Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0203

Summary of the risk assessment of genetically modified petunias

(*Petunia hybrida*) T16

carried out by the German Competent Authority

within the framework of a proposed deliberate release

Berlin, 27 July 2009

Explanatory note to this document:

The following text is a summary of the risk assessment of genetically modified organisms intended for use in an experimental field trial (deliberate release) in Germany. The text forms part of the official authorisation issued in response to an application for the deliberate release of genetically modified organisms in Germany in accordance with Directive 2001/18/EC and the German Genetic Engineering Act (Gentechnikgesetz, GenTG). The authorisation was issued by the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL [Federal Office of Consumer Protection and Food Safety], as the German Competent Authority under the law on genetic engineering, and comprises the chapters:

I. Authorisation

II. Provisions

III. Justification

III.1. Authorisation requirements according to § 16 GenTG [German Genetic Engineering Act]

III.1.1. Authorisation requirements according to § 16 (1) No. 1 GenTG

III.1.2. Authorisation requirements according to § 16 (1) No. 3 GenTG

III.1.3. Authorisation requirements according to § 16 (1) No. 2 GenTG

III.1.4. Authorisation requirements according to § 16 (4, 5) GenTG

III.2 Appraisal of and response to objections

IV. Costs

V. Legal instruction

Only the original German authorisation document is legally binding. The following extract is a courtesy translation of chapter III.1.2., prepared for the Biosafety Clearing House.

Dienstsitz Braunschweig
Bundesallee 50, Geb. 247
38116 Braunschweig
Tel: +49 (0)531 21497-0
Fax: +49 (0)531 21497-299

Abt. Pflanzenschutzmittel
Messeweg 11/12
38104 Braunschweig
Tel: +49 (0)531 299-5
Fax: +49 (0)531 299-3002

Dienstsitz Berlin
Mauerstraße 39-42
10117 Berlin
Tel: +49 (0)30 18444-000
Fax: +49 (0)30 18444-89999

Referatsgr. Untersuchungen
Diedersdorfer Weg 1
12277 Berlin
Tel: +49 (0)30 18412-0
Fax: +49 (0)30 18412-2955

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

(a) The *aadA* gene

The *aadA* gene [*ant*(3´´)-Ia; Strep^R/Spec^R] which was transferred to the genetically modified (GM) plants originates from the plasmid R538-1 from *E. coli*. This gene encodes an aminoglycoside adenyltransferase which modifies the 3”-hydroxyl position of streptomycin and the 9-hydroxyl position of spectinomycin. The transferred *aadA* gene is controlled by the 16S rrn promoter from *B. napus* and the ribosome-binding site of the *N. tabacum rbcL* gene, as well as the terminator of the *psbC* gene from *B. napus*.

The *aadA* gene confers resistance to streptomycin and spectinomycin. The presence of the *aadA* gene has been demonstrated in numerous bacteria in different media such as soil, waste water, seawater, foodstuffs, clinical specimens and faeces. Bacteria which are resistant to streptomycin/spectinomycin are widespread in the environment. Therefore, resistance to these antibiotics can also be spread through horizontal gene transfer from non-GM microorganisms.

These antibiotics have only limited uses in human medicine, but they are still therapeutically relevant in the treatment of tuberculosis (streptomycin) and gonorrhoea (spectinomycin) when drugs with less toxic potential cannot be applied.

The GM petunias are only to be released on a limited area for a specified period. These plants may not be used in the production of feed or food. Given the very low probability of horizontal gene transfer from plant DNA to microorganisms and the absence of selection pressure on the release sites, the presence of the *aadA* gene in the GM petunia plants is not expected to lead to a significant increase in the overall frequency of this resistance mechanism in microorganisms.

In its statement of December 2008 concerning the *aadA* gene the Central Committee on Biological Safety (ZKBS) determined that in view of the improbability of horizontal gene transfer between plants and microorganisms and the already existing distribution of the *aadA* gene in the environment, the presence of the *aadA* gene in the genome of GM plants will not have any effect on the spread of this antibiotic resistance gene in the environment.

In its opinion of 26 March 2009 on the use of antibiotic resistance genes as marker genes in GM plants, the GMO Panel of the European Food Safety Authority (EFSA) found that, based on the current state of knowledge, the transfer of *aadA* gene from plants to bacteria is not expected to have adverse effects on human health or the environment.

Expression of the *aadA* gene from *E. coli* in plants is not expected to result in a selective advantage, since the plants are unlikely to be exposed to streptomycin or spectinomycin in agricultural or natural ecosystems.

(b) The *uidA* gene

The *uidA* gene from the bacterium *E. coli,* which was transferred to the GM plants*,* encodes the enzyme β-glucuronidase. The *uidA* gene is controlled by the 16S *rrn* promoter and the ribosomal binding site of the *rbcL* gene from *N. tabacum* as well as the terminator of the *psbA* gene from *N. tabacum*. The β-glucuronidase enzyme splits glucuronide and is found in tissues of vertebrates and invertebrates and in bacteria. After adding the corresponding substrate, enzyme activity can be demonstrated in the GM tissue by colour reaction. The *uidA* gene was inserted as a marker gene for the identification of transformed plant cells.

