHD-27531-4 and its conventional counterpart (parental variety) were grown in two pot trials in Australia in 2010. The objective of the studies was to identify any morphological differences, which could affect the vegetative or reproductive fitness of the transgenic line SHD-27531-4. The trials comprised 20 replicates per line. Details regarding experimental design and statistical analysis of the pot trials have not been included in the technical dossier from the notifier. The VKM GMO Panel considers this a short-coming in the application. However, since the carnation SDH-27531-4 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 SHD-27531-4 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 using high-performance liquid chromatography (HPLC) in accordance with the method of Fukui et al. (2003). Roots and stems were not assayed.

The pink petals of the parental variety contained mainly pelargonidin (1.34 mg/g fresh weight [fw]) complemented with small amounts of cyanidin (0.01 mg/kg fw), whereas the purple petals of the carnation SHD-27531-4 contained delphinidin (1.18 mg/g fw), cyanidin (0.51 mg/g fw), pelargonidin (0.26 mg/g fw) and petunidin (0.01 mg/g fw).

EFSA (EFSA 2015, Appendix I) considered that since the intended uses of carnation SHD-27531-4 did not include cultivation or human or animal consumption, compositional analysis limited to the newly synthesised anthocyanidins in petals was sufficient for the risk assessment. Reported differences in anthocyanidin content were not expected to influence the risk scenario upon accidental release to the environment or intake of the GM carnation.

Furthermore, EFSA concluded that the compositional data provided by the applicant confirmed the intended effects of the genetic modification.

3.3 Morphological traits and GM phenotype

In total, 26 different quantitative morphological characteristics were measured in the two Australian pot trials. Different morphological characters were observed in the trials and a statistical test of difference (single-factor ANOVA) was applied to 23 of those characteristics. Six significant differences between carnation SHD-27531-4 and conventional counterpart (parental variety) were found in the first trial (leaf length, petal length, number of internodes per stem, number of viable anthers, filament number and filament length) and one in the second trial (number of petals per flower).

EFSA (EFSA, 2015) 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.

Studies on pollen morphology and viability were performed on pollen collected from flowers in the first Australian pot trial. Pollen viability was assessed after acetocarmine staining, and by studying pollen germination. Both methods identified reduced pollen viability in carnation SHD-27531-4 compared to the parental variety. Pollen diameter was not influenced.

The notifier has also published results from observations of 27 qualitative morphological characteristics of carnation SHD-27531-4. 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 SHD-27531-4 and the parental variety.

3.4 Conclusion

Based on current knowledge and considering the intended use of carnation SHD-27531-4, 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 SHD-27531-4 and its conventional counterpart do not raise safety concerns.

4 Food and feed safety assessment

4.1 Previous evaluations by the VKM and EFSA GMO Panels

The VKM GMO Panel has previously not performed a risk assessment of carnation SHD-27531-4, however three other GM-carnations expressing the same inserted genes as SHD-27531-4 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 SHD-27531-4 in December 2015 (EFSA, 2015. Appendix I). The Panel identified no safety concerns to human health or the environment with the intended use of Carnation SHD-27531-4.

4.2 Product description and intended uses

The scope of the notification C/NL/13/01 is restricted to the import of cut flowers for ornamental use only. Accordingly, if approved, products of Carnation SHD-27531-4 will be marketed with an accompanying label or document that states that it is genetically modified and not for human or animal consumption, or for 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 SHD-27531-4 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 SHD-27531-4 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 new anthocyanidin pigments in carnation SHD-27531-4, 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 on the amino acid sequences of the newly expressed proteins in Carnation SHD-27531-4 (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 2015 a,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 cyanidin and delphinidin are naturally present in foods like aubergines, blueberries and blackcurrants at rather higher levels than in the petals of carnation SHD-27351-4 (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 individuals 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 SHD-27531-4 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 reasons for concern (EFSA 2006b; EFSA 2008; EFSA 2014a; EFSA 2014b; VKM 2008, VKM 2015 a,b,c).

