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Notification (Reference C/NL/06/01) for the placing on the market of the genetically modified carnation Moonaqua 123.8.12 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Florigene[1]

Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2007-177)

Adopted on 12 March 2008

PANEL MEMBERS

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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 Moonaqua 123.8.12, genetically modified (GM) for flower colour (Unique Identifier FLO-40689-6). The GM carnation also contains a gene conferring tolerance to sulfonylurea herbicides. Cut flowers of carnation Moonaqua 123.8.12 are intended to be imported in the European Union for ornamental use only.

The present opinion is based on a question raised by the European Commission related to a notification to place the GM carnation Moonaqua 123.8.12 on the market under Directive 2001/18/EC (Notification reference C/NL/06/01). 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

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Moonaqua 123.8.12 was requested by the European Commission because of outstanding objections raised by some Member States following the evaluation 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 Moonaqua 123.8.12 for import only is likely to cause any adverse effects on human health and the environment.

In delivering its opinion, the GMO Panel considered the full notification, additional information provided by the notifier and the specific outstanding objections raised by the Member States. The carnation Moonaqua 123.8.12 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 compounds intended to be produced by the GM carnation. Furthermore, the potential environmental impact of carnation Moonaqua 123.8.12, including a monitoring plan, was assessed in the context of the restricted intended use of carnation Moonaqua 123.8.12.

Carnation Moonaqua 123.8.12 has a modified flower colour, a shade of light mauve, whereas the non-GM parent has cream-white flowers. The colour has been achieved by introducing into white carnation two genes of the anthocyanin biosynthesis pathway from *Petunia* and *Viola* sp. These genes, encoding dihydroflavonol 4-reductase (*dfr*) and flavonoid 3'5' hydroxylase (*f3'5'h*), together with other genes of the anthocyanin biosynthesis pathway already present in the non GM 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 Moonaqua 123.8.12 is also tolerant to sulfonylurea herbicides conferred by a mutated *SuRB (als)* gene used as marker gene for the selection of genetically modified plants but not for plant protection purposes. Other Florigene GM carnation varieties Moondust™, Moonshadow™ and Moonlite™ 123.2.38, which have also been genetically modified to express a specific blue-violet colour, were authorised to be placed on the market within the EU in 1997, 1998 and 2007, respectively.

The molecular analysis of the DNA inserts confirms that the three genes expressing the intended traits (light mauve flower colour encoded by *dfr* and *f3'5'h* genes and herbicide tolerance encoded by the mutated *SuRB (als)* gene) are present in carnation Moonaqua 123.8.12. Results of bioinformatic analyses of the three newly expressed proteins in carnation Moonaqua 123.8.12 did not indicate relevant homologies with known toxins or allergens. No new open reading frames were created in the flanking regions between the inserts and the carnation genome.

Given the intended use of carnation Moonaqua 123.8.12 (excluding human or animal consumption and cultivation), the GMO Panel considers that a compositional analysis limited

to the newly synthesised anthocyanins is sufficient for the risk assessment of the intended modification. The GMO Panel concludes that there is no indication of increased toxicity of the carnation Moonaqua 123.8.12 compared to the recipient variety.

The carnation Moonaqua 123.8.12 was assessed for imported cut flowers for ornamental use only. Scientific information on potential environmental effects associated with the cultivation of carnation Moonaqua 123.8.12 was therefore not required. Carnation Moonaqua 123.8.12 cut stems and flowers have marginal viability, negligible pollen production and little or no viable seed. However, in the very unlikely event of escape into the environment via seeds or rooted plants, the GMO Panel considers that the carnation Moonaqua 123.8.12 would not show enhanced fitness characteristics, except in the presence of sulfonylurea herbicides. The consequences of the potential transfer of the three genes into bacteria or plants would be negligible in terms of adverse effects on the environment. The GMO Panel concludes that there is no indication that GM carnation Moonaqua 123.8.12 will have adverse effects on the environment in the context of the intended use.

The GMO Panel is of the opinion 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 provided in the notification.

In conclusion, the GMO Panel considers that the information available for carnation Moonaqua 123.8.12 addresses the outstanding objections raised by the Member States and considers that, in the context of its intended use, carnation Moonaqua 123.8.12 is unlikely to have adverse effects on human and animal health or the environment.

