Australian Government
Department of Health
Office of the Gene Technology Regulator

RISK ANALYSIS

Inadvertent dealings with petunia genetically modified for altered flower colour

1. Introduction to the GMOs

Petunias (*Petunia hybrida*) are widely grown in Australia as ornamental plants (Harden 1992). Petunia cultivars African Sunset, Trilogy Red, Trilogy Deep Purple and Trilogy Mango were imported into Australia between 2013-2017, and were recently discovered to be genetically modified. Testing has found that the GM petunia cultivars contain an introduced gene conferring altered flower colour and an introduced antibiotic resistance marker gene (Table 1).

Table 1: Genetic elements detected in the GM petunias

Genetic element	Encoded protein	Function	Source
p35S	N/A	Constitutive promoter	Cauliflower mosaic virus
A1	Dihydroflavonol 4-reductase (DFR)	Altered flower colour	*Zea mays*
pNOS	N/A	Constitutive promoter	*Agrobacterium tumefaciens*
nptII	Neomycin phosphotransferase II (NPTII)	Antibiotic resistance marker gene	*Escherichia coli*

Source: testing by university researchers (Bashandy & Teeri 2017) and Finnish Food Safety Authority.

The cultivars Raspberry Blast, Candy Blast (also known as Rose Blast Charm) and Colourworks Homare were also imported. Cultivars Raspberry Blast and Candy Blast have been tested by the United States Department of Agriculture and found to be GMOs (APHIS guidance GE petunias accessed on 25/5/17). Cultivar Colourworks Homare is a suspected GMO. USDA testing covers the p35S, pNOS and *nptII* genetic elements (APHIS guidance petunia testing accessed on 26/5/17). It is assumed, as cultivars Raspberry Blast and Candy Blast share three introduced genetic elements with the GM petunias African Sunset, Trilogy Red, Trilogy Deep Purple and Trilogy Mango, that they have been genetically modified in the same way. This risk analysis document is based on currently available information regarding the introduced genetic modifications as outlined above in Table 1.

Altered flower colour trait

Petunia flower colour is due to anthocyanin pigments. The native DFR enzyme in petunia favours the conversion of the anthocyanin precursor dihydrokaempferol to the pigments cyanidin (red) and delphinidin (blue). The maize DFR enzyme can also drive conversion of dihydrokaempferol to the pigment pelargonidin (orange), and thus alter flower colour (Meyer et al. 1987; Griesbach 1993). The total anthocynanin content of GM petunias containing an introduced *A1* gene was measured and is similar to non-GM petunias (Griesbach 1993). Humans are naturally exposed to anthocyanins through ingestion of fruit and vegetables (Wu et al. 2006), and anthocyanins are authorised food additives used for colouring in Australia (FSANZ 2014).

2. Potential for toxicity or allergenicity

Petunias are not grown for human food or animal feed in Australia. Non-GM petunias are not considered toxic if accidentally ingested by children or pets (Safe and Poisonous Garden Plants, ASPCA Toxic and Non-Toxic Plants List), and are recommended plants for low-allergen gardens (Asthma Foundation Victoria 2013). This suggests that petunias do not produce any native toxins or allergens that cause problems when petunias are grown in gardens and not intentionally consumed. Based on experience to date with a range of GM plants, it is unlikely that the genetic modifications in the GM petunia would alter levels of native toxins or allergens, and, even if this were to occur it is not expected to lead to harm.

The introduced *A1* gene is derived from maize and is naturally expressed in all maize tissues (Bernhardt et al. 1998). Maize kernels have a long history of safe consumption by people and animals, whole maize plants are used as grazing for livestock, and maize flowers are regularly visited by insect pollinators without ill effects. In addition, the encoded maize DFR protein is homologous to the geranium and petunia DFR proteins, which have been previously assessed by the Regulator as being of negligible risk to people and the environment. GM torenias containing geranium DFR were approved for field trials (DIR 068/2006). GM carnations containing petunia DFR were approved for commercial releases and also placed on the GMO Register for uncontained release without a licence (DIR 030/2002, DIR 134, GMO Register). Thus, the maize DFR protein is not expected to be toxic or allergenic to people or other organisms, including insect pollinators.

The introduced *nptII* gene from *E. coli* is used extensively as a selectable marker in the production of GM plants and is present in many GMOs approved for commercial release in Australia and other countries. Further information about this gene can be found in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website. Regulatory agencies in Australia and other countries have found no evidence that the NPTII protein is toxic or allergenic to people or other organisms, including insect pollinators.

3. Potential for weediness

Petunias are typically planted in gardens as annuals; they are killed by frost but may survive mild winters (Small 2014). Petunias can also self-seed in humid subtropical climates such as Florida and South Carolina (Burch & Demmy 1995; Russ 2007). However, most petunias will die if not watered for 14 days (Estrada-Melo et al. 2015). Thus, unwanted petunias could be

minor weeds in gardens, but probably only if the gardens are in a warm climate and frequently watered.

Petunias may spread outside gardens by several mechanisms, including wind transport of their tiny seeds, which weigh around 0.1 mg each (Jauron 2013). Seeds are initially dormant and require light (cannot be buried), warm temperatures and moisture in order to germinate (Jauron 2013; Small 2014; Petruzzelli et al. 2003).