Expression of the *uidA* gene from *E. coli* is not expected to confer a selective advantage to the plants, since there is no evidence that the β-glucuronidase enzyme might play a role in plant metabolic pathways that influence persistence or dispersal abilities.

(c) Additional elements located on the transferred DNA segment

In addition to the above-mentioned genes, the plasmid used to transform the petunia plants also contains the necessary expression regulation sequences within the DNA segment intended for homologous recombination. These are the 16S rrn promoters from tobacco and oilseed rape, as well as the ribosomal binding site of the *rbcL* gene from tobacco and the terminators of the *psbC* and *psbA* genes from oilseed rape and tobacco, respectively. These are non-coding sequences and they regulate expression of the DNA sequences located between them in the GM plants. More complex functions are not known and additional effects on the GM plants are not expected.

(d) Sequences located outside the DNA segment intended for homologous recombination

Transformation of the T16 petunias took place by particle bombardment, whereby the plasmid pUM73(AD) was used to coat the particles. A possible transfer of additional DNA sequences from this plasmid cannot be completely ruled out.

The transformation plasmid pUM73(AD) is a derivative of the vector pBR322 and contains the following genetic elements outside the DNA segment intended for homologous recombination:

- the tetracycline resistance gene *tet*A
- the ampicillin resistance gene *bla*$_{TEM-1}$
- the replication start point *ori*
- repression of plasmid replication *rop* gene

The sequences intended for homologous recombination comprise the following elements:

- the gene for the ε– and β-subunits of ATP-synthase from tobacco *atp* ε *-atp*β
- the gene for the large subunit of ribulosebiphosphate carboxylase from tobacco *rbcL*
- the gene for a subunit of acetyl-CoA carboxylase from tobacco *accD*

Transfer by simple crossover of fragments of the plasmid pUM73(AD) located outside the homologous sequeneces of the *rbcL* and *accD* genes was ruled out by Southern blot analyses. At the same time it was shown that the T16 petunia is homoplasmic.

Thus it is most likely that the vector fragments *atp* ε *-atp*β, *tet*A, *bla*$_{TEM-1,}$ *ori* and *rop* were not transferred to the DNA plastids of the petunia T16.

The applicant provided PCR analyses to prove that neither the bacterial ampicillin resistance gene *bla*$_{TEM-1}$ nor the tetracycline resistance gene *tet*A were transferred into the nuclear genome of the plant. These analyses showed that the transformants proposed for release contain neither a complete *bla*$_{TEM-1}$ gene nor a complete *tet*A gene.

Tests to prove the presence or absence of the remaining vector plasmid sequences in the GM plants were not carried out. As a result, the risk assessment is performed on the assumption that these sequences may be contained in the plants.

The aforementioned segments located outside the region for homologous recombination regulate expression in bacteria and have no function in plants. The formation of significant amounts of functional gene products based on these sequences is not anticipated in the GM plants, since they are not driven by plant-specific promoters nor are they adapted to plant codon usage.

(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 integration on the chromosome and on the nucleotide sequences neighbouring the integration site (“position effect”). Under field conditions the level of expression may be influenced by environmental factors, for instance, by temperature. In this particular case this could mean that the characteristics of the GM petunia plants are not modified to the same degree in the field as under climate-chamber

or greenhouse conditions. This is not expected to pose a risk to the environment or to human or animal health.

The insertion of foreign genes may influence the expression or regulation of native plant 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 on the GM plants no observations were made that would suggest 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 plants. The inactivation of genes or changes in gene regulation also take place in a range of other naturally occurring processes such as point mutations, deletions or translocations and are traditionally used in plant breeding. Therefore, even in non-GM plants such events can always influence plant metabolic pathways. With regard to these properties the GM plants do not differ fundamentally from non-GM plants.

Given the current state of knowledge, it is impossible to make reliable predictions about the possible allergenic action of a protein on the basis of its amino acid sequence. Since the GM plants are not intended for use in food or feed and are to be cultivated on a limited area only, a potential allergenic effect is not expected to pose risks.

III.1.2.2. Evaluation of the ability of the GM plants to persist or establish in the environment

Petunias are not winter-hardy. Despite isolated reports of their survival through the winter period, naturalisation of petunias has not been observed in Central Europe. In trials aimed at increasing frost tolerance in petunias, frost tolerance of -4°C in the wild type was slightly increased to -6°C to -8°C in GM petunias. Therefore a substantial increase in frost tolerance due to unexpected effects resulting from the genetic modification of the petunias proposed for release here is considered unlikely.

Trials conducted by the Max Planck Institute for Plant Breeding Research also demonstrated that after being incubated in a wet medium with frost temperatures below -4°C petunia seeds were no longer capable of germination.

Furthermore, from observations made during field trials conducted by the Max Planck Institute for Plant Breeding Research we know that after ploughing the soil in which petunias had been cultivated, regardless of whether this took place before or after the first frost, germinating petunias were not observed in the following year.

Given the characteristics mentioned above and taking the genetic modification into consideration, petunias are not expected to have the capacity to persist or establish on these sites. However, should petunias emerge on the release sites from overwintered seeds following a

mild winter, these would be identified and destroyed during the course of post-trial monitoring.