Key words: acetolactate synthase (SuRB/ALS), anthocyanin, carnation, C/NL/06/01, cyanidin, 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-40689-6.

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BACKGROUND

The Dutch Competent Authority forwarded the notification (Reference C/NL/06/01) to the European Commission on 1st of March 2007, together with a positive assessment report.

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 notifier, Florigene, provided the Member States with additional information in response to the objections raised during the 60-day period. The Member States had until 21 September 2007 to confirm or lift their objections. Where these objections are maintained, the Commission is required under Article 28 of Directive 2001/18/EC to consult the relevant Scientific Committee(s) for opinion, now 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 6 November 2007, after receipt of the complete background information (request from the Commission, full notification and final objections maintained by the Member States). During the 90-day period, EFSA requested further clarifications from the notifier. Therefore the deadline set for the delivery of this opinion was extended.

In delivering its opinion the GMO Panel considered the original notification, additional information provided by the notifier and the specific objections raised by three Member States.

The scope of notification C/NL/06/01 is restricted to the import of cut flowers of carnation Moonaqua 123.8.12 for ornamental use only. The progeny derived from sexual crosses with Moonaqua 123.8.12 variety are not covered under notification C/NL/06/01.

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 Moonaqua 123.8.12 for import is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC.

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 political 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.

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ASSESSMENT

1. Introduction

The genetically modified (GM) carnation Moonaqua 123.8.12 (Unique Identifier FLO-40689-6) 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, 2006a). In its evaluation the GMO Panel focused in particular on the issues raised by the Member States during the initial assessment of the notification (Reference C/NL/06/01) introduced under Directive 2001/18/EC (EC, 2001). The evaluation presented here is based on the original notification, additional information provided by the notifier and the specific objections raised by three Member States and further scientific literature identified by the GMO Panel.

Carnation Moonaqua 123.8.12 is a new variety which contains a mutated herbicide tolerance *SuRB* (*als*) gene coding for an acetolactate synthase (ALS) variant protein, used to facilitate selection during the genetic transformation process. The light mauve 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 biosynthesis pathway, enable the biosynthesis of delphinidin and cyanidin in the petals.

The same transformation vector (pCGP1991) was used to produce the GM carnation variety Florigene Moonshadow™ (Notification reference C/NL/97/13), which was authorised within the EU for placing on the market in 1998 (http://europa.eu.int/comm/environment/biotechnology/authorised_prod_1.htm). This authorisation included cultivation and was issued by the Dutch Competent Authority. The new carnation Moonaqua 123.8.12 differs in the shade of flower colour and the morphology of the flower.

Another transformation vector with similar genes (pCGP1470) has been used in GM carnation varieties Florigene Moondust™ (Notification reference C/NL/96/14) and Florigene Moonlite™ (Notification reference C/NL/04/02) to modify the flower colour. Florigene Moondust™ was authorised for placing on the market in 1997. Following the opinion of the GMO Panel (EFSA, 2006b), Florigene Moonlite™ 123.2.38 was authorised by the European Commission for placing on the market in 2007 (EC, 2007). This authorisation did not include cultivation. The slight differences between the vectors pCGP1991 and pCGP1470 come from the source of the *f3'5'h* gene and some regulatory elements.

Upon request of the European Commission, EFSA is requested to make specific references to scientific objections from Member States. The objections as regards traceability, labelling and

validation of detection methods fall outside the remit of the GMO Panel. In addition, with respect to the objections related to post-market monitoring, the GMO Panel gave its opinion on the scientific quality of the monitoring plan provided by the notifier although a final adoption of the monitoring plan falls outside the mandate of the GMO Panel.

2. Molecular characterisation

2.1. Issues raised by Member States

No objection raised by a Member State remained at the end of the 45-day Member States consultation period. Therefore, notwithstanding its own risk analysis, the GMO Panel had no specific concerns to address from Member States on the molecular characterization of GM carnation Moonaqua 123.8.12.

