Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0166

Summary of the risk assessment of the genetically modified potato (Solanum tuberosum: Baltica) within the framework of a proposed deliberate release carried out by the German Competent Authority

Berlin, 30. May 2006

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]
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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.

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

(a) The zeaxanthin epoxidase gene from *S. tuberosum*

The cDNA fragment of zeaxanthin epoxidase transferred to the genetically modified potato plants is expressed primarily in the potato tuber under the control of the tuber-specific GBSS promoter of the granule-bound starch synthase gene of potato and the terminator sequence of the nopaline synthase gene from *A. tumefaciens*. The gene fragment was transferred in sense or antisense orientation.

In plants and animals xanthophylls act as buffers against excessive light irradiation. The xanthophylls that come into play here - violaxanthin and zeaxanthin - are also synthesised in potato tubers. Zeaxanthin, however, is immediately converted to violaxanthin by the zeaxanthin epoxidase present in the tubers. Inhibition of zeaxanthin epoxidase leads to a build-up of zeaxanthin, a parent compound for the synthesis of carotinoids.

In one line, expression of the inserted gene fragment occurs in antisense orientation. This causes the formation of an antisense RNA in the GM plants which inactivates the endogenous transcript of the gene, thus inhibiting the formation of the corresponding enzyme.
In another line, the expression of the inserted gene fragment occurs in sense orientation. This usually leads to an increase in the transcript level of the specific gene. If, however, the transferred gene displays homology to an endogenous gene, mutual inactivation of both the foreign and the endogenous gene can result. This effect is known as “homology-dependent gene silencing“, “cosuppression“ or “sense-suppression”.

In both approaches, the genetic modification leads to a reduction in the synthesis of endogenous zeaxanthin epoxidase. Northern blot analyses show that zeaxanthin epoxidase mRNA is considerably reduced in the tubers of the GM potatoes, whereas in the leaves only a moderate reduction is observed. The genetic modification provokes a shift in the metabolic equilibrium in the tubers from violaxanthin to zeaxanthin. Since violaxanthin is necessary for the biosynthesis of the plant hormone abscisic acid - a phytohormone that controls various physiological processes in the plant (seed maturation, drought tolerance, leaf wilt) - a drop in the production of this hormone can be expected. Therefore, a shift in metabolic concentrations in other metabolic pathways cannot be ruled out. In previous greenhouse and field trials, the applicant observed no phenotypic differences to non-GM potatoes, apart from a variation in tuber colour. It can thus be assumed that the genetic modification leads to alterations in the concentration of various endogenous plant substances.

Since fluctuations in the zeaxanthin content and the contents of other violaxanthins occur naturally, the modification of the GM plants conducted in the course of the proposed deliberate release is not expected to pose any risks to human or animal health, or to the environment.

(b) The *npt*II gene

The *npt*II gene codes for a neomycin phosphotransferase. It was transferred to the genetically modified plants as a marker gene for selecting transformed plant cells.

The neomycin phosphotransferase gene is a type II aminoglycoside 3'-phosphotransferase (APH(3')II), which catalyses the 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, and paromomycin belong to the APH(3')II enzyme substrates. Clinically relevant gentamicins 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 widely used in veterinary medicine. The enzyme is non-toxic for plants, animals, microorganisms and humans.

(c) Border sequences fromTi plasmids and regulation sequences

The GM plants contain sequences of the left and right border regions of the T-DNA of the plasmid pTiT37 from *A. tumefaciens*. These sequences, in conjunction with the gene products of the *vir* region of the helper plasmid present in the *Agrobacterium* strain LBA4404 which was used in the transformation but was not transferred to the plants, are responsible for integrating the genes located between the border regions into potato plant chromosomes. The border regions of the Ti plasmid are non-functional in the GM plants and are not expected to effect any changes in the plants.

The following regulation sequences have been integrated into the genome of the GM plants:

- the GBSS promoter of the granule-bound starch synthase gene from *S. tuberosum*,
- the promoter and terminator of the nopaline synthase gene (*nos*) from *A. tumefaciens*.

These promoter and termination sequences control the 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. (cf. III.1.2.4.)

(d) Sequences located outside the T-DNA

As a rule only DNA located within the border regions is integrated into the plant genome in Agrobacterium-mediated transformation events. However, the transfer of DNA fragments outside the borders has been documented in the literature.

The vector pPGB121S used to produce the GM plants is a derivate of the vector pBIN19 and contains the *npt*III gene outside the T-DNA border regions. This gene codes for an aminoglycoside-3'-phosphotransferase type III that confers resistance to aminoglycoside antibiotics. Based on the PCR and Southern blot investigations cited in the application, it can be assumed that neither a functional *npt*III gene nor the *IS1* transposon integrated into the non-functional part were transferred into the genomes of the GM potato lines.

