Notification 6786-01-0065

Summary of the risk assessment of genetically modified petunia (*Petunia hybrida*) (*Linie RL01-17*) carried out by the German Competent Authority within the framework of a proposed deliberate release, Berlin, 29 April 1997

Explanatory note to this document:

The following text reflects the summary of the risk assessment of (a) genetically modified organism(s) to be used for experimental field trials (deliberate releases) in Germany. The text forms part of the official authorisation regarding applications for the permit of deliberate releases (field trials) of genetically modified organisms in Germany under the legal framework of Directive 2001/18/EC and the German Gene Technology Act (Gentechnikgesetz, GenTG). The authorisation is issued by the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL [*Federal Office of Consumer Protection and Food Safety*], as the German Competent Authority. It comprises the chapters

I. Consent [to the application]
II. Provisions [to be respected in execution of the field trials]
III. Justification
III.1. Requirements for approval according to section 16 GenTG [German Gene Technology Act]
III.1.1. Requirements for approval according to section 16 (1) Nr. 1 GenTG
III.1.2. Requirements for approval according to section 16 (1) Nr. 3 GenTG
III.1.3. Requirements for approval according to section 16 (1) Nr. 2 GenTG
III.1.4. Formal requirements according to section 16 (4, 5) GenTG
III.2 Appraisal of and reply to objections
IV. Costs
V. Legal instruction

Only the original German document is legally binding. The following passage is a courtesy translation of the chapter III.1.2. and was prepared for the Biosafety Clearing-House.

III.1.2.1 Evaluation of changes in the genetically modified plants effected by the transferred nucleic acid sequences

(a) The *A1* gene

The active gene product is a dihydroflavonol 4-reductase which converts the dihydrokaempferol that has accumulated due to a mutation in the anthocyanin metabolism into leucopelargonidin, thereby leading to salmon-red coloured blossoms (pelargonidin). The enzymes and substrates of this metabolism are widespread in nature.

After the end of the trial, the genetically modified plants will be mechanically destroyed and shallowly incorporated into the soil. They are not intended for human or animal consumption. Even in the event of unintentional consumption by animals or humans, no adverse health effects would be expected.

(b) The *nptII* gene

The *nptII* gene transferred to the genetically modified plants encodes the enzyme neomycin phosphotransferase. It was inserted as a marker gene for the selection of transformed plant cells.

The neomycin phosphotransferase is a type II aminoglycoside 3'-phosphotransferase (APH(3')II) which catalyses ATP-dependent phosphorylation of the 3'-OH group of the aminohexose ring of specific aminoglycoside antibiotics, causing these to become inactivated. The enzyme is characterised by its high substrate specificity. The antibiotics kanamycin, neomycin, geneticin, butirosin, gentamicin A and B as well as paromomycin belong to the APH(3')II enzyme substrates. The clinically relevant antibiotic gentamicin and other aminoglycosides and aminocyclitoles used in human medicine do not belong to the substrate spectrum of the APH(3')II enzyme. Kanamycin and neomycin are, however, widely used in veterinary medicine. Due to the substrate specificity of neomycin phosphotransferase, in the absence of substrate under field conditions no new metabolic products are expected to arise in the genetically modified petunias. Since high concentrations of the relevant antibiotics are not present in the soil, the neomycin phosphotransferase does not confer any selective advantage to the genetically modified plants under field conditions. There is no evidence to suggest that this enzyme is toxic to plants, animals, microorganisms or humans.

(c) Other DNA fragments which may have been transferred

The genetically modified petunias were created by transformation with the vector p35*A1*. This vector also contains the fragment from pBR322 required for bacterial replication and selection. It contains an origin of replication and the gene for ampicillin resistance, but both with deletions. This sequence does not code for any gene product that is functional in plants.

(d) Deletion

Roughly 200 base pairs of the recipient genome are deleted at the site of integration. There is no evidence to indicate that this may be associated with functional changes or risks. Integrated into the genome, the genetically modified plants contain the following regulatory sequences:

- the 35S promoter and terminator of Cauliflower mosaic virus (CaMV),
- the promoter of the nopaline synthase gene from *Agrobacterium tumefaciens*,
- the termination region of the octopine synthase gene from *A. tumefaciens*.

The promoter and termination sequences regulate the expression of the cDNAs located between them which, in the genetically modified plants, encode an enzyme for anthocyanin metabolism from maize

and for the *nptII* gene. Further information on the effects of the expression of these sequences in the plants can be found under III.1.2.1.(a) to (b).

(e) Position effects and context changes; allergenicity

Genes which have been integrated into the plant genome by genetic engineering methods are expressed at different levels, depending on the site of insertion on the chromosome, and on the environment at the site of insertion (position effect). Under field conditions, the level of expression may be additionally influenced by environmental factors, for instance by temperature. In the present case this could mean that the traits of the genetically modified petunia plants might not be altered to the same extent under field conditions as under greenhouse conditions. Experience gained from previous deliberate release experiments can be summarised as follows: “The activity of the *A1* gene is subject to fluctuations due to internal circumstances (e.g. age) and to external circumstances (e.g. abiotic factors). It is regulated through methylation.” This is not expected to pose any risks to the environment or to human or animal health.

The insertion of foreign genes may influence the expression or regulation of the plant’s own genes at or near the site of insertion. Such processes may alter plant metabolic pathways. However, during the course of the work carried out to date with the genetically modified plants in the greenhouse and in the field, no observations were made that would indicate such an event. Mobile genetic elements (transposable elements), which when transposed within the genome can exert effects on existing plant genes at the target site, occur naturally in petunias. The inactivation of genes or alterations in gene regulation also take place in a range of other naturally occurring processes, e.g. point mutations, deletions or translocations, and are traditionally used in plant breeding. Therefore, even in non-genetically modified plants such events can always have an effect on plant metabolic pathways. In this respect the genetically modified plants proposed for release here do not differ fundamentally in those characteristics from non-genetically modified plants.