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

2.2 Evaluation of relevant scientific data

2.2.1. Transformation process and vector constructs

To develop carnation Moonaqua 123.8.12, new genetic material was introduced into carnation line FE123 (which is a DFR mutant and so does not contain the *f3'5'h* gene) by *Agrobacterium*-mediated transformation using disarmed *Agrobacterium tumefaciens* strain AGL0 carrying the transformation vector pCGP1991 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 outside the T-DNA.

The vector pCGP1991 contained the following three expression cassettes between the left (LB) and right (RB) borders that are commonly considered to define the region to be transferred to the plant: 1) the promoter from a snapdragon (*Antirrhinum majus*) gene encoding chalcone synthase, cDNA encoding flavonoid 3'5' hydroxylase (F3'5'H) from *Viola* sp., the D8 terminator from the petunia gene encoding a phospholipid transfer protein homologue; 2) the entire petunia gene that encodes dihydroflavonol-4-reductase (DFR), including its promoter and terminator. These two cassettes were needed to obtain the desired flower colour. The third cassette contained a chimeric gene consisting of the cauliflower mosaic virus 35S promoter, 5' untranslated region (*ca*. 60 bp) from the cDNA corresponding to the petunia gene encoding chlorophyll a/b binding protein, and the mutated *SuRB* (*als*) gene coding for a acetolactate synthase (ALS) variant protein derived from *Nicotiana*

tabacum, including its terminator. The *als* gene provided tolerance to sulfonylurea herbicides used as marker trait in the selection of genetically modified plants but not intended for plant protection purposes. In addition, small stretches (*ca*. 530 bp total) of *Escherichia coli* plasmid pBluescript/pUC were included in the region between the LB and RB.

The entire sequence of the transformation vector pCGP1991 and a description of the function of all genes present were provided.

2.2.2. Transgenic constructs in the genetically modified plant

Carnation Moonaqua 123.8.12 contains three transgenic loci. Integration Locus 1 (14433 bp) contains an intact construct between LB and RB. Integration Locus 2 (5140 bp) contains a fragment starting from RB and extending to D8 terminator, which is linked to another fragment containing an almost complete *f3'5'h* cassette (missing ca. 40 bp from the promoter). Integration Locus 3 (1741 bp) contains an incomplete *f3'5'h* cassette. Southern analysis of *Eco*R1-digested genomic DNA with seven probes covering the whole plasmid backbone outside the LB and RB indicated that none of these sequences had been integrated into carnation Moonaqua 123.8.12. Sequences have been provided for all three inserts including their flanking regions.

Bioinformatic analysis of amino acid sequences encoded by the introduced genes indicated no homologies to known toxin or allergen coding genes. The analysis was carried out by comparing the translated sequences encoded by the three introduced genes with the GenBank and SwissProt databases by using the search program BLAST2.2.9 (FAO/WHO, 2001; Codex Alimentarius, 2003).

On the request from the GMO Panel the notifier performed sequence homology search using 80-amino-acid long sliding window, looking for a minimum of 35% non-contiguous identical amino acids. No matches were found. The notifier also performed a similarity search for short identical stretches of six contiguous amino acids. Several identities were found for each newly expressed protein. However, the GMO Panel notes that a number of reports in scientific literature indicate that the 6-amino-acid threshold is likely to give rise to many false positives. The GMO Panel therefore concludes that no relevant homologies exist between the newly expressed proteins in carnation Moonaqua 123.8.12 and known allergens.

Bioinformatic analysis of the flanking regions was carried out using the following criterion: the open reading frame (ORF) should be larger than 50 amino acids and start with methionine. No ORFs were found at the six junctions of the integrated DNA and genomic DNA of carnation.

2.2.3. Information on the expression of the insert

The expression of the three genes, encoding F3'5'H, DFR and ALS enzymes in petals, was demonstrated by northern analysis. Confirmation of the expression of functional enzymes was obtained from metabolite analysis using liquid chromatography (HPLC analysis). The levels of delphinidin and cyanidin in a single assay of bulked petal samples were 0.07 and 0.02 mg/g fresh weight, respectively. It was estimated that the concentration of delphinidin in the genetically modified carnation flowers is approximately one-fiftieth of that in blueberry. Delphinidin is not produced in stems, nodes, leaves or roots of carnation Moonaqua 123.8.12. Cyanidin is not a novel metabolite in carnation.

