Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/NL/04/02) for the placing on the market of the genetically modified carnation Moonlite 123.2.38 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Florigene[1]

(Question No EFSA-Q-2005-282)

Opinion adopted on 17 May 2006

SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on the notification to import carnation Moonlite 123.2.38 variety, genetically modified (GM) for flower colour (Unique Identifier FLO-40644-4). The GM carnation also contains a gene conferring tolerance to sulfonylurea herbicides. Cut flowers of carnation Moonlite 123.2.38 are intended to be imported within the European Union for ornamental use only.

The present opinion is based on a question raised by the Commission related to a notification to place carnation Moonlite 123.2.38 on the market under Directive 2001/18/EC (Reference C/NL/04/02). The question followed a scientific assessment that was initially made by the competent authority of the Netherlands and evaluated subsequently by all other Member States. An assessment of the GM carnation Moonlite 123.2.38 was requested by the Commission because of questions raised by several Member States following the evaluations at the national level. When this is the case, the EU legislation requires that EFSA carries out a further assessment and provides an opinion. The GMO Panel was, therefore, asked to consider whether there is any scientific reason to believe that the placing on the market of the GM carnation Moonlite 123.2.38 for import is likely to cause any adverse effects on human health and the environment.

In delivering its opinion, the GMO Panel considered the notification, additional information provided by the applicant and the specific questions and concerns raised by the Member States. The carnation Moonlite 123.2.38 was assessed with reference to its intended use and the appropriate principles described in the 'Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed'. The scientific assessment included examination of the DNA inserted into the GM carnation using *Agrobacterium*-mediated transformation and the nature and safety of the new products intended to be produced by the GM variety. Furthermore, the potential environmental impact of carnation Moonlite 123.2.38, including a monitoring plan, was assessed in the context of the restricted intended use of carnation Moonlite 123.2.38.

The carnation Moonlite 123.2.38 has a modified flower colour, a shade of violet. The colour has been achieved by introducing into white carnation two genes of the anthocyanin biosynthesis

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pathway from petunia. These genes, encoding dihydroflavonol 4-reductase (*dfr*) and flavonoid 3'5' hydroxylase (*f3'5'h*), in combination with other genes of the anthocyanin biosynthesis pathway already present in the carnation, give rise to the anthocyanins delphinidin and cyanidin, the same compounds that give colour to blueberry, blackcurrant and red grape. Both anthocyanins are present in the petals of the GM carnations. Carnation Moonlite is also tolerant to sulfonylurea herbicides conferred by a mutated *SuRB (als)* gene used as marker trait in the selection of genetically modified plants but not for plant protection purposes. Another GM carnation variety, Florigene Moondust™, which is genetically modified with the same transformation vector, received the consent for placing on the market, including cultivation, within the EU in 1997.

The molecular analysis of the DNA inserts confirms that the three genes expressing the intended traits (violet flower colour encoded by *dfr* and *f3'5'h* genes and herbicide tolerance encoded by the mutated *SuRB (als)* gene) are present into carnation Moonlite 123.2.38. The carnation Moonlite 123.2.38 does not contain a functional antibiotic resistance marker gene. Bioinformatic analysis shows that two new open reading frames (ORFs) were created but that neither shows homologies to any toxic or allergenic proteins. Results of bioinformatic studies of the three newly expressed proteins in carnation Moonlite 123.2.38 did not indicate relevant homology with known toxins or allergens.

Given the intended use of carnation Moonlite 123.2.38 (excluding human or animal consumption and cultivation), the GMO Panel considers that the comparative analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment. The GMO Panel concludes that there is no indication of increased toxicity of the carnation Moonlite 123.2.38 compared to the recipient variety.

The carnation Moonlite 123.2.38 was assessed for imported cut flowers for ornamental use only. Scientific information on potential environmental effects associated with the cultivation of carnation Moonlite 123.2.38 was therefore not required. Carnation Moonlite 123.2.38 cut stems and flowers have very restricted viability, very low pollen emission and little or no viable seed. However, in the very unlikely event of accidental release into the environment, the GMO Panel considers that the carnation Moonlite 123.2.38 would not show enhanced fitness characteristics, except in the presence of sulfonylurea herbicides. The consequences of the potential transfer of the three genes would be negligible in terms of adverse effects on the environment. The GMO Panel concludes that there is no indication that GM carnation Moonlite 123.2.38 will have adverse effects on the environment in the context of the intended use.

The GMO Panel agrees with the applicant that the environmental risk assessment did not identify risks that require a case-specific monitoring plan. The GMO Panel also agrees with the general methods and approaches of the general surveillance plan.

In conclusion, the GMO Panel considers that the information available for carnation Moonlite 123.2.38 addresses the outstanding questions raised by the Member States and considers that, in the context of its intended use, carnation Moonlite 123.2.38 is unlikely to have adverse effects on human and animal health or the environment.

Key words: acetolactate synthase (SuRB/ALS), anthocyanin, carnation, C/NL/04/02, delphinidin, *Dianthus caryophyllus*, dihydroflavonol 4-reductase (DFR), Directive 2001/18/EC, environment, feed safety, flavonoid 3'5' hydroxylase (F3'5'H), Florigene, flower colour, GMO, health, herbicide tolerance, import, sulfonylurea, Unique Identifier FLO-40644-4.

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BACKGROUND

The Commission received the notification (Reference C/NL/04/02) from Florigene, on 9 December 2005, together with a positive assessment report, from the lead Member State (The Netherlands).

In accordance with Directive 2001/18/EC (EC, 2001), the notification was then transmitted to the Competent Authorities of the other Member States, a number of which have raised objections during the statutory 60-day period. The applicant provided the Member States with additional information in response to the objections raised during the 60-day period. The Member States had until 6 November 2005 to confirm or lift their objections. Where these objections are maintained, the Commission is required to consult the relevant Scientific Committees for opinion, now represented by EFSA.