The ALS, DFR and F3'5'H proteins do not show sequence resemblance to known IgE-bound 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 SHD-27531-4 is not intended for food or feed purposes. Although dermal and respiratory allergies to carnations in workers handling cut flowers/carnations has been described (Cistero-Bahima et al., 2000; Sanchez-Fernandez et al., 2004; Sanchez-Guerrero et al., 1999; Stefanaki and Pitsios, 2008), the source of which appears to be multifaceted. These allergies appear to be caused by the flower, mites such as *Tetranychus urticae* infesting the carnations, or a combination of the two. Notably, case reports of occupational allergies to carnations are rare. Interestingly, a case report of an individual with a respiratory allergy to carnations with no occupational exposure was published recently (Brinia et al., 2013). However, according to the applicant, no adverse allergenic reactions to GM carnation cut flowers used for ornamental purposes have been reported in the human populations handling the flowers.

4.5 Nutritional assessment

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 SHD-27531-4 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 SHD-27531-4 (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 *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 SHD-27531-4, a cyanidin concentration of 0.51 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 SHD-27531-4 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 SHD-27531-4, a delphinidin concentration of 1.18 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 SHD-27531-4 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 SHD-27531-4 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 SHD-27531-4 compared to the conventional counterpart or other non-GM carnations.

5 Environmental risk assessment

5.1 Introduction

This assessment applies to carnation SHD-27531-4 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. Cultivars used in Norway are frost sensitive and do not survive in regions with temperatures lower than -5°C. There is no greenhouse production of carnation for cut flowers in Norway. Thus, all the cut flowers of carnation are imported. According to Statistics Norway import of carnation in 2014 was about 427 metric tonnes (www.sbb.no).

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

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

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

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

5.2 Unintended effects on plant fitness due to the genetic modifications

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

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

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 SHD-27531-4 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 cultivars.

5.3 Potential for gene transfer

5.3.1 Plant to micro-organisms gene transfer

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

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

5.3.2 Plant to plant gene flow

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

5.4 Interaction between the GM plant and target organisms

Considering the scope of notification C/NL/13/01 and the absence of target organisms, potential interactions of carnation SHD-27531-4 with target organisms were not considered a relevant issue by the VKM GMP Panel.

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 SHD-27531-4 will have any adverse effect on abiotic environment or biochemical cycles.

5.7 Conclusion

Considering the intended use of SHD-27531-4, 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, SHD-27531-4 has no altered survival, multiplication or dissemination characteristics compared to conventional carnation cultivars, and there are no indications of an increased likelihood of spread and establishment of feral carnation plants in the case of accidental release into the environment. Carnations are cultivated in Norway but plant to plant gene flow is not considered to be an issue due to low pollen spread and viability and low likelihood of seed development from cut flowers.

Based on current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that carnation SHD-27531-4 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 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 PMEM plan proposed by the applicant includes (1) a questionnaire for the European importers and operators, including questions on unexpected adverse effects; (2) the consultation of a network of taxonomists and botanists to report on any wild populations or unusual *Dianthus* hybrids that might originate from the GM carnation; (3) European

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

The VKM GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the restricted intended uses of carnation SHD-27531-4. No specific environmental impact of genetically modified carnation SHD-27531-4 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 SHD-27531-4. Thus, the general surveillance plan is sufficient and there is no need for a specific surveillance plan.

7 Conclusions (with answers to terms of reference)

Molecular characterisation

The molecular characterisation shows that carnation SHD-27531-4 only has one transgenic insert in its genome, located at a single locus. Flanking sequences indicate no disruption of endogenous genes by the insert. The insert consists only of the intended T-DNA sequence comprised by single copies of each of the three genes *dfp*, *f3'5'h* and *als*, and sequences necessary for their proper expression. Bioinformatic analyses performed by the applicant show no significant homologies between the DFR, F3'5'H and ALS proteins and known toxins or allergens. Consistency of the intended new flower colour was observed over multiple vegetative generations indicating stability of the insert in carnation SHD-27531-4.

Based on current knowledge and considering the intended use of carnation SHD-27531-4 as cut flowers the VKM GMO Panel finds the molecular characterisation of carnation SHD-27531-4 sufficient.