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 Moonaqua 123.8.12, which includes the production of over seven million of flowers. In 2003, two off-types with white streaks were found among 1000 flowers assessed. No off-types were found during flower assessment in 2005 and 2006.

2.3. Conclusion

The molecular characterisation data establish that the carnation Moonaqua 123.8.12 contains, in one locus, the complete cassettes containing the genes responsible for the intended traits (light mauve flower colour encoded by *dfr* and *f3'5'h* genes and herbicide tolerance encoded by the mutated *SuRB (als)* gene). In addition, two other loci contain incomplete *f3'5'h* cassettes.

Results of bioinformatic analyses of the three newly expressed proteins in carnation Moonaqua 123.8.12 did not indicate relevant homologies with known toxins or allergens. No new open reading frames were created in the flanking regions covering the inserted DNA and the carnation genome. The GMO Panel concludes that the molecular characterisation of carnation Moonaqua 123.8.12 does not raise any safety concern for humans, animals or the environment.

3. Comparative analysis

3.1 Issues raised by Member States

No objection remained among Member States concerning the comparative analysis of carnation Moonaqua 123.8.12 to its non-GM parent at the end of the 45-day Member States consultation period.

3.2. Evaluation of relevant scientific data

3.2.1. Choice of comparator and production of material

Carnation Moonaqua 123.8.12 was compared with the parental variety FE123 which does not produce the anthocyanins, delphinidin and cyanidin, and has cream-white petals.

3.2.2. Compositional analysis

Petals of carnation variety Moonaqua 123.8.12 and the parental variety FE123 were analyzed for three anthocyanins, namely delphinidin, cyanidin and petunidin. Roots, leaves and stems were not assayed. The GMO Panel reviewed the liquid chromatography (HPLC analysis) data provided on the concentrations of these three anthocyanins (Fukui *et al*., 2003). While petunidin was not detected in either the GM variety, or in the non-GM parent, delphinidin and cyanidin were detected in petals of carnation Moonaqua 123.8.12 at levels of 0.07 mg/g and 0.02 mg/g fresh weight, respectively (see Section 2.2.3). These anthocyanins were not present in petals of the white-flowered variety FE123.

The GMO Panel considers that the compositional analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment of the intended modification since the intended use of carnation Moonaqua 123.8.12 excludes cultivation and human or animal consumption.

3.2.3. Agronomic traits and GM phenotype

Carnation Moonaqua 123.8.12 and the parental variety FE123 were grown in field trials in The Netherlands in 2000 and in Australia in 2005 and compared for several morphological characteristics. The comparison of data from these field trials identified significant differences between the GM carnation and the parental variety FE123. The GM carnation has smaller

flowers, reduced stem thickness at the 5th node, and reduced numbers of stamens, styles and anthers and stamen length. According to the notifier, the observed differences are most likely attributable to somaclonal variation and/or environmental effects.

3.3. Conclusion

On the basis of the data provided by the notifier and in consideration of the intended use of carnation Moonaqua 123.8.12 (excluding cultivation and human or animal consumption), the GMO Panel considers that a compositional analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment of the intended modification. In addition to confirming the introduced traits, the field trials identified significant morphological differences in some of the phenotypic characteristics observed between the GM carnation and the parental variety. The GMO Panel concludes that the GM carnation Moonaqua 123.8.12 is not agronomically equivalent to the parental variety FE123, as indicated by the morphological changes.

4. Safety assessment of GM carnation Moonaqua 123.8.12 for humans and animals

4.1. Issues raised by Member States

A need for further assessment of the allergenic potential of carnation Moonaqua 123.8.12 was identified by one Member State at the end of the 45-day Member States consultation period.

4.2. Evaluation of relevant scientific data

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 grown in gardens and are available in the cut flower market as ornamental plants.

The scope of notification C/NL/06/01 is restricted to the import of cut carnations Moonaqua 123.8.12 for ornamental use only. Carnation Moonaqua 123.8.12 is a new variety with specific light mauve flower colour that results from the synthesis of delphinidin and cyanidin due to introduced *dfr* and *f3'5'h* genes. The GM carnation Moonaqua 123.8.12 also contains a mutated *SuRB* (*als*) gene conferring tolerance to sulfonylurea herbicides and used to facilitate selection during the transformation process *in vitro*.