Article 18(1) of Directive 2001/18/EC states that the period of time during which the Commission is awaiting the opinion of the Scientific Committee shall not exceed 90 days. The evaluation by EFSA started on 4 January 2006, after receipt of the complete background information (request from the Commission, dossier of the applicant and final objections maintained by the Member States). During the 90-day period, EFSA requested further clarifications from the applicant. This procedure extended the final deadline set for the delivery of this opinion.

In delivering its opinion the GMO Panel considered the original notification, additional information provided by the applicant and the specific questions and concerns raised by the Member States.

The scope of notification C/NL/04/02 is restricted to the import of cut flowers of carnation Moonlite 123.2.38 for ornamental use only, produced by vegetative propagation. The progeny derived from sexual crosses with Moonlite 123.2.38 variety is not covered under notification C/NL/04/02.

TERMS OF REFERENCE

EFSA was requested, under Article 29(1) and in accordance with Article 22(5)(c) of Regulation (EC) No 178/2002 (EC, 2002a), to provide a scientific opinion as to whether there is any scientific reason to believe that the placing on the market of the GM carnation Moonlite 123.2.38 for import is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC (EC, 2001).

In particular, EFSA was requested to take account of the scientific objections raised by the Competent Authorities of the Member States in this context, to highlight diverging scientific views, if any, and how these are resolved in the opinion.

EFSA was not requested to give an opinion on the non-scientific objections raised by the Competent Authorities in their replies, in the context of the entry into force of forthcoming legislation or requests for further legislative/implementing measures.

ASSESSMENT

1. Introduction

The genetically modified (GM) carnation Moonlite 123.2.38 (Unique Identifier FLO-40644-4) was assessed with reference to its intended use, taking account of the appropriate principles described in the 'Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed' (EFSA, 2004) which was updated with a new version of chapter 11.4 on General Surveillance as part of the post market environmental monitoring (EFSA, 2006). In its evaluation the Panel focused in particular on the issues raised by the Member States during the initial assessment of the notification (Reference C/NL/04/02) introduced under Directive 2001/18/EC. The evaluation presented here is based on the information provided in the original notification related to carnation Moonlite 123.2.38 submitted to the Competent Authority of the Netherlands including additional information from the applicant in reply to the Member States questions. This information was provided to the Member States via EFSA-net.

The scope of notification C/NL/04/02 is restricted to the import of cut flowers of carnation Moonlite 123.2.38 for ornamental use only, produced by vegetative propagation. The progeny derived from sexual crosses with Moonlite 123.2.38 variety is not covered under notification C/NL/04/02.

Carnation Moonlite 123.2.38 is a new variety which contains the herbicide tolerance *SuRB* (*als*) gene coding for a mutant acetolactate synthase protein (ALS), used to facilitate selection during the genetic transformation process *in vitro*. The violet colour of the flowers results from the expression of two new genes encoding dihydroflavonol 4-reductase (DFR) and flavonoid 3'5' hydroxylase (F3'5'H) which, together with endogenous genes in the anthocyanin biosynthetis pathway, enable the biosynthesis of delphinidin in the petals.

The same transformation vector (pCGP1470) was used to produce the GM carnation variety Florigene Moondust™ (Notification reference C/NL/96/14) which was approved for placing on the market on December 1st 1997 (http://europa.eu.int/comm/environment/biotechnology/authorised_prod_1.htm). The consent for placing on the market in EU, including cultivation, was issued by the Dutch Competent Authority (see http://www.vrom.nl/ggo-vergunningverlening).

2. Molecular characterisation

2.1. Issues raised by Member States

Questions were raised regarding (1) the sequences of the inserts and flanking regions, (2) the presence/absence of an intact tetracycline (*tet*(A)) gene in carnation Moonlite 123.2.38, (3) the the expression levels of the three newly inserted genes and (4) the analysis of open reading frames (ORFs).

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

Question (1) regarding the flanking sequences of the inserts is considered under section 2.2.2 of the present opinion. Question (2) regarding the presence/absence of an intact *tet*(A) gene is considered under section 2.2.2.1. Question (3) regarding the expression levels of the three newly inserted genes is considered under section 2.2.3. Question (4) regarding the analysis of ORFs is considered under section 2.2.2.

2.2 Evaluation of relevant scientific data

Having considered the information provided in the original notification and the Member States comments, the GMO Panel requested from the applicant further data on the nucleotide sequence(s) of the insert(s) and of the associated flanking sequences as well as on appropriate bioinformatic analysis.

2.2.1. Transformation process and vector constructs

Genetic material was introduced into carnation Moonlite 123.2.38 by *Agrobacterium*-mediated transformation using disarmed *Agrobacterium tumefaciens* strain AGL0 carrying the transformation vector pCGP1470 described below. *Agrobacterium* was subsequently eliminated with ticarcillin and its absence was confirmed by PCR using *virG* gene primers; this gene is located in the Ti plasmid.

Details of the construction of the vector pCGP1470 used in the genetic modification of carnation Moonlite 123.2.38 are provided. The vector contains the following three expression cassettes ligated to the plasmid pWTT2132 backbone: 1) the promoter from a snapdragon gene encoding chalcone synthase, petunia flavonoid 3'5' hydroxylase (F3'5'H) cDNA, the terminator from the petunia gene encoding a phospholipid transfer protein homologue; 2) the constitutive promoter Mac, the petunia dihydroflavonol 4-reductase (DFR) cDNA, the terminator from the *Agrobacterium* gene encoding mannopine synthase (Mas); 3) the cauliflower mosaic virus 35S promoter, an untranslated region from the cDNA corresponding to the petunia gene encoding chlorophyll a/b binding protein 5, the *SuRB* (*als*) gene coding for a mutant acetolactate synthase protein (ALS) derived from *Nicotiana tabacum*, including its terminator. The first two cassettes were needed to obtain the desired flower colour.