Comparative assessment

Considering the intended use of carnation SHD-27531-4, which excludes cultivation and use in food and feed, compositional studies were limited to analyses of the anthocyanidins delphinidin, cyanidin, petunidin and pelargonidin. The altered anthocyanidin content between the GM and non-GM parent cultivar, measured by HPLC, confirmed the intended effects of the genetic modification. Other morphological traits were assessed by pot trials and revealed that carnation SHD-27531-4 differed significantly in several traits compared to the parent cultivar. 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 SHD-27531-4, 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 SHD-27531-4 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 SHD-27531-4 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 SHD-27531-4 compared to the conventional counterpart or other non-GM carnations.

Environmental risk assessment

Considering the intended use of SHD-27531-4, 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, SHD-27531-4 has no altered survival, multiplication or dissemination characteristics compared to conventional carnation cultivars, and there are no indications of an increased likelihood of spread and establishment of feral carnation plants in the case of accidental release into the environment. Carnations are cultivated in Norway but plant to plant gene flow is not considered to be an issue due to low pollen spread and viability and low likelihood of seed development from cut flowers.

Based on current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that carnation SHD-27531-4 does not represent an environmental risk in Norway.

Post-market environmental monitoring

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

Based on current knowledge and considering its import, distribution and intended use as cut ornamental flowers, the VKM GMO Panel concludes that the environmental risk assessment did not identify any potential adverse environmental effects of the transgenic line of carnation SHD-27531-4. 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 SHD-27531-4 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 SHD-27531-4 and the parent cultivar 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 SHD-27531-4 compared to the conventional counterpart or other non-GM carnations.

Likewise, the VKM GMO Panel concludes that carnation SHD-27531-4, based on current knowledge and the intended use as cut ornamental flowers, does not represent an environmental risk in Norway.

8 Data gaps & Uncertainties

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 SHD-27531-4 is lacking.

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Part C notification (reference C/NL/13/01) from Suntory Holdings Limited for the import, distribution and retailing of carnation SHD-27531-4 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 (EFSA GMO Panel) has evaluated the overall safety of genetically modified (GM) carnation SHD-27531-4 cut flowers to be imported into the European Union (EU) for ornamental use. The genetic modification results in the flowers having purple petals. The stability of the new colour trait 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 EFSA 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 SHD-27531-4 for humans. The EFSA 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 EFSA GMO Panel did not identify any environmental safety concerns and agrees with the scope of the post-market environmental monitoring plan. The EFSA 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-2015-00126

Correspondence: gmo@efsa.europa.eu

Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Achim Gathmann, Mikolaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Hanspeter Naegeli, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal.

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Summary

Following a request from the European Commission, the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on notification C/NL/13/01 from Suntory Holdings Limited submitted under Part C of Directive 2001/18/EC¹. The scope of notification C/NL/13/01 covers the import, distribution and retailing in the European Union (EU) of genetically modified (GM) carnation SHD-27531-4 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 EFSA GMO Panel was, therefore, asked to consider if there is any scientific reason to believe that the placing on the market of carnation SHD-27531-4 is likely to cause any adverse effects on human health and the environment.

In delivering the present scientific opinion, the EFSA GMO Panel considered the full notification C/NL/13/01, including e.g. additional information provided by the notifier, the assessment report of the Dutch competent authority, the concerns raised by Member States, relevant scientific publications and the experience gained in assessing GM carnations with similar traits.

During its safety evaluation, the EFSA 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/13/01.

Carnation SHD-27531-4 has a modified flower colour, a shade of purple, whereas the parental line has a pink 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 SHD-27531-4 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 EFSA GMO Panel concludes that the molecular characterisation data establish that carnation SHD-27531-4 contains one insert, consisting of three expression cassettes responsible for the intended trait (purple flower colour) conferred by the dihydroflavonol 4-reductase (*dfh*) 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 was observed over multiple vegetative generations.

Carnation flowers have a long history of use as ornamentals. Carnation SHD-27531-4 differs from its parental variety in that it synthesises different levels of anthocyanins in the petals, e.g. an increased content of delphinidin, cyanidin and petunidin (common pigments in many ornamental flowers and food plants). The altered levels of anthocyanins in carnation SHD-27531-4 confer a purple colour to the flowers. It is not expected that accidental intake of carnation SHD-27531-4 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 EFSA 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/13/01 and the possible routes of exposure, the EFSA GMO Panel identified no reasons for any safety concerns of carnation SHD-27531-4 for humans related to the genetic modification.

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.