4.2.2. Stability during processing

Since carnation Moonaqua 123.8.12 is intended to be imported as cut flower like other non-GM carnations, the petals of carnation Moonaqua 123.8.12 are not expected to be processed and used as food and feed. Consequently, the GMO Panel did not consider stability of the GM carnation during processing as an issue.

4.2.3. Toxicological assessment of expressed novel proteins

General BLAST searches were performed in order to compare the amino acid sequences of the proteins encoded by the three inserted genes with proteins from the GenBank and SwissProt databases. No homologies were observed with known toxic proteins using general BLAST searches (see Section 2.2.2).

4.2.4. Toxicological assessment of new constituents other than proteins

Given that carnation Moonaqua 123.8.12 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.

According to Directive 94/36/EC on colours for use in foodstuffs (EC, 1994), anthocyanins (E 163), including delphinidin and cyanidin, are authorised food additives in the EU. Anthocyanins have been evaluated by the previous 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 (SCF, 1984). Therefore the GMO Panel sees no reason for concern regarding the presence of delphinidin and cyanidin in petals from carnation Moonaqua 123.8.12.

The anthocyanins delphinidin and cyanidin are present in many foods and in some of them at much higher concentrations than in the petals of carnation Moonaqua 123.8.12, particularly high concentrations being found, for example, in blackcurrants and red grapes (Cachio *et al*., 1992). Many other delphinidin-containing species (e.g. *Dampiera* spp., *Delphinium* spp., *Lisianthus* spp., *Wisteria* spp.) contain a higher concentration of delphinidin (as a percentage of total anthocyanins) than does carnation Moonaqua 123.8.12. 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) and *Vicia villosa* (Catalano *et al*., 1998).

4.2.5. Toxicological assessment of the whole GM plant

The GMO Panel has considered the possible effects of the genetic modification on human and animal health of accidental consumption of carnation Moonaqua 123.8.12 petals.

4.2.5.1 Acute toxicity testing

The notifier conducted an acute oral toxicity study in mice for the purpose of assessing the impact of accidental consumption of carnation Moonaqua 123.8.12 on human or animal health.

Groups of five male mice received by gavage water extracts from leaves or petals (corresponding to a single dose of 4 g per kg body weight) of carnation Moonaqua 123.8.12. As anthocyanins are water soluble, the extracts from carnation Moonaqua 123.8.12 contained delphinidin and cyanidin. Control groups received either aqueous extracts from leaves or petals of the parental variety FE123 or water. There were no indications of adverse effects in mice administered aqueous extracts from carnation Moonaqua 123.8.12 compared with the non-GM controls at the end of the 14-day observation period.

4.2.5.2 Gene mutation assay

The notifier performed a study on gene mutations in bacteria using *Salmonella enterica Typhimurium* (Ames test) with water extracts of leaves or petals of carnation Moonaqua 123.8.12 and the parental variety FE123. The water extracts did not show mutagenic activity under the conditions of the assay.

4.2.6. Allergenicity

The notifier performed general BLAST searches comparing the amino acids sequences of proteins encoded by the three inserted genes with proteins found in the GenBank and SwissProt databases (FAO/WHO, 2001; Codex Alimentarius, 2003). No homologies were observed with known allergens.

The notifier performed a search for short identical stretches of at least six contiguous amino acids. Various positive outcomes consisting of solely six identical contiguous amino acids shared by the three transgenic proteins and allergens have thus been found. The GMO Panel, however, notes that a number of reports in the scientific literature indicates that stipulating only 6-amino-acid long stretches in the homology search is likely to give rise to many false positive outcomes. Therefore, for those proteins identified in the search, as well as for the transgenic proteins, hydrophilicity plots were drawn to predict the possible antigenic sites using a window of six amino acids. The prediction is based on the assumption that relatively hydrophilic residues are more exposed on the protein surface and thus likely to be bound by

antibodies. The scientific literature was also screened for data on IgE-binding epitopes in the identified allergens. No indication of potential allergenicity was found.