The third cassette provided tolerance to sulfonylurea herbicides used as marker trait in the selection of genetically modified plants but not for plant protection purposes. Between the left (LB) and right (RB) borders that are commonly considered to define the region to be transferred, the vector also includes small stretches (*ca*. 400 bp total) of *Escherichia coli* plasmid pBluescript/pUC. Outside the LB and RB, the transformation vector pCGP1470 contained: 1) *ca*. 1.5 kb from *E. coli* for replication of the transformation vector in *E. coli*; 2) *ca*. 8 kb from

Pseudomonas aeruginosa for replication of the transformation vector in *A. tumefaciens*; 3) *ca*. 2 kb of a *tet* gene complex from *E. coli* for the selection of transformed bacterial cells based on tetracycline (tet) resistance. The complex includes *tet*(A) and *tet*(R) genes.

The entire sequence of the transformation vector pCGP1470 and a description of the function of all genes present were provided. The same transformation vector was used to produce the GM carnation variety Florigene Moondust™ (C/NL/96/14).

2.2.2. Transgenic constructs in the genetically modified plant

Carnation Moonlite 123.2.38 contains two transgenic loci:

- Locus 1: The genetic material located in the transformation vector between the partial LB and RB regions is stably integrated in the carnation Moonlite 123.2.38. In addition, Southern analysis of *Eco*RI-digested genomic DNA with 12 probes covering the entire *ca.* 25 kb transformation vector pCGP1470 indicated that some sequences outside the border regions have been integrated in the GM variety. The probe which partly overlapped the *tet* resistance gene complex showed weak hybridisation with plant DNA. Further studies using TAIL-PCR indicated that only a partial *tet*(A) gene is incorporated into the plant DNA. This sequence consists of *ca*. 190 nucleotides from the 3' end of the gene, representing less than 20% of the entire gene. No sequence corresponding to the *tet*(R) gene is incorporated into the carnation genome;

- Locus 2: Further Southern analysis was performed to understand the organization of the integrated sequences better. In contrast to all other probes used, the DFR and RB probes gave additional bands which would not be expected from a single copy of an intact T-DNA integrated in a single locus of the plant genome. The applicant concluded that, in addition to the sequence spanning from partial *tet*(A) gene through LB to RB, the carnation variety contains another integration site. Further sequence analysis indicated that the second integration site contained a truncated *dfr* gene and the *Mas* terminator as well as partial RB region.

Bioinformatic analysis showed that two new open reading frames (ORFs) were created at the junction region of locus 1. General BLAST searches were performed in order to compare the hypothetical protein sequences encoded by the three inserted genes and, the sequences of putative proteins that might be encoded by the two ORFs at the junction between the inserted DNA fragment and the plant DNA, with proteins from the GenBank and SwissProt databases. No relevant homologies were observed with known allergens and toxins using general BLAST searches. Additional searches for sequences homologies of at least six identical contiguous amino acids of the transgenic proteins with peptide sequences of identical length in known allergens were performed by the applicant (see sections 4.2.3 and 4.2.6).

2.2.2.1 Absence of plasmid backbone sequences

Some plasmid backbone sequences were present in carnation Moonlite 123.2.38. These include the modified pACYC184 sequence necessary for replication of the transformation vector in *E. coli*, and part (ca. <20%) of the *tet(A)* resistance gene are integrated into locus 1 of the GM carnation. None of these sequences raise any concern (see section 2.2.3 regarding safety impact of *tet*(A) gene).

2.2.3. Information on the expression of the insert

The expression of the three genes, encoding F3'5'H, DFR and ALS enzymes, was demonstrated at the mRNA level by northern analysis. The expression analysis also included quantification of the resulting new metabolites by liquid chromatography. The levels of delphinidin and cyanidin in a single assay of bulked petal samples were 0.093 and 0.031 mg/g fresh weight, respectively. It was estimated that the amount of delphinidin in 200 genetically modified carnation flowers corresponds to that in 100 g blueberries.

The partial *tet*(A) gene incorporated into the carnation genome is unlikely to confer tetracycline resistance. Yamaguchi and co-workers (1993) found that a larger fragment of *tetA* corresponding to the C-terminal half of the TetA protein, which also comprises the smaller fragment encoded by the *tet(A)* fragment inserted into the topical GM carnation, was unable to convey antibiotic resistance to recipient bacteria. This was further confirmed by the applicant by cloning the *tet*(A) sequence present in the GM carnation into a bacterial vector which included a ribosome binding site necessary for transcription in *E. coli* and by adding an upstream ATG start codon in-frame with the *tet*(A) sequence and a terminal stop codon for translation. The correctness of the construct in the resulting plasmid pCGP3128 was confirmed by sequence analysis. In the tetracycline resistance assay appropriate positive and negative controls were used. Tetracycline resistance was studied by plating the bacteria on media containing tetracycline concentrations ranging from 0.5 to 12.5 mg/l. The cloned *TetA* fragment failed to confer resistance to tetracycline.

2.2.4. Inheritance and stability of inserted DNA

Carnations are propagated vegetatively. No instability in the introduced trait, *i.e.* the particular flower colour, has been reported during the commercial cultivation of the carnation Moonlite 123.2.38, which includes approximately seven generations and the production of millions of flowers.

2.3. Conclusion

The molecular characterisation data establish that the carnation Moonlite 123.2.38 contains in one locus the cassettes containing the genes responsible for the intended traits (violet flower colour encoded by *dfr* and *f3'5'h* genes and herbicide tolerance encoded by the mutated *SuRB (als)* gene).

Some vector backbone sequences were shown to be present at this locus. An additional locus was detected that does not express any functional protein. The carnation Moonlite 123.2.38 does not contain a functional antibiotic resistance marker gene. The bioinformatic analysis showed that two new ORFs were created at the first locus. These new ORFs do not share homology with any toxic or allergenic proteins. The GMO Panel concludes that, considering the intended use of the GM carnation, the molecular characterisation of carnation Moonlite 123.2.38 does not raise any safety concern for humans, animals or the environment.

3. Comparative analysis

3.1 Issues raised by Member States

A question was raised regarding the need for further information on sample preparation for the HPLC analysis of anthocyanins. This question is considered under section 3.2.2 of the present opinion.

