FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF GENETICALLY MODIFIED CARNATIONS (*Dianthus caryophyllus L.*) FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/8

The objective of the Biosafety Act is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by the Suntory Holdings Ltd. to import and release products of genetically modified carnations, *Dianthus caryophyllus L*.

1. What is this application for?

This application is to commercial import and release of cut flowers of novel colour varieties of genetically modified carnation (*Dianthus caryophyllus* L.) by Suntory Holdings Ltd. The varieties are as below:-

(a) FLORIGENE® Moonaqua™,
(b) FLORIGENE® Moonlite™,
(c) FLORIGENE® Moonshade™,
(d) FLORIGENE® Moonvista™,
(e) FLORIGENE® Moonberry™,
(f) FLORIGENE® Moonvelvet™,
(g) FLORIGENE® Moonique™, and
(h) FLORIGENE® Moonpearl™.

2. What is the purpose of the import and release?

The aim of the import and release is to supply or offer to supply for sale/ placing on the market.

3. How has the genetically modified carnations *Dianthus caryophyllus L* been modified?

Genetically modified carnations (*D. caryophyllus L*.) has been modified in terms of colour as well as resistance to the sulfonylurea herbicide chlordulfuron. Flower colour is produced primarily as a result of anthocyanin and carotenoid biosynthesis in the petal tissue. The anthocyanins cyanidin, pelergonidin and delphinidin 3-glucosides are pigments that produce pink, mauve, red and blue shades of flowers. In order to produce flower colours in the violet spectrum it was necessary to identify white or pink carnations that possessed the necessary genes and enzymes of the anthocyanin biosynthetic pathway without producing flower colour. Once such cultivars were identified, the insertion of additional genes that result in the creation and conversion of colourless

dihydroflavanols to coloured delphinidin-related anthocyanins could be incorporated to produce mauve, violet and blue flowers.

4. Characteristics of the Genetically Modified Carnations, *Dianthus caryophyllus L*.

(a) Details of the parent organism

All eight varieties applied for approval are derived from the same parent organism carnation (*Dianthus caryophyllus*). Carnation is probably native to the Mediterranean region and belongs to the taxonomic family Caryophyllaceae. It has been extensively cultivated for the last 2,000 years. It is an herbaceous perennial plant growing to 80 cm tall. The leaves are glaucous greyish green to blue-green, slender, up to 15 cm long. The flowers are produced singly or up to five together in a cyme and are sweetly scented. The original natural flower colour is bright pinkish-purple.

(b) Details of the donor organisms

The flower colours of the carnations has been genetically modified by insertions of genes originated from snapdragon (*Antirrhinum majus*), Petunia (*Petunia hybrida*) and black pansy (*Viola* sp.). In addition to the genes responsible for the production of flower colour, a marker gene *SuR*B from tobacco conferring tolerance to ALS inhibiting herbicides was also incorporated.

The snapdragon belongs to family Plantaginaceae and also a native to the Mediterranean region. Like carnation, the snapdragon is also herbaceous perennial plant and growing to 0.5-1 m tall. Petunia on the other hand, belongs to family Solanaceae and is a flowering plant of South American origin, closely related to tobacco. Petunias can tolerate relatively harsh conditions and hot climates. They grow well in low humidity or moist soil.

Pansy belongs to family Violaceae and can be found mainly in the northern hemisphere. However, pansy can also be found in Hawaii, Australasia as well as in South America. Most pansy are perennials but some can be annual plants. All the above mentioned species are often planted in gardens for their beautifully coloured flowers.