Carnation SHD-27531-4 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 EFSA GMO Panel considers that carnation SHD-27531-4 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides. Considering the scope of notification C/NL/13/01 and the low level of exposure to the environment, interactions with the biotic and abiotic environment are not considered to be relevant issues by the EFSA GMO Panel. The EFSA GMO Panel also concludes that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation SHD-27531-4 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 SHD-27531-4. The EFSA GMO Panel agrees with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

The EFSA GMO Panel therefore concludes that there is no scientific reason to consider that the import, distribution and retailing in the EU of carnation SHD-27531-4 cut flowers for ornamental use will cause any adverse effects on human health or the environment.

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1. Introduction

Carnation SHD-27531-4 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 SHD-27531-4 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 SHD-27531-4 is evaluated by the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) in light of the scope of notification C/NL/13/01, i.e. import, distribution and retailing in the European Union (EU) of GM carnation SHD-27531-4 cut flowers for ornamental use only.

Both intentional and accidental oral intake of GM carnation flowers by animals were excluded from this opinion, as carnation SHD-27531-4 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 EFSA GMO Panel did not assess the possible consequences of the intentional consumption of GM carnations by humans². Nevertheless, the EFSA GMO Panel evaluated the safety of carnation SHD-27531-4 for humans considering three possible routes of exposure: (1) dermal contact, (2) inhalation and (3) accidental oral intake³.

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 July 2013, the European Commission received the full notification (reference C/NL/13/01), 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 (i.e. Cyprus) maintained objections 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 May 2014, the European Commission consulted the EFSA for a scientific opinion in response to the three objections raised by Cyprus. In October 2014, the EFSA GMO Panel issued a scientific opinion addressing the objections of Cyprus (EFSA, 2014a).

In February 2015, the EFSA received an additional request from the European Commission to provide a consolidated scientific opinion as to *'whether there is any scientific reason to believe that the placing on the market of carnation line SHD-27531-4 is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC.'*

² The EFSA GMO Panel is aware of a food habit in certain populations to intentionally consume carnation petals as garnish; however, this intentional use is outside the scope of this notification.

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

2. Data and Methodologies

2.1. Data

The present safety evaluation of GM carnation SHD-27531-4 by the EFSA GMO Panel is based on the information provided in notification C/NL/13/01, including e.g. additional information⁵ provided by the notifier, the assessment report of the Dutch competent authority, the concerns raised by Member States, relevant scientific publications and the experience gained in assessing GM carnations with similar traits (EFSA, 2006, 2008; EFSA GMO Panel, 2014a,b,c).

2.2. Methodologies

The EFSA GMO Panel performed its safety evaluation of GM carnation SHD-27531-4 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 environmental risk assessment (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 SHD-27531-4 remained at the end of the 45-day Member States' consultation period.

3.1.2. Evaluation of relevant scientific data

Transformation process and vector constructs

To develop the carnation line SHD-27531-4, the conventional carnation *Dianthus caryophyllus* L. 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 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*. This acetolactate synthase provided tolerance to sulfonylurea herbicides and was used as a marker in the selection of transformants.

Transgene constructs in the genetically modified plants

Carnation SHD-27531-4 contains one insert consisting of the T-DNA region of the transformation vector pCGP1991.

Southern blot and polymerase chain reaction (PCR) analyses indicated that no plasmid backbone sequences had been integrated into carnation SHD-27531-4. The sequences of the insert and the flanking regions were provided.

⁵ See section 'Documentation provided to EFSA'

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

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

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

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.

Inheritance and stability of the inserted DNA

Genetic stability of carnation SHD-27531-4 was studied by visual observation of flower colour in vegetatively propagated plants grown since 2007. There were no incidents reported of a flower colour change that would indicate genetic instability.

3.1.3. Conclusion

The molecular characterisation data establish that carnation SHD-27531-4 contains one insert, consisting of three expression cassettes responsible for the intended trait (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 SHD-27531-4 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 SHD-27531-4 remained at the end of the 45-day Member States' consultation period.

3.2.2. Evaluation of relevant scientific data

The EFSA 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).

Choice of comparator

Carnation SHD-27531-4, having purple-coloured petals, was compared with the parental non-GM carnation variety which is characterised by pink-coloured petals.