Petunia populations (often classified as *P. axillaris*, which is one parent of the hybrid *P. hybrida*) are occasionally naturalised in Australia, primarily in northern NSW and southern Queensland (Harden 1992)(Atlas of Living Australia). Naturalised *P. axillaris* plants are considered a minor problem warranting control in NSW and are present but not considered to warrant control in Queensland (Groves et al. 2003). Petunias are not classified as noxious weeds or weeds of national interest (National weeds lists).

Neither the altered flower colour trait conferred by the *A1* gene, nor the antibiotic resistance conferred by the *nptII* gene are expected to increase the weediness potential of the GM petunias.

4. Potential for gene transfer

Petunias are insect pollinated. They are sexually compatible with cultivated petunias, other species from the genus *Petunia* and possibly species from the closely related genus *Calibrachoa* (Small 2014; Vandenbussche et al. 2016). No plants from *Petunia* or *Calibrachoa* are native to Australia. Cultivated petunias and *Calibrachoa* species are grown as ornamental garden plants in Australia. Petunias are occasionally naturalised as discussed in section 3. The species *C. parviflora* is sparsely naturalised but not considered to warrant control in northern NSW (Groves et al. 2003; Harden 1992)(Atlas of Living Australia).

As discussed in sections 2 and 3 of this document, the introduced *A1* and *nptII* genes are not expected to increase toxicity, allergenicity or weediness in GM petunia. For the same reasons, if gene transfer to related species occurred, these genes would not be expected to increase the toxicity, allergenicity or weediness of the related species.

5. Consideration of proposed dealings

The dealings with GMOs proposed in the licence are:

(a) disposing of the GMOs;
(b) conducting experiments with the GMOs for purposes relating to disposing of the GMOs;
(c) growing the GMOs for purposes relating to disposing of the GMOs;
(d) transporting the GMOs for purposes relating to disposing of the GMOs; and
(e) possession or supply of the GMOs for purposes relating to disposing of the GMOs.

The GM petunia cultivars are already present in the Australian environment, as they have been imported and grown since 2013. The effect of the proposed dealings would be to reduce the number of GM petunias present in the environment, thus reducing exposure of people and animals to the GMOs and reducing the potential for spread and persistence of the GMOs. The proposed dealings are not expected to pose any new risks that do not already exist due to the presence of the GMOs in the environment.

Methods of disposal

Methods of disposal for GM petunia plants listed in the licence include herbicide application, uprooting, desiccation and incineration. Methods such as mowing or shredding are not listed due to some uncertainty regarding their effect, as petunias can regrow from cuttings under favourable conditions (Ahkami et al. 2009). Listed methods of disposal for GM petunia seeds include various types of heat treatment, which are expected to denature proteins and render the seeds non-viable, or deep burial. Rationales for the recommended methods can be found in Appendix A to this document.

6. Conclusions of risk analysis document

Based on the information currently available, the GM petunias are considered to pose negligible risks to human health and safety or the environment. Should new and/or different information become available, then further risk analysis may be required.

7. References

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Appendix A GM petunia disposal guidelines

Seedless petunia plants may be destroyed by any of the following methods:

- Herbicide treatment. Tips on safe herbicide use can be found in the web page of Australian Pesticides and Veterinary Medicines Authority
- Burning. Burn only in accordance with all federal, state, and local laws and ordinances and permits. Monitor weather conditions prior to ignition to avoid hazardous fires (Rowe 2013)
- Composting. Plants can be pulled and left with roots exposed to dry out. This material can be then composted. Composting at 55°C for three continuous days kills most plant propagules, but note that this is not 100% effective if seeds or flowers are present (Rowe 2013)
- Burial. Dig a hole of at least 1 meter deep and bury the plants. Such a pit is best located in an out-of-the-way spot in the yard where it will not be disturbed (Timber Press 2013).
- Desiccation. Stop watering the petunia plants. Water-deprived plants usually die after 14 days in greenhouse conditions (Estrada-Melo et al. 2015)
- Plants can be placed into plastic bags and stored in a lidded waste bin for at least a month. Non-viable plant material can then be disposed of via standard methods (eg landfill). This method has been previously used to destroy GM-Torenia plants, another herbaceous ornamental, after a field trial (OGTR 2008).
- Plants may also be bagged in a black plastic bag, sealed and then 'baked' in the sun until destroyed (Australian Department of Environment).

If petunia plants bear seeds the preferred method of disposal is burning. If this is not possible, seeds need to be harvested prior applying any of the other methods to kill the plants. Harvested seeds and seeds in packets may be destroyed by:

- Boiling in water for 20 min. Petunia seeds do not have hard seed coats (Sink 1984), therefore they are sensitive to hot water. Even plants with hard seed coats that require scarification treatments to germinate can be killed by boiling them in water for prolonged periods (Fulbright & Flenniken 1987; Kimura & Islam 2012)
- Heating in the oven at 150°C for 30 minutes. Petunia seeds are not known for their resistance to heat (Sink 1984). Seeds of plant species that live in fire prone environments and are built to withstand high temperatures, have their germination completely inhibited by exposure to 150°C for 5 minutes (Gashaw & Michelsen 2002)
- Microwaving at 800 watt or more for 10 minutes. Microwaving annual rye grass seeds at 800 watt for 240 seconds was sufficient to stop completely the germination of seeds (GRDC 2015)
- Deep burial at a biosecurity waste class 8.2 site approved by the Department of Agriculture and Water Resources. This approved arrangement for deep burial requirements ensures that the burial site will be undisturbed. Petunia seeds require light (cannot be buried) in order to germinate (Jauron 2013; Small 2014; Petruzzelli et al. 2003).

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