In response to a request from the GMO Panel, the notifier performed an additional sequence homology search this time between the three newly expressed proteins and known allergens using a 80 a.a. long sliding window looking for a minimum of 35% non-contiguous identical amino acids. No matches were found. The GMO Panel therefore concludes that no relevant homologies exist between the newly expressed proteins in carnation Moonaqua 123.8.12 and known allergens.

Sanchez (1999; 2004) has described occupational allergy (skin and respiratory allergy) to carnation in workers handling cut flowers/carnation over a long time. This allergy could be caused either by the flower, by mites such as *Tetranychus urticae* infesting carnations or by both simultaneously. According to the notifier, no adverse reaction to carnation Moonaqua 123.8.12 cut flowers used for ornamental purpose has been reported in the general populations where it is marketed. The notifier also reported to the GMO Panel that there have never been any reports of allergenicity or contact dermatitis from growers, distributors and purchasers in over 6 years due to production and processing in Ecuador and Colombia or from export of flowers to the United States.

Considering the scope of this notification and the limited exposure to carnation Moonaqua 123.8.12, 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.

Therefore the GMO Panel is satisfied with the data provided in the notification and is of the opinion that, in this specific case, no further tests are required with respect to allergenicity.

4.3. Conclusion

Carnation flowers have a long history of use as ornamentals. Carnation Moonaqua 123.8.12 differs from the parental variety FE123 by the synthesis of delphidinin and cyanidin in the petals, which confers a light mauve colour to the flowers. Delphinidin and cyanidin, which are common pigments in many ornamental flowers and food plants such as red grapes, blackcurrants, egg plants and blueberries, are produced as a result of the combined expression of the introduced *dfr* and *f3'5h* genes together with endogenous genes in the anthocyanin biosynthesis pathway.

The possibility of accidental consumption of carnation Moonaqua 123.8.12 petals cannot be ruled out. However the amount of delphinidin and cyanidin consumed will be negligible in comparison with the amount of delphinidin and cyanidin present in fruits containing high levels of delphinidin and cyanidin such as blackcurrant or red grapes.

No toxicity of water extracts of carnation Moonaqua 123.8.12 petals was observed in an acute oral toxicity study and no mutagenicity of aqueous extracts was indicated by a bacterial mutagenicity assay (Ames test). The amino acid sequences of the newly expressed proteins showed no similarity to known toxins or allergens.

Considering the intended use of carnation Moonaqua 123.8.12, 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

There was a question from a Member State on possible naturalization of carnation Moonaqua 123.8.12. Considering the scope of the notification, there will be a very limited environmental exposure with respect to viable plant parts of carnation Moonaqua 123.8.12.

A need for a more detailed post-market monitoring plan was identified by a Member State at the end of the 45-day Member States consultation period.

Monitoring is clearly related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of the GMO Panel. However, the GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the notifier under Section 5.2.4 of the present scientific opinion.

5.2. Evaluation of relevant scientific data

The GMO Panel considered the information provided in the original notification, the Member State objection and further scientific literature in the assessment of the potential for environmental risks and the requirement for a more detailed monitoring plan. As the notification concerns only import of cut flowers, no scientific information on potential environmental effects associated with the cultivation of carnation Moonaqua 123.8.12 was required. Considering the scope of the notification, there will be a very limited environmental exposure with respect to viable plant parts of carnation Moonaqua 123.8.12. 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 notifier, 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 to encourage root growth. Rooted plants may be planted in soil or grown hydroponically, and are kept for 1-2 years. Flowers are produced in flushes, beginning 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 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 only produces a negligible amount of 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. However there are no known reports of insect pollinators of *D. caryophyllus*, in particular. 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. *Dianthus* species 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 in Europe and occasionally naturalized in some Mediterranean countries but appears to be restricted to the coastal Mediterranean regions of Greece, Italy, Sicily, and Sardinia (Tutin *et al.,* 1993).

Carnation Moonaqua 123.8.12 are imported as cut flowers and thus have no roots and only occasional vegetative buds. The cut stems with vegetative shoots could be propagated by rooting or by micro-propagation.

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, to cover the very unlikely event of escape into the environment, the fitness of the GM plants was considered by the GMO Panel.