Compositional analysis

The comparative analysis of the composition of carnation SHD-27531-4 was limited to the anthocyanin content, in order to identify the intended changes. The content of the anthocyanin colour pigments delphinidin, cyanidin, petunidin and pelargonidin was determined in acetonitrile extracts of freeze-dried petals using high-performance liquid chromatography (HPLC) in accordance with the method of Fukui et al. (2003).

The pink petals of the parental variety contained mainly pelargonidin pigments (1.34 mg/g fresh weight [fw]) complemented with small amounts of cyanidin pigments (0.01 mg/kg fw), whereas the purple petals of the carnation SHD-27531-4 contained delphinidin (1.18 mg/g fw), cyanidin (0.51 mg/g fw), pelargonidin (0.26 mg/g fw) and petunidin (0.01 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 SHD-27531-4 explain the intended phenotypic change in the flower colour.

Morphological traits and genetically modified phenotype

Flower colour differed between carnation SHD-27531-4 (purple) and the parental variety (pink). In the comparison of 27 qualitative morphological characteristics, no differences were found between carnation SHD-27531-4 and its comparator (i.e. the parental variety). In two trials performed in Australia in 2010, 26 quantitative morphological characteristics were measured for carnation SHD-27531-4 and its comparator, and a statistical test of difference (single-factor ANOVA) was applied to 23 of those characteristics⁶. Six significant differences between carnation SHD-27531-4 and the comparator were found in the first trial (for leaf length, petal length, number of internodes per stem, number of viable anthers, filament number and filament length) and one in the second trial (number of petals per flower).

Studies on pollen morphology and viability were performed on pollen collected from flowers in the first Australian trial. Pollen viability was assessed after acetocarmine staining, and by studying pollen germination. Both methods identified reduced pollen viability in carnation SHD-27531-4. Pollen diameter was not influenced.

3.2.3. Conclusion

The altered levels of anthocyanins in carnation SHD-27531-4 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 SHD-27531-4 for humans remained at the end of the 45-day Member States' consultation period.

3.3.2. Evaluation of relevant scientific data

Toxicology

(a) Toxicological assessment of newly expressed proteins

Bioinformatic analyses of the amino acid sequences of the three proteins newly expressed in carnation SHD-27351-4 (ALS, DFR and F3'5'H) reveal no significant similarities to known toxins to humans (see Section 3.1.3).

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

⁶ Of the characteristics tested for significant differences, eleven were measured in both trials (plant height at flowering, length of 5th node, leaf length, 3rd from flower, flower diameter, calyx length, number of petals per flower, petal length, petal width, number of styles, style length and days to flowering), eight only in the first trial (number of internodes per stem, thickness of 5th node, height of corolla, calyx diameter, number of lobes per calyx, number of viable anthers, filament length and number of filaments), and four only in the second trial (stem length, stem diameter, leaf width and flower height). The three characteristics not formally tested were measured only in the first trial (pollen diameter, % pollen viability (acetocarmine) and % pollen viability (germination)).

(b) Toxicological assessment of new constituents other than proteins

As intended, the anthocyanin profile of carnation SHD-27351-4 differs from that of parental variety used as comparator (see Section 3.2.2). Delphinidin and petunidin are present in carnation SHD-27351-4 and not in its comparator, and a higher level of cyanidin is found in carnation SHD-27351-4. These anthocyanins can also be found in many foods and, in some of them, at much higher concentrations than in the petals of carnation SHD-27351-4. Particularly high concentrations can be found, for example, in blueberries, blackcurrant, black plum and red cabbage (Wu et al., 2006). According to Regulation⁷ 1333/2008 on food additives, anthocyanins (E 163) are authorised food additives in the EU. Anthocyanins have been evaluated by the Scientific Committee on Foods (SCF), which concluded that anthocyanins prepared by physical processes from natural foods are acceptable for use in food without further investigations. The SCF indicated that anthocyanins derived from natural sources are only acceptable as food additives if the quantities ingested do not differ substantially from the amounts that are likely to be ingested as a result of the normal consumption of the foods in which they occur naturally (SCF, 1975). In the re-evaluation of anthocyanins, the Scientific Panel on Food Additives and Nutrient Sources Added to Food of EFSA (EFSA ANS Panel, 2013) concluded that, provided that exposure from the use of food colours is 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 SHD-27351-4 petals would contribute substantially to the overall intake of anthocyanins from foods. Therefore, the EFSA GMO Panel sees no reason for concern regarding the anthocyanin profile in petals of carnation SHD-27351-4.

