Advisory Committee on Releases to the Environment

Advice of the Advisory Committee on Releases to the Environment (ACRE) under S.124 of the Environmental Protection Act 1990 (Part VI) to UK ministers and ministers in the Devolved Administrations

Advice on a notification for the marketing of a GM carnation FLO-40685-2[1]

Details of notification

Notifier:	Suntory Holdings Ltd.
Notification reference:	C/NL/13/02
Product:	A GM carnation line genetically modified for petal colour and herbicide tolerance. This line (FLO-40685-2) is modified to contain F3’5'H and DFR proteins, which confer the ability to produce a violet pigment, and a mutated ALS protein which confers tolerance to sulfonylurea herbicides.
Scope:	To import, distribute and retail cut carnation flowers containing event FLO-40685-2 into the EU. The scope of this notification does not include cultivation or use as food or feed.
Date:	10 October 2014

45 Day Assessment Period Advice: ACRE has not altered its previous advice in concluding that the import and distribution of cut flowers from GM carnation line FLO-40685-2 does not pose an increased risk to human health or the environment as compared with non-GM carnation varieties.

ACRE did not request further information from the notifier during the initial (60 day) assessment period. However, we have considered the further information provided by the notifier in response to requests from other Member States. This does not alter our previous advice (please see below).

[1] Previously referred to by the identifier FLO-40685-1

Comment

This notification is to import, distribute and retail one line of a GM carnation (FLO-40685-2) onto the EU cut flower market. The scope of the notification does not include cultivation or use as food or as feed.

ACRE has considered the molecular characterisation of FLO-40685-2 carnation and the risks associated with importing and retailing cut flowers, including the potential impacts on human health. We have considered the suitability of the plan for post-market monitoring in the light of the risks posed by this GMO.

ACRE is familiar with the transgenes/ GM traits associated with FLO-40685-2 carnation as we have considered them in previous notifications to market GM carnations in accordance with Directive 2001/18/EC (please refer to notifications C/NL/13/01 and C/NL/06/01). Our conclusions on the risks posed to human health and the environment are consistent. These were reached after a case-specific assessment of each GMO.

Molecular Characterisation

The non-GM parental line of this GM carnation line has white flowers. It was modified with two enzymes in the anthocyanin biosynthetic pathway *i.e.* dihydroflavonol-4-reductase (DFR) and flavonoid 3'5'- hydroxylase (F3'5'H). The *DFR* gene is a genomic clone derived from Petunia x hybrida and it is under the control of its own regulatory elements. The *F3'5'H* gene is a complementary DNA derived from Viola species and is under the control of the antirrhinum *CHS* promoter. Simultaneous expression of the proteins encoded by these genes in the carnation results in modified flavonoid synthesis and formation of the violet pigment delphinidin.

In addition, FLO-40685-2 carnation has been modified to contain a mutated version of the enzyme acetolactate synthase (ALS). The gene encoding this enzyme is derived from *Nicotiana tabacum* (cultivated tobacco) and is under the control of the 35S cauliflower mosaic virus promoter. There are two unlinked *ALS* genes in tobacco, *SuRA* and *SuRB*. This GM carnation contains a version of the *SuRB* gene (i.e. *S4-Hra*) that has been mutated to confer resistance to sulfonylurea herbicides. This trait was used to select for transformed plants during the genetic transformation process.

ACRE considers that the notifier has submitted good quality, detailed molecular data. The notifier has used Southern hybridisations and DNA sequence analysis to characterise the transformation event. The results support the notifier's conclusion that FLO-40685-2 contains up to five copies of each integrated component of the T-DNA, and integration of T-DNA has occurred at four loci. This DNA was introduced into the carnation using an Agrobacterium vector. The notifier has demonstrated (using a number of different probes spanning the backbone of the Agrobacterium vector) that no backbone DNA (including the tetA antibiotic resistance marker gene) from Agrobacterium is present in the GMO.

The notifier has analysed the full DNA sequence of each insertion locus, as well as 150 base pairs of DNA flanking each insertion locus, to search for newly created, chimeric

open reading frames (ORFs). The notifier also searched databases of known allergens and toxins with the deduced amino acid sequences of the inserted genes (i.e. *DFR, F3'5'H* and *ALS*), and each potential chimeric ORF identified. ACRE is content that, in addition to the information provided on toxicity and allergenicity elsewhere in the notification, these bioinformatics analyses demonstrate that this GMO is unlikely to have a greater allergenic or toxic potential as compared to its non-GM counterparts.

Risks to Human Health

The notification does not include food use within its scope. However, the notifier has considered the consequences of individuals using petals or leaves from FLO-40685-2 carnation to garnish salads.

DFR, F3'5'H and ALS proteins are common in plants. DFR and F3'5'H are present in foods that contain delphinidin and these include a number of fruits and vegetables *e.g.* blackcurrants, aubergines and blueberries. ALS protein is common to plants, bacteria and fungi and there are a number of commercial crop varieties that contain ALS mutations conferring herbicide resistance *e.g.* imidazolinone resistant maize, wheat, oilseed rape, lentil and rice. The notifier has not identified a commercial crop variety that contains the *SuRB* gene mutation (i.e. *S4-Hra*) that is present in FLO-40685-2 carnation. However, the notifier has searched databases of known allergens and toxins for short stretches of homology with the deduced amino acid sequence of the *S4-Hra* gene inserted into FLO-40685-2 carnation. None were detected.

This lack of significant homology between the deduced amino acid sequences of the inserted genes (i.e. *DFR, F3'5'H and ALS*) and known allergens along with the results of bioinformatic analysis of insertion loci and flanking DNA, supports our conclusion that any pollen produced by this GMO is unlikely to have a greater allergenic potential as compared to its non GM counterparts. We note that there is no indication that the low viability observed for standard cultivars of the cultivated carnation is increased in FLO-40685-2.

FLO-40685-2 carnation has been grown commercially outside of the EU since 2000 and flowers have been shipped to the US, Canada and Japan, and grown and sold in Australia since 1997. There have been no reports of adverse effects by wholesalers, retailers or consumers exposed to this product.

Environmental Exposure and Environmental Impact

ACRE agrees with the notifier that it is very unlikely that cut carnation flowers will:

1. produce vegetative structures (without human assistance) and survive in the environment after disposal;

2. set viable seed or

3. release pollen that will generate hybrids with wild carnations, other *Dianthus* species or other members of the *Carophyllaceae* family.

Consequently, environmental exposure and therefore, the risk that FLO-40685-2 carnations pose to the environment is negligible. However, it is possible that individuals will propagate leaf and/or stem cuttings in glasshouses or in gardens. This is not legal as carnation FLO-40685-2 is not approved for cultivation in the EU. If this does happen, ACRE considers that there is sufficient information in this notification to conclude that the impact on the environment will be minimal. There is no evidence that gene flow from garden-cultivated carnations into related species would occur or that these plants will become invasive or weedy.

Post-market monitoring

ACRE considers that the post-market monitoring plan for FLO-40685-2 carnation is compatible with the environmental risk assessment provided in the notification. We are content that no risks have been identified in this assessment and as such, case-specific monitoring is not required. Consequently, it is appropriate that the monitoring plan for this GMO is based on general surveillance for unanticipated adverse effects. The notifier will submit annual monitoring reports if the GMO is approved for import, distribution and retail in the EU.

10 October 2014