3.2. Evaluation of relevant scientific data

Having considered the information provided in the original notification and the Member States comments, the GMO Panel considered the additional information provided by the applicant to the Member States with respect to the HPLC sample preparation.

3.2.1. Choice of comparator and production of material

Carnation Moonlite 123.2.38 was compared with the parental variety 123 which does not produce anthocyanins and has white petals consequently.

3.2.2. Compositional analysis

Freeze dried petals of carnation variety Moonlite 123.2.38 and the control variety 123 were analyzed for three anthocyanins, namely delphinidin, cyanidin and petunidin. Roots and stems were not assayed. The GMO Panel reviewed the HPLC data provided on the concentrations (mg/g fresh weight petal) of these three anthocyanins (Fukui *et al*., 2003). While petunidin was not detected in either the GM variety or the non-GM control, delphinidin and cyanidin were detected in carnation Moonlite 123.2.38 at levels of 0.093 mg/g and 0.031 mg/g fresh weight respectively. These anthocyanins were absent from the white-flowered variety 123.

The GMO Panel considers that the comparative analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment considering the intended use of carnation Moonlite 123.2.38 (excluding human or animal consumption and cultivation).

3.2.3. Agronomic traits and GM phenotype

Carnation Moonlite 123.2.38 and the control variety 123 were grown in field trials and compared for several morphological characteristics including stem length, leaf length and width, bud shape, flower diameter and fragrance, number of petals, number of styles, and the height of the calyx and corolla. The two varieties showed no significant differences in any of these characteristics, except for the introduced traits and the mean height of the corolla of carnation Moonlite 123.2.38 (3,5 cm), which was higher than in the control variety (2,7 cm).

3.3. Conclusion

On the basis of the data provided by the applicant and in consideration of the intended use of carnation Moonlite 123.2.38 (excluding human or animal consumption and cultivation), the GMO Panel considers that the comparative analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment. The compositional data available in the application confirm the intended effects of the genetic modification (namely, the modified colour of flowers). The GMO Panel considers that the observed differences in the corolla height are not of significance with respect to the safety assessment of carnation Moonlite 123.2.38 for humans and animals in the unlikely event that carnation Moonlite 123.2.38 petals are consumed.

4. Safety assessment of carnation Moonlite 123.2.38 for humans and animals

4.1. Issues raised by Member States

Questions were raised regarding (1) the need for further explanations with respect to the outcomes of the acute toxicity study to analyze the anthocyanin content (in particular, the cyanidin content), (2) the limitation of the acute toxicity assay which administers petal extracts rather than feeding whole petals as part of the diet and (3) possible risk related to increasing use of GM carnation petals in food.

Questions (1) and (2) regarding the acute toxicity study are considered under section 4.2.4 of the present opinion and question (3) regarding the accidental consumption of carnation Moonlite 123.2.38 petals by humans under section 4.2.5.

4.2. Evaluation of relevant scientific data

Having considered the information provided in the original notification and the Member States comments, the GMO Panel requested from the applicant further clarifications and data with respect to the assessment for potential toxicity and allergenicity.

4.2.1. Product description and intended use

The genus *Dianthus* comprises species that have been cultivated for ornamental uses for hundred of years (Office of the Gene Technology Regulator, 2005). Carnations are present in gardens and in the cut flower market as ornamental plants.

The scope of notification C/NL/04/02 is restricted to the import of cut carnations Moonlite 123.2.38 for ornamental use only. The progeny derived from sexual crosses with Moonlite 123.2.38 is not covered under notification C/NL/04/02. Carnation Moonlite 123.2.38 is a new variety with specific violet flower colour that results from the synthesis of delphinidin due to the introduction of the *dfr* and *f3'5'h* genes. The GM carnation variety also contains a *SuRB* (*als*) gene, coding for a mutant acetolactate synthase protein (ALS), which confers herbicide tolerance used to facilitate selection during the transformation process *in vitro*.

4.2.2. Stability during processing

Since carnation Moonlite 123.2.38 is intended to be imported for the cut flower market, as is the case for non GM carnations, the petals of carnation Moonlite 123.2.38 are highly unlikely to be processed and used as processed food and feed. Consequently, the GMO Panel did not consider stability of the GM carnation during processing as an issue.

4.2.3. Toxicology assessment of the newly expressed proteins

General BLAST searches were performed in order to compare the hypothetical protein sequences encoded by the three inserted genes and, the sequences of putative proteins that might be encoded by the two ORFs at the junction between the inserted DNA fragment and the plant DNA, with proteins from the GenBank and SwissProt databases. No homologies were observed with known toxins using general BLAST searches.

4.2.4. Toxicology assessment of new constituents other than proteins

(a) Acute toxicity testing

The purpose of an acute toxicity study is to determine the impact of accidental exposure to carnation Moonlite 123.2.38 on human or animal health.

A 14-day acute toxicity study was performed on four-week old mice fed with water extracts of frozen petals (2 g petals/kg body weight) from carnation Moonlite 123.2.38 and water extracts of the non-GM control variety 123, respectively. Acute toxicity studies on plant materials are commonly carried out with extracts made thereof (see section 3.2.2). As anthocyanins are water soluble, the extract from carnation Moonlite 123.2.38 contains delphinidin and cyanidin. Mice were split into two groups of five each for each exposure. No mortalities were observed. A slight body weight increase of 4% was observed in the group supplied with extracts from GM carnations compared to the group supplied with extracts from non-GM carnations.

(b) Additional in vitro studies

The applicant performed an Ames test and a cytotoxicity study on human embryonic intestinal cells *in vitro* with water extracts of leaves of carnation Moonlite 123.2.38 and control variety 123. The water extract showed neither mutagenicity nor toxicity.

4.2.5. Toxicological assessment of the whole GM plant

Carnation flowers have a long history of use as ornamentals. The genus *Dianthus* comprises species that have been cultivated for ornamental uses for hundred of years (Office of the Gene Technology Regulator, 2005).

