SCIENTIFIC OPINION

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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-27351-4 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

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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/EC[1]. 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 (*dfr*) 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-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.

[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 humans[2]. 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 intake[3].

Moreover, a very limited environmental exposure with respect to viable plant parts of the GM carnation is expected. Hence, the environmental risk assessment (ERA) is mainly concerned with the consequences of exposure through: (1) unintended release into the environment of GM carnations obtained by vegetative multiplication, (2) pollen dispersal from GM cut flowers to other carnations and wild relatives, (3) dispersal of seeds produced by GM cut flowers and possible progeny and (4) discarded GM carnation cut flowers resulting in possible exposure of environmental bacteria to recombinant DNA.

1.1. Background and Terms of Reference as provided by the requestor

In 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/EC[4], 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 information[5] 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 caryoplyllus* 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 characteristics[6]. 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 Regulation[7] 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 plant[8]

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 assessment[9]

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 effects 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 report[10]; 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 information[11] 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 monitoring[12]

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

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the notifier (EFSA GMO Panel, 2011). The potential exposure to the environment of carnation SHD-27531-4 would be mainly through (1) unintended release into the environment of GM carnations obtained by vegetative multiplication, (2) pollen dispersal from GM cut flowers to other carnations and wild relatives, (3) dispersal of seeds produced by GM cut flowers and possible progeny and (4) discarded GM carnation cut flowers resulting in possible exposure of environmental bacteria to recombinant DNA. The scope of the PMEM plan provided by the notifier is in line with the restricted intended use of GM carnation cut flowers.

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

The EFSA GMO Panel is of the opinion that the scope of the PMEM plan proposed by the notifier is in line with the limited intended use of carnation 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 *dfr* 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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