(c) Toxicological assessment of the whole genetically modified plant

Given that carnation SHD-27351-4 is not intended for human consumption as food but is intended for ornamental use only, the EFSA GMO Panel considered the possible effects of the genetic modification on human health in the case of accidental intake (EFSA, 2009a). Considering the assessment of the newly expressed proteins and of the new constituents other than proteins, the EFSA GMO Panel identified no reasons for food safety concern.

Allergenicity

(a) Allergenicity assessment of newly expressed proteins

Bioinformatic analyses of the amino acid sequence of the newly expressed proteins in carnation SHD-27351-4 using the criterion of more than 35 % identity in a segment of 80 or more amino acids (Codex Alimentarius, 2003) revealed no significant similarities to known allergens. In addition, the notifier performed analyses searching for matches of eight contiguous identical amino acid sequences between these newly expressed proteins and known allergens, which confirmed the outcome of the above-mentioned bioinformatic analyses showing no similarities to known allergens.

The EFSA GMO Panel has previously assessed the 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, 2014b,c).

(b) Allergenicity assessment of the whole genetically modified plant⁸

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.

⁷ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33

⁸ Additional information: 26 November 2015

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

According to the notifier, no adverse reactions (including contact dermatitis) to carnation SHD-27351-4 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/13/01, 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.

3.3.3. Conclusion

Carnation flowers have a long history of use as ornamentals. Carnation SHD-27351-4 differs from its parental variety in that it synthesises different levels of anthocyanins, e.g. an increased content of delphinidin, cyanidin and petunidin (common pigments in many ornamental flowers and food plants) in the petals. The altered levels of anthocyanins in carnation SHD-27531-4 confer a purple colour to the flowers. It is not expected that accidental intake of carnation SHD-27351-4 petals would contribute substantially to the overall intake of anthocyanins from foods.

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/13/01 and the possible routes of exposure, the EFSA GMO Panel identified no reasons for safety concerns of carnation SHD-27351-4 for humans related to the genetic modification.

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

3.4.1. Objections raised by Member States

At the end of the 45-day Member States' consultation period, Cyprus maintained the following objections:

- *human aided propagation of carnation line SHD-27531-4 cannot be excluded;*
- *the risk of potential spread of pollen by Lepidoptera insects in the endemic species *Dianthus* occurring in Cyprus cannot be eliminated;*
- *a non-negligible potential for gene transfer would exist if all imported cut flowers were kept outside for the duration of their use.*

The EFSA GMO Panel already addressed these objections in its scientific opinion adopted on 22 October 2014 (EFSA, 2014a).

3.4.2. Evaluation of relevant scientific data

Considering the scope of notification C/NL/13/01, 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.

3.4.3. Environmental risk assessment⁹

Potential unintended effects on plant fitness due to the genetic modification

Carnation is the common name of *Dianthus caryophyllus* (i.e. cultivated carnation). Members of the genus *Dianthus*, including wild and domesticated species, are fairly diverse, as their origins range from southern Russia to the Alpine region of Greece and the Auvergne mountains of France. *Dianthus* spp. are adapted to the cooler Alpine regions of Europe and Asia, and are also found in Mediterranean coastal regions. *D. caryophyllus* is a widely cultivated ornamental plant in Europe both in glasshouses and outdoors (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 one week or more after anthers have shed pollen. Cultivated carnations require pollination by hand to set seed (Bird, 1994). As a result of the long history of use of vegetative propagation and selection for flower characteristics, the carnation produces only a negligible amount of pollen, and consequently seed set is low or absent (Galbally and Galbally, 1997). The quantity and quality of pollen varies with the cultivar (Kho and Baer, 1973; Galbally and Galbally, 1997). Carnation pollen is heavy and sticky and has low viability. Wind plays little role in pollen dispersal (OGTR, 2006). In the wild, cross-pollination of *Dianthus* spp. is by insect pollinators, in particular by Lepidoptera, which have probosces of sufficient length to reach the nectaries at the base of the flowers.