Given that carnation Moonlite 123.2.38 is not intended for human or animal consumption as food or feed but for ornamental use only, the GMO Panel does not consider it necessary to perform a comprehensive food/feed safety assessment of the whole GM plant.

The GMO Panel has, nevertheless, considered the possible effects of the genetic modification on human and animal health of accidental consumption of carnation Moonlite 123.2.38 petals. The GMO Panel notes that the data on acute toxicity studies and on the two *in vitro* studies (see section 4.2.4) do not give any indication of increased toxicity of the carnation Moonlite 123.2.38

petals compared to the parental variety in the unlikely event of accidental consumption of GM petals.

In addition, delphinidin and cyanidin, belonging to the group of anthocyanins are present in many foods and at much higher concentrations than in the petals of carnation Moonlite 123.2.38, particularly high concentrations being found e.g. from blackcurrants. Many other delphinidin-containing species (e.g. *Dampiera* spp., *Delphinium* spp., *Lisianthus* spp., *Wisteria* spp.) show a higher concentration of delphinidin (as a percentage of total anthocyanins) than does carnation Moonlite 123.2.38. Cyanidin and its derivatives are commonly found in a number of plants including *Petunia* (Ando *et al*., 1999), carnation (Bloor, 1998), rose (Biolley and Jay, 1993), apple (Lancaster, 1992), sunflower seeds (Mazza and Gao, 1994), chrysanthemum (Schwinn *et al*., 1993; Andersen *et al*., 2000), *Vicia villosa* (Catalano *et al*., 1998) and *Vitis* spp. (Cachio *et al*., 1992).

4.2.6. Allergenicity

General BLAST searches comparing the hypothetical protein sequences encoded by the three inserted genes and, the sequences of putative proteins that might be encoded by the two ORFs at the junction between the inserted DNA fragment and the plant DNA, with proteins from the GenBank and SwissProt databases were performed. Additional searches for sequences homologies of at least six identical contiguous amino acids of the transgenic proteins with peptide sequences of identical length in known allergens were performed by the applicant. No homologies were observed with known allergens in using general BLAST searches. Various identical sequences of six amino acids were found in the three expressed proteins and known allergens, but there is no further indication of the allergenicity of these transgenic proteins.

Carnation Moonlite 123.2.38 is not intended to be used as food or feed. No adverse reaction to carnation Moonlite 123.2.38 cut flowers for ornamental purpose has been reported in the general populations. However Sanchez (1999; 2004) has described occupational allergy to carnation in workers handling cut flowers/carnation over a long time. This allergy could be caused either by the flower, by mites (*Tetranychus urticae* infesting carnations) or by both simultaneously.

Considering the limited exposure to carnation Moonlite 123.2.38 in the scope of this notification, the GMO Panel is of the opinion that, considering the rare reports of cases of occupational allergies, the issue of potential allergenicity is unlikely to be a safety concern.

4.3. Conclusion

Carnation flowers have a long history of use as ornamentals. Carnation Moonlite 123.2.38 differs from control variety 123 by the presence of delphidinin, which confers a violet colour to the flowers. Delphinidin, a common pigment in many ornamental flowers and food plants such as red grapes, black currants, egg plants, blueberries, is produced as a result of the combined expression of the introduced *dfr* and f*3'5h* genes together with endogenous genes in the anthocyanin biosynthesis pathway. Delphinidin is not known to be a toxic compound.

Furthermore no evidence for toxicity of the products of the three newly inserted genes (Petunia *dfr* gene ; Petunia *f3'5'h* gene and *SuRB* (*als*) gene) was reported based on a 14-day acute toxicity study, an Ames test and a cytotoxicity study on human embryonic intestinal cells *in vitro*. From BLAST searches using the GenBank and SwissProt databases, the GMO Panel concludes that no relevant homologies exist between the newly expressed proteins in carnation Moonlite 123.2.38 and known toxins or allergens.

The possibility of accidental consumption of carnation Moonlite 123.2.38 petals cannot be ruled out. However the amount of delphinidin consumed will be negligible in comparison with the amount of delphinidin present in fruits containing high levels of delphinidin such as blackcurrant or bilberry.

Considering the intended use of carnation Moonlite 123.2.38, the GMO Panel concludes that this carnation is unlikely to have adverse effects on human or animal health.

5. Environmental risk assessment and monitoring plan

5.1 Issues raised by the Member States

Questions were raised regarding (1) the possibility of gene transfer to wild carnations, (2) the need to consider more clearly the presence of cyanidin in the environmental risk assessment, (3) the need for a case specific monitoring plan focusing on hybridization of cut carnation flowers with wild *Dianthus* plants and (4) more details on general surveillance methods.

Question (1) regarding the possibility of gene transfer to wild carnations is considered under section 5.2.2 of the present opinion. Question (2) regarding the presence of cyanidin in the environmental risk assessment is considered under section 5.2.4 whereas questions (3) and (4) regarding the case specific monitoring plan and the general surveillance methods respectively, are considered under section 5.2.5.

5.2. Evaluation of relevant scientific data

The GMO Panel considered the information provided in the original notification, the Member State comments and further scientific literature in the assessment of the potential for environmental risks and the requirements of a monitoring plan. It was concluded that scientific information on potential environmental effects associated with the cultivation of carnation Moonlite 123.2.38 was not required. As the notification concerns import of cut flowers there will be a very limited environmental exposure with respect to viable plant parts of carnation Moonlite 123.2.38. The GMO Panel only considered this restricted exposure when evaluating the potential environmental impact of imported cut flowers and not issues associated with plant cultivation. In addition, the GMO Panel gave its opinion on the scientific quality of the environmental monitoring plan provided by the applicant, including the general surveillance (see section 5.2.4).