Given the current state of knowledge, it is not possible to make reliable predictions about the possible allergenic action of a protein on the basis of its amino acid sequence. However, in previous experiments with the genetically modified plants, as well as in deliberate release trials carried out in other countries with plants which express the *nptII* gene under the control of non-tissue-specific promoters, no evidence of increased allergenicity of the plants was found.

In any case, the pollen of petunias is dispersed only to a limited extent by wind and plays no role in triggering pollen allergies.

III.1.2.2. Evaluation of the ability of the genetically modified plants to persist or become established in the environment

The petunia has long been cultivated in Central Europe as a popular balcony plant. During this time, establishment of petunia in natural ecosystems in Europe has not been observed. Because “petunia seeds are very sensitive to moist cold”, as tested by the applicant in a careful experimental arrangement, their permanent establishment does not occur anywhere. In previous deliberate releases, genetically modified petunias with the *A1* gene exhibited a higher susceptibility to fungal pathogens than the commercial varieties.

After the end of the trial, the genetically modified petunias will be worked into the soil, as planned. In the following growing season, plants are to be cultivated which enable post-trial monitoring for potentially emerging petunias. During this time, any regrowth is to be eliminated. However, according to the applicant's systematic experiences, the likelihood that the genetically modified plants will establish after the end of the trial as a result of seeds potentially remaining in the soil is very low. Any establishment of these plants can be ruled out.
For the reasons stated above, no establishment or uncontrolled persistence of the genetically modified plants is expected.

III.1.2.3. Assessment of the possibility of a pollen-mediated transfer of the inserted genes from the genetically modified plants to other plants

Unlike all other *Solanaceae* (n=12), petunia has a chromosome set of n=7. This explains why it is not cross-compatible with other members of the *Solanaceae* family. The applicant has tested a range of *Solanaceae* as possible crossing partners without any success. The pollen of petunia is transferred by moths and possibly some other insects. Apart from the control plants, no potential crossing partners are present on the release site. Therefore, the likelihood of outcrossing of the transgenes is considered negligible.

III.1.2.4. Assessment of the possibility of transfer of the inserted foreign genes from the genetically modified plants to microorganisms by horizontal gene transfer

The inserted sequences are stably integrated into the chromosomes of the recipient organisms. Studies on the transformation ability of soil bacteria under natural conditions suggest that the transfer of plant genetic material to soil bacteria is, in principle, possible, although it is assumed that a gene transfer of this nature would constitute an extremely rare event. With regard to this question, the applicant has conducted systematic analyses, the results of which can be summarised as follows:
The DNA of the transgenes was not detectable in the soil for long: Of the 400 soil samples taken over a period of two years, DNA fragments of genetically modified plants were detected in only four cases - during the petunia trial and during the first two months after the plants had been ploughed under. No evidence of gene transfer in soil bacteria was found.
Insofar as we assume that an exchange of genetic material between organisms which are so distantly related in terms of taxonomy as plants and bacteria actually occurs, it follows that the occurrence of an exchange of heterologous genetic material does not in itself represent a safety criterion, since such an exchange could always result in the uptake of all forms of heterologous genetic material, including all forms of plant DNA. The gene for an enzyme of anthocyanin synthesis derives from maize and is frequently found in the environment. Therefore, it could also – with a far higher probability – enter microorganisms the environment via horizontal gene transfer from non-genetically modified organisms.
As already elaborated under III.1.2.1.(b), the antibiotics inactivated by the neomycin-phosphotransferase are of little relevance in human medicine but are widely used in veterinary medicine. Therefore, it was necessary to examine whether a potential horizontal gene transfer of the *nptII* gene would affect the therapeutic use of the relevant antibiotics.

The inactivation of aminoglycoside antibiotics by phosphorylation has been demonstrated as a natural resistance mechanism in microorganisms in a range of different environments. APH(3')II enzymes have also been found in human clinical isolates. The prevalence of genes which confer resistance to aminoglycoside antibiotics can be explained by the frequent application of these antibiotics, and by the fact that these genes are often located on plasmids, enabling their effective transfer between microorganisms by conjugation. Even in the event of a horizontal gene transfer from the genetically modified petunias to microorganisms, the overall frequency of this resistance mechanism would not be noticeably increased.

The genetically modified petunias probably contain an origin of replication and an ampicillin resistance from *E. coli*. These sequences are completely or partially deleted and are therefore non-functional. In general, also for these sequences, the likelihood of a spread by transfer between bacteria is far greater than the likelihood of a spread by horizontal gene transfer from the genetically modified plants to microorganisms.

Even in the event of a transfer of the other regulatory sequences used in the construct, there are no grounds for concern that the overall frequency of the corresponding DNA fragments would increase. These regulatory sequences derive from *A. tumefaciens* and CaMV.

A. tumefaciens is widespread in soils and the specified sequences are found in wild-type agrobacteria on Ti plasmids, which can be exchanged via conjugation. Regarding a horizontal gene transfer of these sequences to microorganisms, it should be noted that a transfer of the relevant sequences from *Agrobacterium* is far more likely than their transfer from the genetically modified plants.

The theoretical possibility of a transfer of the CaMV sequences from the genetically modified plants would not constitute a new situation compared to the naturally occurring situation because CaMV, as a double-stranded plant-infecting DNA virus, is already present in plants.