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 one to two years. Flowers are produced in flushes, beginning three to five months after rooted cuttings are planted. Plants can also be multiplied by tissue culture techniques.

Carnation SHD-27531-4 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 affects plant fitness of these species.

Carnation SHD-27531-4 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 seeds of some tolerant weed biotypes germinate more rapidly, especially in cool temperatures. No seeds have been found in cut flowers of carnation SHD-27531-4 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 EFSA 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.

⁹ Notification C/NL/13/01, Section B

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 since these herbicides are not known to be used on cultivated carnations. The notifier provided data on 26 quantitative morphological characteristics of carnation SHD-27531-4 compared with its parental variety from two trials in Australia in 2010 (see Section 3.2.2 for more details). Statistically significant differences between the GM carnation and its parental variety were observed for leaf length, petal length, number of internodes per stem, number of viable anthers, filament number, filament length and number of petals per flower; but not consistently throughout the two trials. The reduced number of viable anthers in carnation SHD-27531-4 observed from one of the trials resulted in reduced pollen production and this pollen had reduced viability. 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. Therefore the EFSA 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 (EFSA, 2014a).

No evidence has been found that that the flower colour and herbicide tolerant traits introduced by the genetic modification into carnation SHD-27531-4 would result in increased persistence and invasiveness of this or any other *Dianthus* species.

Moreover, the EFSA GMO Panel is not aware of any scientific reports of increased spread and establishment of (GM) carnations or of any change in survival capacity, including overwintering (COGEM report¹⁰; EFSA, 2006, 2008, 2014a,b,c). 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/13/01 and the data available, the EFSA GMO Panel considered that there would be no changes in plant characteristics of any ecological significance. Carnation SHD-27531-4 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 EFSA GMO Panel also concludes that the propagation of the GM carnation (e.g. by rooting) cannot be excluded. However, should this occur, carnation SHD-27531-4 would not show any potential for increased survival, fitness or weediness compared with its parental variety.

Potential for gene transfer

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

(a) Plant-to-bacteria gene transfer

Considering the scope of notification C/NL/13/01, 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 gastro-intestinal tract bacteria and microbial decomposers of faecal material will be very low.

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

Successful horizontal gene transfer would require the stable insertion of the transgene sequences into a bacterial genome and a selective advantage conferred on the transformed host. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. This requires the presence of stretches of DNA sequences

¹⁰ 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>

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 SHD-27531-4 does not contain genetic elements with identity or high similarity to those of bacteria. The recombinant genetic elements used for the construction of carnation SHD-27531-4 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 SHD-27531-4 to environmental bacteria is expected. The EFSA GMO Panel could not identify any selective advantage which would be provided to environmental bacteria when receiving the recombinant DNA of carnation SHD-27531-4.

Considering the scope of notification C/NL/13/01, the EFSA GMO Panel therefore concluded that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation SHD-27531-4 to environmental bacteria does not give rise to environmental safety concerns.

(b) Plant-to-plant gene transfer

Considering the scope of notification C/NL/13/01, the ERA is mainly concerned with indirect exposure through (1) unintended release into the environment of GM carnations obtained by vegetative multiplication, (2) pollen dispersal from GM cut flowers to other carnations and wild relatives and (3) dispersal of seeds produced by GM cut flowers and possible progeny.

Carnation SHD-27531-4 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 EFSA GMO Panel is of the opinion that this technique is unlikely to be used by individuals (e.g. amateur gardeners) to propagate GM carnations. However, the GM carnation could be propagated by rooting and then released into the environment (e.g. gardens). The EFSA GMO Panel therefore considered the consequences of such potential releases and concluded that, should this occur, carnation SHD-27531-4 would not show any potential for increased survival, fitness or weediness compared with its parental variety (EFSA, 2014a).

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 information¹¹ provided by the notifier suggest that pollen production by flowers and pollen viability are low. The data indicate that pollen transfer to other carnations is very unlikely to occur owing to very low fertility levels in most carnations. Therefore EFSA 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 SHD-27531-4, most probably because of its limited life time (i.e. three weeks) in comparison with the time needed for complete seed development (i.e. five weeks).

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

¹¹ Notification C/NL/13/01, Attachment A11

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

Potential interactions of the genetically modified plant with target organisms

Considering the scope of notification C/NL/13/01 and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered a relevant issue by the EFSA GMO Panel.