Carnations are double-flowered cultivars and in the general trade and botanical and horticultural literature carnation cultivars are considered to belong to the species *Dianthus caryophyllus*. The cultivated carnation is vegetatively propagated to produce plants for cut flower production. Cuttings are taken from vegetative 'mother plants' which are continually pruned to produce a high number of vegetative cuttings from axillary buds. These cuttings are rooted in conditions of high humidity, after treatment with rooting powder. Rooted plants may be planted in soil or grown hydroponically, and are kept for 1-2 years. Flowers are produced in flushes, beginning 3-5 months after rooted cuttings are planted. Picking of all flowers is essential and flowers are harvested in tight bud (or closed bud for spray types) for distribution and marketing.

The majority of *Dianthus* species are self-sterile because the stigma is not receptive to pollen until one week or more after anthers have shed them. The 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 does not produce much

pollen, and consequently seed set is low or absent (Galbally & Galbally, 1997). The quantity and quality of pollen varies according to the cultivar (Kho & Baer, 1973; Galbally & Galbally, 1997). Carnation pollen is heavy and sticky and has low viability. Wind plays little role in pollen dispersal (Office of the Gene Technology Regulator, 2005).

In the wild, cross-pollination of carnation relies on insect pollinators. There are no known reports of insect pollinators of *D. caryophyllus*, in particular. However, pollination is likely to be affected by lepidopteran pollinators. Lepidopteran species of the genera *Aphantopus, Aporia, Cyaniris, Hesperia, Macroglossum, Melanargia, Mesoacidalia, Ochlodes, Pieris, Plusia, Polyommatus, Sartyrus,* and *Thymelicus* are documented pollinators of other *Dianthus* species in the EU (Office of the Gene Technology Regulator, 2005; Bloch *et al*., 2006).

Members of the genus *Dianthus* are fairly diverse, as their origins range from southern Russia to Alpine Greece and the Auvergne mountains of France. The *Dianthus* species are adapted to the cooler Alpine regions of Europe and Asia, and are also found in Mediterranean coastal regions. *D. caryophyllus* is widely cultivated for ornament in Europe and occasionally naturalized, but apparently not known in the wild, except perhaps in some Mediterranean countries, indicating that the distribution of naturalized *D. caryophyllus* carnation is restricted to the Mediterranean regions of Greece, Italy, Sicily, and Sardinia (Tutin *et al.,* 1993).

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

Carnation varieties in general compete poorly outside their cultivated environment. However, in the very unlikely event of accidental release into the environment, the fitness of the GM plants was considered.

The carnation Moonlite 123.2.38 has a modified flower colour achieved by introducing two genes of the anthocyanin biosynthesis pathway from petunia. These genes, encoding dihydroflavonol 4-reductase and flavonoid 3'5' hydroxylase, give rise to the anthocyanins delphinidin and cyanidin. These anthocyanins are widely found in flowers like *Petunia* (Ando *et al*., 1999), rose (Biolley and Jay, 1993), chrysanthemum (Schwinn *et al*., 1993; Andersen *et al*., 2000). There is no evidence that the presence of delphinidin and cyanidin would lead to effects on plant fitness.

Carnation Moonlite 123.2.38 contains a mutated *SuRB* (*als)* gene conferring tolerance to sulfonylurea herbicides. Given that the ALS enzyme is needed for the biosynthesis of some branched-chain amino acids like isoleucine, ALS-inhibiting herbicides cause the death of the plant by interfering with this biosynthesis pathway. Against this background Tranel & Wright (2002) reported that tolerance to ALS-inhibiting herbicides was widespread among weeds and mostly due to a mutated *SuRB* (*als)* gene. In addition the ALS-tolerant biotype was shown to be less sensitive to feedback inhibition by branched-chain amino acids. This results in greater accumulation of branched-chain amino acids in tolerant biotypes, which may allow seeds from tolerant biotypes to germinate more rapidly, especially under cool temperatures. This may indicate a possible change in behaviour of the tolerant plants in the absence of herbicide selection, in the very unlikely event of escape into the environment. Wild *Dianthus* populations exhibit a diversity of phenotypes occupying niches in a wide geographical range in Europe (Tutin *et al.*, 1993). The GMO Panel considered that a small change in seed germination characteristics induced by ALS tolerance is unlikely to be outside the current range of seed germination characteristics currently expressed by non GM carnations and thus is unlikely to have an ecological impact. In addition, because of the intended use of carnation Moonlite 123.2.38 and therefore of the very low exposure of recipient populations, the GMO Panel considers this to be of no ecological significance. The carnation Moonlite 123.2.38 plant would not show changed fitness characteristics except in the presence of sulfonylurea herbicides and this herbicide is not used in habitats where wild carnation might occur.

In the very unlikely event of gene transfer to cultivated carnations, they may express the mutated *SuRB* (*als)* gene conferring tolerance to sulfonylurea herbicides. This could result in a possible fitness advantage and higher weediness of the tolerant plants in the presence of these herbicides and those with a similar mode of action. Such herbicide tolerant plants can be managed by a range of measures (Tranel & Wright, 2002).

The GMO Panel is of the opinion that the carnation Moonlite 123.2.38 is unlikely to have adverse effects on the environment in comparison with non GM carnations.

5.2.2. Potential for gene transfer

(a) Plant to bacteria gene transfer

The carnation Moonlite 123.2.38 contains a mutated acetolactate synthase (*SuRB/als*) gene conferring tolerance to sulfonylurea herbicides as well as a *dfr* gene, coding for dihydroflavonol 4-reductase (DFR), and the petunia *f3'5'h* gene, coding for flavonoid 3' 5' hydroxylase (F3'5'H) (see section 2.2.1 for further details on the molecular characterisation). Delphinidin is produced as a result of the combined expression of the introduced genes *dfr* and *f3'5'h* together with endogenous genes in the anthocyanin biosynthesis pathway. These genes are already present in other plant communities and thus in soil decomposition processes. Plant to bacteria gene transfer of the genes was not considered to pose an environmental risk by the Member States or the GMO Panel. In the very unlikely event that a plant to bacteria gene transfer would take place, no adverse effects on human and animal health or the environment are expected as no new genes from decomposing plants would be introduced into microbial communities.