(c) Description of the traits(s) and characteristics which have been introduced or modified

In all varieties of the genetically modified carnations, the modification is basically phenotypic (flower colour). Manipulation of the anthocyanin biosynthesis pathway results in accumulation of delphinidin-related anthocyanins in flower petals. Genes from various flowering plants and

enzymes were transferred to carnations to give desired colours. The necessary enzymes are Flavonoid 3', 5' hydroxylase (F3'5'H) and Dihydroflavonol-4 reductase (DFR). The addition of genes responsible for encoding these enzymes results in the production of specific delphinidin pigments only. Other genes that are also involved are *Cytochrome b5*, *ds Dihydroflavonol 4-reductase* and the *Acetolactate synthase* (ALS) gene *SuR*B DNA encoding the ALS protein. The mutation of *SuR*B gene that has been used also confers the genetically modified carnations resistance to sulfonylurea herbicide chlordulfuron

(d) Safety of the Expressed Proteins

Carnation has long history of safe use. Carnation flowers are produced and consumed in Malaysia safely, and have been for many decades. Since the development of double flowers varieties suited to the cut flower industry in the 1940s to 1950s, billions of flowers are grown and sold each year. Carnation is one of the major flower types produced in the Malaysia flower industry. Today, major centres of carnation production are Colombia, China, Spain and Italy. However, carnation is grown in virtually every non-equatorial country where there are suitable climates such as Brazil, Ecuador, California, Netherlands, Russia, Ethiopia, Kenya, Morocco, Israel, Japan, Vietnam and Australia, as well as Malaysia.

Carnation is not known to be a toxic plant. Surveys on several on-line databases for poisonous and toxic plants indicated that *Dianthus caryophyllus* has no toxicity. Carnation is also not known as an allergenic plant, and does not produce wind-dispersed pollen. There are isolated cases in which occupational allergy were associated with long term handing of cut flowers of carnation, possibly due to association with insect pests.

5. Assessment of Risks to Human Health

Carnation has been used safely by humans for ornamental purposes for centuries. The modification in the genetically modified higher plants (GMHP), such as production of delphinidin, is novel for carnation, but there are many flowers and other ornamental species that produce delphinidin. Delphinidin is also present in many common foods. Carnation is not reported to be a poisonous plant, or to cause allergic reactions, and there is no evidence that the transgenic line has, or could, cause an adverse reaction. There is now an extensive history of safe use of the product overseas. Carnation is not used as a food but there is a slight possibility that some home consumers may decide to eat flower petals, or garnish foods with flower petals. In the event that this did occur we do not believe the transgenic carnation poses any health risk because the novel products in the GMHP are found naturally in many foods. Open reading frame analysis of introduced regions of DNA reveals that the deduced

amino acid sequences of the transgenic carnation lines in this application appear not to be homologous to any known toxic or allergenic proteins. Direct tests of potential toxicity indicate no potential for harm to plant, animal or human health.

(a) Toxicological Information

Delphinidin is not known to be a toxic compound, when consumed or when handled. There is no toxicity data in the Merck Index for the aglycone, the mono-glucoside or the 3'5'-glucoside of delphinidin. Anthocyanins have a low acute toxicity of ca. 20,000 mg/kg BW in rodents, and a very low order of toxicity (WHO, 2001). Delphinidin is found in many raw foods. Major health benefits attributed to anthocyanin consumption include improved cardiovascular health, anti-viral capacity and treatment of infection (Broadhurst, 2001). The health properties of anthocyanins, including delphinidin, are further described in Sterling (2001) and Lila (2004). The concentration of delphinidin we have measured in the transgenic flowers is approximately one-fiftieth to one-half the level in blueberry, for example, which may have up to 5 mg anthocyanin per g FW, 40% of which is delphinidin (Kalt *et al.*, 1999). In summary, delphinidin is non-toxic, and is found in many commonly consumed and handled plants. There is considerable evidence that delphinidin-derived anthocyanins are in fact health-promoting.

(b) Carcinogenicity and Pathogenicity

Two other studies also point to the positive health effects of delphinidin;

- No apparent genotoxicity and mutagenicity effects were found for either the anthocyanin or delphinidin extracts in mice fed eggplant skin or pure delphinidin (Azevedo *et. al*., 2007)
- Delphinidin 3-sambubioside (Dp3-Sam), a hibiscus anthocyanin, could induce a dose-dependent apoptosis in human leukaemia cells (HL-60) measured by changes in cell morphology, DNA fragmentation, activation of caspase-3, -8, and -9, and inactivation of poly(ADP)ribose polymerase (Hou *et. al*., 2005b).