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

Considering the scope of notification C/NL/13/01 and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered a relevant issue by the EFSA GMO Panel.

Potential interactions with the abiotic environment and biogeochemical cycles

Considering the scope of notification C/NL/13/01 and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the EFSA GMO Panel.

3.4.4. Post-market environmental monitoring¹²

According to Annex VII of Directive 2001/18/EC, the objectives of a post-market environmental monitoring (PME) plan are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus a final adoption of the PME plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the PME 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 PME plan provided by the notifier is in line with the restricted intended use of GM carnation cut flowers.

The PME 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 PME report on an annual basis. The report will include, for example, the number of imported GM cut flowers and a report of the identified hybrids and of feral carnation populations, if any.

The EFSA GMO Panel is of the opinion that the scope of the PME plan proposed by the notifier is in line with the limited intended use of carnation SHD-27531-4. As no potential adverse environmental effects were identified during the ERA, no case-specific monitoring is required.

3.4.5. Conclusion

Carnation SHD-27531-4 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

¹² Notification C/NL/13/02, Section D

via viable seeds, pollen or rooted plants, the EFSA GMO Panel considers that carnation SHD-27531-4 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides. Considering the scope of notification C/NL/13/01 and the low level of exposure to the environment, interactions with the biotic and abiotic environment are not considered to be relevant issues by the EFSA GMO Panel. The EFSA GMO Panel also concluded that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation SHD-27531-4 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 SHD-27531-4. The EFSA GMO Panel agreed with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

4. Conclusions

In response to the request from the European Commission to assess notification C/NL/13/01, the EFSA GMO Panel adopted the present scientific opinion on the import, distribution and retailing in the EU of carnation SHD-27531-4 cut flowers for ornamental use only.

The EFSA 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 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/13/01, additional datasets, initial assessment report by The Netherlands), the EFSA GMO Panel concludes that the molecular characterisation data establish that carnation SHD-27531-4 contains one insert, consisting of three expression cassettes responsible for the intended trait (purple flower colour) conferred by the *dfrr* and *f3'5'h* genes, and 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 SHD-27351-4 differs from its parental variety in that it synthesises different levels of anthocyanins in the petals, e.g. an increased content of delphinidin, cyanidin and petunidin (common pigments in many ornamental flowers and food plants). The altered levels of anthocyanins in carnation SHD-27531-4 confer a purple colour to the flowers. It is not expected that accidental intake of carnation SHD-27351-4 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 EFSA 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/13/01 and the possible routes of exposure, the EFSA GMO Panel identified no reasons for any safety concerns of carnation SHD-27351-4 for humans related to the genetic modification.

Carnation SHD-27531-4 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 EFSA GMO Panel considers that carnation SHD-27531-4 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides. Considering the scope of notification C/NL/13/01 and the low level of exposure to the environment, interactions with the biotic and abiotic environment are not considered to be relevant issues by the EFSA GMO Panel. The EFSA GMO Panel also concludes that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation SHD-27531-4 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 SHD-27531-4. The EFSA GMO Panel agrees with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

The EFSA GMO Panel therefore concludes that there is no scientific reason to consider that the import, distribution and retailing in the EU of carnation SHD-27531-4 cut flowers for ornamental use will cause any adverse effects on human health or the environment.

Documentation provided to EFSA

1. Notification C/NL/13/01 under Part C of Directive 2001/18/EC submitted by Suntory Holdings Limited to the European Commission, and received from the European Commission on 20 February 2015.
2. Letter from the European Commission, dated 19 February 2015, to the EFSA Executive Director concerning a request for the placing on the market of genetically modified carnation SHD-27531-4 under Directive 2001/18/EC by Suntory Holdings Limited.
3. Acknowledgement letter, dated 3 March 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 EFSA to the notifier, dated 3 August 2015, requesting additional information.
7. Letter from EFSA to the notifier, dated 12 October 2015, requesting additional information.
8. Letter from the notifier to EFSA, received on 28 August 2015, providing additional information.
9. Letter from the notifier to EFSA, received on 26 November 2015, providing additional information.
10. Letter from EFSA to the notifier, dated 7 December 2015, restarting the clock.

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