(b) Plant to plant gene transfer

The reproductive biology of *Dianthus* (Office of the Gene Technology Regulator, 2005), including the low production and low viability of the pollen, and the limited information provided by the applicant suggesting that the proportion of flowers carrying pollen is low if at all, indicate that pollen transfer is very unlikely to occur. In addition, viable seed set on cut flowers is very unlikely given the limited life time in comparison to the time needed for complete seed development.

The GMO Panel considered the possibility of natural exchange of genetic material with other carnation varieties, *Dianthus caryophyllus* L., and some wild *Dianthus* species. Although hybridisation is mentioned in some floristic surveys, the GMO Panel is not aware of reports of gene flow between wild *Dianthus* spp. and cultivated carnations in the literature. The probability of spontaneous hybridisation between GM carnation and other cultivated carnations and establishment of a viable plant is considered to be very low. Therefore, the GMO Panel concludes that plant to plant gene transfer of the introduced genes is unlikely to be of environmental concern.

5.2.3. Potential interactions of the GM plant with non-target organisms

There are several herbivorous pests of the carnation and they could be affected by a change in delphinidin/cyanidin ratio. However, the scope of this notification does not include cultivation and therefore the exposure of herbivores to this GM carnation will be extremely limited and the exposure to detritivores would be localised (e.g. in waste processing). Thus the GMO Panel considered that carnation Moonlite 123.2.38 is unlikely to have adverse effects on non-target organisms in the context of the intended use.

5.2.4. Monitoring

The GMO Panel is of the opinion that the structure of the environmental monitoring plan provided by the applicant complies with the requirements defined in Directive 2001/18/EC, in Council Decision establishing guidance notes supplementing Annex VII (EC, 2002b) and in the Guidance document provided by EFSA (EFSA, 2004). The monitoring plan describes objectives, responsibilities and tasks, flow of information and monitoring methods. The GMO Panel gives its opinion on the scientific quality of the environmental monitoring plan provided by the applicant, including the general surveillance.

The GMO Panel agrees with the applicant that the environmental risk assessment did not identify risks that require case-specific monitoring.

The GMO Panel considered the general surveillance methods as provided in the notification (a.o. questionnaire to European importers). It was also noted that the applicant requested taxonomists and botanists to inform them of hybrids that might originate from their GM carnation. The GMO Panel additionally suggests that national botanic survey networks and plant protection services should also be considered.

In the light of the very low environmental exposure of viable forms of carnation line 123.2.38 due to the restricted intended use of the GM carnation, the GMO Panel concludes that the proposal of the applicant for general surveillance is in line with the EFSA Guidance on post-market environmental monitoring (EFSA, 2006). The GMO Panel recommends the adoption of the proposals for annual reporting made in the EFSA guidance document (EFSA, 2006).

5.3. Conclusion

The GMO Panel based its environmental risk assessment on cut flowers of carnation Moonlite 123.2.38 to be imported for ornamental use only. From the information supplied by the applicant, and from studies of relevant literature, there is no indication that this GM carnation will have adverse effects on the environment in the EU.

The carnation Moonlite 123.2.38 was assessed for imported cut flowers for ornamental use only. Scientific information on potential environmental effects associated with the cultivation of carnation Moonlite 123.2.38 was therefore not required. Carnation Moonlite 123.2.38 cut stems and flowers have very restricted viability, very low pollen emission and little or no viable seed. However, in the very unlikely event of accidental release into the environment, the GMO Panel considers that the carnation Moonlite 123.2.38 would not show enhanced fitness characteristics, except in the presence of sulfonylurea herbicides. The consequences of the potential transfer of the three genes would be negligible in terms of adverse effects on the environment. Exposure of non-target organisms to GM carnation would be very low and the GMO panel concludes that there is no indication that GM carnation Moonlite 123.2.38 will have adverse effects on the environment in the context of the intended use.

The GMO Panel agrees with the applicant that the environmental risk assessment indicates that there is no need for a case-specific monitoring plan. The GMO Panel also agrees with the general methods and approaches of the general surveillance plan.

CONCLUSIONS

The GMO Panel was asked to consider whether there is any scientific reason to believe that the placing on the market of the GM carnation Moonlite 123.2.38 for import is likely to cause any

adverse effects on human health and the environment within the scope of Directive 2001/18/EC.

The carnation Moonlite 123.2.38 has a modified flower colour, a shade of violet, which is achieved by introducing into white carnation two genes of the anthocyanin biosynthesis pathway from petunia. Carnation Moonlite 123.2.38 also expresses sulfonylurea herbicide tolerance.

The GMO Panel has evaluated the molecular analysis of the genetically modified variety. The carnation Moonlite 123.2.38 does not contain a functional antibiotic resistance marker gene. From the bioinformatic analysis, there is no reason to assume that the DNA regions transferred code for toxic and/or allergenic products.

Given the intended use of carnation Moonlite 123.2.38 (excluding human or animal consumption and cultivation), the GMO Panel considers that the comparative analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment. Furthermore, based on the results of toxicity and allergenicity studies, there is no evidence that any of the three proteins expressed is toxic or allergenic. The GMO Panel concludes that carnation Moonlite 123.2.38 is unlikely to have adverse effects on human or animal health in the unlikely event that carnation Moonlite 123.2.38 petals are consumed.

Considering the low environmental exposure due to the restricted scope of the notification, this is very unlikely that gene transfer and escape into the environment would occur and, if any, the consequences of the three genes would be negligible for the environment in line with the intended use of Moonlite 123.2.38 cut flowers. The GMO Panel agrees with the general methods and approaches of the general surveillance plan provided in the notification.

DOCUMENTATION PROVIDED TO EFSA

1. Note to Mr. Koëter and the annexes, dated 2 December 2005 with ref. DG ENV/B.4/KT D(05)25125, from Mr. Ladislav Miko – Notification C/NL/04/02 (Carnation Moonlite 123.2.38), under Directive 2001/18/EC - request for EFSA opinion.