Several studies have compared the relative efficacy of the major anthocyanins and have shown that delphinidin or delphinidin-derived anthocyanins has greater activity than other anthocyanins.(Khan *et. al*., 2002; Noda *et. al*., 2002; Hou *et. al*., 2005a; Lazze *et. al*., 2006; Lamy *et. al*., 2006; Lamy *et. al*., 2007).

6. Assessment of Risks to the Environment

There is no environmental impact from the placing on the market of the GMHP which would be different to that of placing flowers from the recipient plant on

the market. Carnation flowers from many varieties, including the recipient, are a commodity in the EU, and several billion non-GM carnation flowers are consumed per annum in the community. There is no evidence the products would have any adverse effects:

- An analysis of the biology of carnation shows no potential for gene dispersal as a result of import of cut-flowers. The flowers are not invasive and there is no opportunity for the cut- flowers to become “weeds”. Carnation is not a weed in Europe and despite hundreds of years of cultivation, and plantings in parks and gardens, it has not become a weed, or escaped from cultivation, anywhere in the world, including Malaysia. No hybrid between carnation and any other *Dianthus* species has ever been recorded in the wild.

7. What is the Emergency Response Plan?

No significant health hazards are currently associated with this product.

(a) First Aid Measures

No special first aid measures are required for exposure to this product. Modified GM carnation now has over a decade of safe use, in several countries. In the countries of production, where the intensity of handling is highest no adverse consequences have been identified. For example, no dermatological reactions have been reported. Where the product has been consumed as an imported cut-flower there have been no reports of adverse consequences of use.

(b) Illegal propagation

Accidental release can happen through illegal propagation. Illegal propagation, though difficult, can be possible from cut flowers using tissue culture methods. Though this illegal import of tissue cultures into Malaysia could have occurred in the past, as the varieties have been available for more than a decade, the import of cut flowers increases the risk of illegal propagation. Illegal propagation is relevant to risk assessment as this is effectively the only way that gene flow could occur from the imported cut flowers. This is because there are virtually no mechanisms for “natural “gene dispersal from imported cut flowers of the GM carnation.

Illegal growers can be readily detected because of the unique characteristics of the varieties described in this document. The unique flower colour would be the primary incentive for illegal propagation but it also allows the varieties to be identified in the marketplace. Unambiguous

identification of specific varieties is possible using molecular analysis techniques.

(c) Handling and Storage

No special handling procedures are required for this product.

(d) Disposal Considerations

There are no specific instructions or recommendations for waste disposal and treatment. The product can be disposed of in the same way as flowers from non-GM varieties of carnation. It is expected that as the flowers will be sold in the general floristry trade, waste product will be discarded with other household and business rubbish and eventually decompose in general refuse.

8. How can I comment on this application?

Any member of the public may submit their comments or queries on publicly notified information about the application. Before submission of comments or queries, the person should review the information provided. Your comments and queries on any possible impacts/risks to the health and safety of the people and the environment that may be posed by the proposed release are appreciated. The submission of the comments or queries should be prepared carefully as it will be given the same scrutiny as the application by the NBB. The submission of comments and clarifications of queries should contribute to the NBB's assessment. Even if the submission is not science-based, and focuses on cultural or other values, it should still be developed in the form of a well-founded argument.

Please note that the consultation period closes on 2nd October 2012 and written submissions are required by that date. Submissions must be addressed to:

Director General
Department of Biosafety
Ministry of Natural Resources and Environment
Level 1, Podium 2, Wisma Sumber Asli
No. 25, Persiaran Perdana, Precinct 4
62574 Putrajaya, MALAYSIA
E-mail: biosafety@nre.gov.my
Fax: 03-88904935

Please indicate your full name, address and contact details in your submission.