This would not entail hazards to human health or the environment.

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

The pollen from petunias is dispersed mainly by insects, in particular moths, and possibly some other insects. Wild varieties of the genus and other wild-growing crossing partners are not known in Europe.

Therefore, it can be assumed that the possibility of unintentional outcrossing only exists for cultivated petunias. In trials, the Max Planck Institute for Plant Breeding investigated outcrossing from GM petunias to directly neighbouring recipient plants, whereby a very low rate of incrossing from neighbouring stocks was demonstrated. The likelihood that the genetically modified characteristic would be spread by pollen transfer is further minimised by the fact that the genetic modification is localised in the plastid genome of the plant. In the *Solanaceae* family, inheritance of the plastid genome occurs exclusively by maternal inheritance.

Apart from the recipient plants no other potential crossing partners are found on the release site. According to the applicant the minimum distance to petunias cultivated outside the release site is 100 m. The next horticultural farm is situated approximately 5 km from the site of the proposed trial. Since this farm grows petunias from seeds and cuttings but does not propagate or cultivate seeds (seed production), the spread of potential crossing products is not expected.

In view of the low probability of crossing in petunias coupled with the low probability of pollen-mediated transfer of genetically modified plastids, the likelihood that the genetically engineered trait would be transferred to petunias outside the experimental facility is considered negligible.

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

The inserted sequences are stably integrated into the plastid genome of the recipient organisms. There is no evidence to suggest that a transfer of genetic information from plants and its expression in microorganisms takes place under natural conditions. Studies on the transformation capacity of soil bacteria under natural conditions do however suggest that a trans-

fer of plant genetic material to soil microorganisms is theoretically possible, although it is assumed that such a gene transfer would constitute an extremely rare event.

Insofar as we assume that a genetic exchange between organisms that are so distantly related in terms of taxonomy as plants and microorganisms is actually possible, it can be concluded 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.

(a) The *aadA* gene

In the opinion of the Central Committee on Biological Safety (ZKBS) from December 2008, all antibiotic resistance marker genes are to be included in the safety assessment of GM plants, without distinguishing between different groups. At the same time, the safety assessment incorporated new scientific findings. This led to the conclusion that the impact of such horizontal gene transfer events, if they do take place, is negligible when compared to the natural processes of transfer and re-emergence of these resistance genes in the existing community of microorganisms.

The scientific opinion of the European Food Safety Authority (EFSA) comes to the conclusion that, based on the current state of knowledge, the transfer of the *aadA* gene from plants to bacteria is not expected to result in adverse effects on human health or the environment.

Because of the widespread distribution of the *aadA* gene in microorganisms, even if horizontal gene transfer were to occur, this would not be expected to result in a discernible increase in the overall frequency of the gene.

(b) The *uidA* gene

The *uidA*-Gen, which originates from *E. coli*, is a component of the transformation plasmid pUM73(AD) and can be used as a reporter gene. The β-glucuronidase enzyme splits glucuronide and is found in tissues of vertebrates and invertebrates and also in bacteria. Plants also exhibit low levels of endogenous β-glucuronidase activity. Glucuronidases are widespread in microorganisms, so that even in the case of a horizontal gene transfer no discernible increase in the overall freqeuncy of the gene would occur.

(c) Additional genetic elements of the transformation vector pUM73(AD)

The complete transfer of the genes for resistance to tetracycline (tetA) and ampicillin (bla_{TEM-1}) localised on the transformation vector pUM73(AD) was ruled out by PCR analysis. In any

case the tetracycline resistance gene on the transformation vector pUM73(AD) is non-functional, since it was interrupted by the insertion of a DNA segment. Therefore, if parts of the antibiotic resistance genes were actually transferred into the nuclear genome of the T16 petunia it can be assumed that they would not be functional.

In its opinion of December 2008 the ZKBS also established that in view of the improbability of a horizontal gene transfer between plants and microorganisms and the existing distribution of these genes in the environment the presence of these genes in the genome of the GM plants would not have an effect on the distribution of these antibiotic resistance genes in the environment.

Transfer of the genetic elements *ori, rop, atpε -atpβ, rbcL* and *accD*, which are located on the transformation vector pUM73(AD) cannot be ruled out. In the case of the origin of replication *(ori)* for the replication of the plasmid and the *rop* gene for regulation of plasmid replication in *E. coli*, the likelihood of spreading by transfer between bacteria is much greater than the likelihood of spreading by horizontal gene transfer from the genetically modified plants to microorganisms.

The genes for the ε– and β-subunits of the synthase *atpε -atpβ,* the large subunit of ribulose-bisphosphate carboxylase *rbcL* and a subunit of acetyl-CoA carboxylase *accD* originate from tobacco and, moreover, only occur in slightly modified form in many higher plants.

Even in the case of a transfer of these genes, there is no reason to fear a significant increase in the overall frequency of the corresponding DNA sequences. These genes are derived from plants and microorganisms that are widespread in the environment. A gene transfer of this type is unlikely to have consequences for ecosystem processes.