2. Submission from Florigene (4 January 2006) to EFSA regarding the notification for the placing on the market of carnation Moonlite 123.2.38 in accordance with Directive 2001/18/EC: Ref C/NL/04/02, and the related annexes.

3. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/SM/jq (2006) 1412310, 8 March 2006).

4. Additional information submitted by Florigene on 31 March 2006 in response to EFSA request for further information.

REFERENCES

Andersen, Ø. M., Cabrata, L., Frøystein, N.A., 2000. Anthocyanin trisaccharides in blue berries of *Vaccinium padifolium.* Food Chemistry, 69, 33-36.

Ando, T., Saitob, N., Tatsuzawau, F., Kakefudau, T., YamakageuE, K., Ohtani, T., Koshi-ishiu, M., MatsusakeuH, Y., Kokubunu, I., Watanabeu, H., Tsukamotou, T., Uedau, Y., Hashimoto, G., Marchesid, E., Asakura, K., Harar, R., Sekir, F., 1999. Biochemical Systematics and Ecology. Floral anthocyanins in wild taxa of Petunia, 27, 623-650.

Biolley, J.P., Jay, M., 1993. Anthocyanins in Modern Roses: Chemical and Colorimetric features in Relation to the Colour Range. Journal of Experimental Botany, 44(268), 1725-1734.

Bird, R., 1994. Border Pinks. Timber Press, Portland, pp 1-174.

Bloch, D., Werdenberg, N., and Erhardt, A., 2006. Pollination crisis in the butterfly-pollinated wild carnation *Dianthus carthusianorum*? New Phytologist, 169, 699–706.

Bloor, S.J., 1998. A macrocyclic anthocyanin from red/mauve carnation flowers. Phytochemistry, 49(1), 225-228.

Cacho, J., Fernández, P., Ferreira, V., and Castells J. E., 1992. Evolution of Five Anthocyanidin-3-Glucosides in the Skin of the Tempranillo, Moristel, and Garnacha Grape Varieties and Influence of Climatological Variables. American Journal of Ecology and Viticulture, 43, 244-248.

Catalano, G., Fossen, T. and Andersen, Ø. M., 1998. Petunidin 3-*O*-r-Rhamnopyranoside-5-*O*-â-glucopyranoside and Other Anthocyanins from Flowers of *Vicia villosa.* Journal Agricultural and Food Chemistry, 49, 4568-4570.

EC, 2001. 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. Official Journal of the European Communities, L106, 1-39.

http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_106/l_10620010417en00010038.pdf

EC, 2002a. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities, L31, 1-24.

http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_031/l_03120020201en00010024.pdf

EC, 2002b. Council Decision (2002/811/EC) of 3 October 2002 establishing guidance notes supplementing Annex VII to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L280, 27-36.

http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_280/l_28020021018en00270036.pdf

EFSA, 2004. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed, The EFSA Journal (2004) 99, 1-94.

http://www.efsa.europa.eu/science/gmo/gmo_guidance/660/guidance_docfinal1.pdf

EFSA, 2006. Opinion of the Scientific Panel on Genetically Modified Organisms on the Post Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 319, 1-27.

http://www.efsa.europa.eu/science/gmo/gmo_opinions/1381/gmo_op_ej319_pmem_en1.pdf

Fukui, Y, Tanaka,Y., Kusumi T.,Iwashita, T. and Nomoto, K., 2003. A rationale for the shift in colour towards blue in transgenic carnation flowers expressing the flavonoid 3',5'-hydroxylase gene. Phytochemistry, 63, 15–23.

Galbally, J., Galbally, E., 1997. Carnations and pinks for garden and greenhouse. Timber Press, Portland, Oregon, USA, pp 1-310.

Kho, Y.O., Baer, J., 1973. The effect of temperature on pollen production in carnations. Euphytica, 22, 467-470.

Lancaster, 1992. Regulation of skin color in apples. Critical reviews in Plant Sciences, 10(6), 487-502.

Mazza, G. and Gao, L., 1994. Malonylated anthocyanins in purple sunflower seeds. Phytochemistry, 35, 237-239.

Office of the Gene Technology Regulator, Department of Health and Ageing, Australian Government, 2005. The Biology and Ecology of *Dianthus caryophyllus* L. (Carnation), 18 p.

http://www.ogtr.gov.au/pdf/ir/biologycarnation.pdf

Sanchez-Fernandez, C., Gonzalez-Gutierrez, M. L., Esteban-Lopez, M. I., Martinez, A., Lombardero M., 2004. Occupational asthma caused by carnation (*Dianthus caryophyllus*) with simultaneous IgE-mediated sensitization to *Tetranychus urticae*. Allergy, 59, 114 –119.

Sanchez-Guerrero, IM, Escudero, AI, Bartolome, B, Palacios, R., 1999. Occupational allergy caused by carnation (*Dianthus caryophyllus*). J Allergy Clin Immunol, 104, 181–185.

Schwinn, KE, Markham, KR, Given, NK, 1994. Floral flavonoids and their potential for pelargonidin biosynthesis in commercial chrysanthemum cultivars. Phytochemistry, 35, 145-150.

Sparnaaij, L.D., Beeger, G.W., 1973. The improvement of seed production for breeding purposes in the glasshouse carnation (*Dianthus caryophyllus* L.). Euphytica, 22, 274-278.

Tranel, P.J., Wright, T.R., 2002. Resistance of weeds to ALS-inhibiting herbicides: What have we learned? Weed Science, 50, 700-712.

Tutin, T.G., Burges, N.A., Chater, A.O., Edmondson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A., 1993. Flora Europaea. Cambridge University Press, Cambridge, pp 227-246.

http://rbg-web2.rbge.org.uk/FE/fe.html

Yamaguchi, A, Someya, Y, Sawai, T., 1993. The *in vivo* assembly and function of the N- and C-terminal halves of the Tn10-encoded TetA protein in *Escherichia coli*. FEBS-Lett., 324(2), 131-135.

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