The carnation Moonaqua 123.8.12 has a modified flower colour achieved by introducing two genes of the anthocyanin biosynthesis pathway from *Petunia* and *Viola sp.*. These genes,

encoding dihydroflavonol 4-reductase and flavonoid 3'5' hydroxylase, give rise to the anthocyanins delphinidin and cyanidin. These anthocyanins are also widely found *e.g.* in flowers of the genus *Petunia* (Ando *et al*., 1999), *Rosa* (Biolley and Jay, 1993) or *Chrysanthemum* (Schwinn *et al*., 1993; Andersen *et al*., 2000). There is no evidence that the presence of delphinidin and cyanidin would lead to effects on plant fitness.

Carnation Moonaqua 123.8.12 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. The GMO Panel took into account the phenotypic characteristics reported in Section 3.2.3. The GMO Panel considered that, because of the intended use of carnation Moonaqua 123.8.12 and therefore the very low exposure of recipient populations, there were no changes in plant characteristics of any ecological significance. The carnation Moonaqua 123.8.12 plant would not show changed fitness characteristics except in the presence of sulfonylurea herbicides and these herbicides are 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. However, these herbicides are not known to be used on cultivated carnations. Such herbicide tolerant plants can be managed by a range of measures (Tranel & Wright, 2002). The consequences of the potential transfer of the three genes would be negligible in terms of adverse effects on the environment.

The GMO Panel is of the opinion that the carnation Moonaqua 123.8.12 is unlikely to have adverse effects on the environment in comparison with non GM carnations.

5.2.2. Potential for gene transfer

5.2.2.1 Plant to bacteria gene transfer

The carnation Moonaqua 123.8.12 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 *Viola 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.

5.2.2.2 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 information provided by the notifier suggest that the proportion of flowers carrying pollen is low. The data indicate that pollen transfer is very unlikely to occur. In addition, viable seed set on cut flowers is very unlikely and has not been observed so far with carnation Moonaqua 123.8.12, most likely because of 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 cause an adverse environmental effect.

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 Moonaqua 123.8.12 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 notifier 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 of the GMO Panel on GM plants (EFSA, 2006a). 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 notifier, including the general surveillance.

The GMO Panel agrees with the notifier 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 which included a questionnaire to European importers. It was also noted that the notifier requested taxonomists and botanists to inform them of hybrids that might originate from the GM carnation. In addition the notifier will involve national botanic survey networks and plant protection services in his monitoring activities.

In the light of the very low environmental exposure of viable forms of GM carnation Moonaqua 123.8.12 due to the restricted intended use of the GM carnation, the GMO Panel concludes that the proposal of the notifier for general surveillance is in line with the Guidance Document of the GMO Panel on GM plants and in particular with its provisions on post-market environmental monitoring (EFSA, 2006a). The GMO Panel agrees with the proposal made by the notifier to report the monitoring activities on an annual basis as suggested in its Guidance Document (EFSA, 2006a).

5.3. Conclusion

The GMO Panel based its environmental risk assessment on cut flowers of carnation Moonaqua 123.8.12 to be imported for ornamental use only. From the information supplied by the notifier, 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 Moonaqua 123.8.12 was assessed for imported cut flowers for ornamental use only. Scientific information on potential environmental effects associated with the cultivation of carnation Moonaqua 123.8.12 was therefore not required. Carnation Moonaqua 123.8.12 cut stems and flowers have marginal viability, negligible pollen production 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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 will have adverse effects on the environment in the context of the intended use.

The GMO Panel agrees with the notifier 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 provided in the notification.

CONCLUSIONS AND RECOMMANDATIONS

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 Moonaqua 123.8.12 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 Moonaqua 123.8.12 has a modified flower colour, a shade of light mauve, which is achieved by introducing into cream-white carnation two genes of the anthocyanin biosynthesis pathway, one from *Petunia* and the other from *Viola* sp. Carnation Moonaqua 123.8.12 also expresses sulfonylurea herbicide tolerance.

The GMO Panel has evaluated the molecular analysis of the genetically modified carnation Moonaqua 123.8.12 and concludes that the molecular characterisation of carnation Moonaqua 123.8.12 does not raise any safety concern for humans, animals or the environment.

Given the intended use of carnation Moonaqua 123.8.12 (excluding cultivation and human or animal consumption), the GMO Panel considers that a compositional analysis limited to the newly synthesised anthocyanins is sufficient for the risk assessment of the intended modification. In the case of accidental consumption of petals from carnation Moonaqua 123.8.12, the amount of delphinidin and cyanidin consumed will be negligible in comparison with the amount present in fruits containing high levels of delphinidin and cyanidin, such as blackcurrant or red grapes. An extract from petals did not induce adverse effects in an acute oral toxicity study and was not mutagenic in bacterial gene mutation tests. Furthermore, based on the results of bioinformatic studies, there is no evidence that any of the three proteins expressed is toxic or allergenic. The GMO Panel concludes that carnation Moonaqua 123.8.12 is unlikely to have adverse effects on human or animal health in the unlikely event that carnation Moonaqua 123.8.12 petals are consumed.

Considering the low environmental exposure due to the restricted scope of the notification, it is very unlikely that gene transfer and escape into the environment would occur. In the event that this did occur, the consequences of the escape of the three genes would be negligible with

regard to environmental impact. 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 Catherine Geslain-Lanéelle, Executive Director EFSA, and the annexes, dated 1st of October 2007 with ref. Directorate B D (2007) 17333, from Director Ladislav Miko – Notification C/NL/06/01 (Carnation Moonaqua 123.8.12), under Directive 2001/18/EC - request for EFSA opinion.
2. Letter from EFSA to the notifier with request for further copies of the notification (ref. SR/SM/shv (2007) 2430887, 9 October 2007).
3. Letter from notifier to EFSA, dated 18 October 2007, in response to EFSA request.
4. Letter from EFSA to the notifier – Acknowledgement of receipt (ref. CGL/SR/SM/shv (2007) 2460197, 6 November 2007).
5. Letter from EFSA to notifier, dated 19 December 2007, with request for clarifications/additional information (Ref. SR/SM/shv (2007) 2586837).
6. Letter from EFSA to notifier, dated 18 January 2008, with request for clarifications/additional information (Ref. SR/SM/shv (2008) 2630645).
7. Letter from notifier to EFSA, dated 24 January 2008 and received on 7 February 2008, providing additional information upon EFSA request.
8. Letter from EFSA to notifier, dated 18 February 2008, about additional data considered satisfactory (Ref. SR/SM/shv (2008) 2695632).

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.

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.

Codex Alimentarius, 2003. Guideline for the Conduct of Food Safety Assessment of Foods derived from Recombinant-DNA Plants. CAC/GL 45-2003. Codex Alimentarius, Annex, section 3.2, item 9.
ftp://ftp.fao.org/codex/Publications/Booklets/Biotech/Biotech_2003e.pdf

EC, 1994. Directive (EC) 94/36 of the European Parliament and of the Council as amended by Regulation (EC) No 1882/2003 on colours for use in foodstuffs. Official Journal of the European Communities, L 237, 13-18.
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31994L0036:EN:HTML

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

EC, 2007. Commission Decision (2007/364/EC) of 23 May 2007 concerning the placing on the market, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, of a carnation (*Dianthus caryophyllus* L., line 123.2.38) genetically modified for flower colour. Official Journal of the European Communities, L138, 50-52. http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_138/l_13820070530en00500052.pdf

EFSA, 2006a. 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 374, 1-115. http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_guidance/gmo_guidance_ej374.Par.0001.File.tmp/gmo_guidance_ej374_gmm.pdf

EFSA, 2006b. 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. The EFSA Journal 362, 1-19. http://www.efsa.europa.eu/EFSA/Scientific_Opinion/gmo_op_ej362_carnation_moonlite_en1.pdf

Food and Agriculture Organization & World Health Organization (FAO/WHO), 2001. Allergenicity of genetically modified foods, a joint FAO/WHO consultation on foods derived from biotechnology, Rome, Italy, 22-25 January 2001. Section 6.1, page 12. http://www.who.int/foodsafety/publications/biotech/en/ec_jan2001.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), 18p.

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.

SCF, 1984. Report of the Scientific Committee for Food, Food-Science and Techniques, Commission of European Communities (Fourteenth series).
http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_14.pdf

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

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