Furthermore, as a result of the integration of other DNA fragments of vector pBIN19 located outside the border regions, the following may have been transferred to the GM potato plants:

(i) the replication origin *oriV* of the plasmid RK2,
(ii) the *traF* region, which contains the *oriT* of the plasmid RK2,
(iii) the *trfA* locus of the plasmid RK2 (codes for two proteins required for replication of the plasmid),
(iv) a non-functional fragment of the *kilA* gene from the plasmid RK2,
(v) the *tetA* gene of the plasmid RK2 (interrupted by insertion of the T-DNA-region),
(vi) the replication origin of the plasmid pMB1 (similar to *Col* E1).

The replication origins *oriV* (i) and *oriT* (ii) of the plasmid RK2 facilitate the replication of the plasmid in a broad host range of gram negative bacteria or its transfer by conjugation, provided the mobilisation functions are supplied by a helper plasmid. There is no evidence to suggest that the replication origins of RK2, the replication origin of pMB1 (vi) or the other DNA fragments of bacterial origin (iii, iv, v, v) have a function in higher plants. Moreover, some of the DNA fragments are incomplete (iv) or interrupted (v).

(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 genetically modified potato 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 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 on the GM plants under greenhouse conditions and within the scope of the field trial 6786-01-0135, 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 and were first detected in maize. 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-genetically modified plants such events can always influence plant metabolic pathways. With regard to these properties the genetically modified plants do not differ fundamentally 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 the amino acid sequence. Nevertheless, none of the trials conducted so far with GM plants or in the numerous deliberate releases of plants, which express the *npt*II gene under the control of non-tissue specific promoters, indicate an increased allergenicity in the plants. The pollen of potato plants is only dispersed over short distances by wind and generally plays a negligible role in triggering pollen allergies.

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

The cultivation of potatoes in Central Europe goes back several hundred years. In Europe the establishment of potatoes in natural ecosystems during this period has not been observed. From time to time potato plants are found beyond cultivated areas, but only on non-natural sites such as verges and other ruderal areas. Owing to a lack of frost hardiness potato does not establish in these areas either. As a result of potato cultivation, volunteer potatoes can, depending on winter temperatures, emerge in the subsequent cultivation period from seeds or tubers that have overwintered in the soil.

The tubers of the genetically modified potato plants will be analysed after harvesting and the berries will also be gathered for analysis.

The leaves and stalks of the potato plants will be incinerated and surplus tuber material will be shredded and spread over the release site where it is left to biodegrade. After analysis residual potatoes will be inactivated by autoclaving. In the vegetation period following the proposed deliberate release, potatoes will not be cultivated on the former release site. Volunteers will also be identified and destroyed during the post-trial period.

Potato plants of the varieties used can flower and bear fruit. However, under Central European climate conditions there is unlikely that potato seeds will overwinter and produce plants. In the event that tubers or seeds remain in the soil, the resulting plant growth would be detected during post-trial monitoring. The berries are gathered in order to minimize the risk of seeds entering the soil. In previous greenhouse and deliberate releases carried out by the applicant, the phenotype of the GM plants did not deviate from the corresponding control plants, except in the colour of the tuber flesh. However, a possible change in the dormancy (premature germination) of the tubers due to the genetic modifications cannot be ruled out. This possibility is adequately addressed by the planned cultivation gap of one year and the post-trial monitoring. Wheat will be planted on the monitored sites, making it easy to locate potential volunteer potatoes.

For the reasons stated above, the genetically modified plants are not expected to persist or establish in the environment.

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

Attempts to crossbreed potatoes with solanaceous plants found in Central Europe were unsuccessful. Under field conditions no incrossing took place from genetically modified potatoes to *Solanum nigrum* (black nightshade). The artificial transfer of pollen to *S. nigrum* also failed to produce viable seeds. Only under conditions that do not occur naturally and with the help of artificial methods (embryo rescue) was it possible to regenerate a small number of hybrids. These, however, turned out to be sterile. The potato and *Solanum dulcamara* (bittersweet or woody nightshade) proved to be strictly bilaterally incompatible; in crossbreeding experiments pollination of the ovule was not achieved. Similarly, the potato does not crossbreed with the tomato (*Lycopersicon esculentum*). In agricultural practice, potatoes are propagated vegetatively via tubers.

The following passage, therefore, deals only with a possible pollen transfer from the genetically modified potato plants to other potatoes .The pollen of the potato plant can be transferred by insects or by wind. However, wind dispersal only takes place over short distances. A transfer is most likely to occur between neighbouring plants.

The proposed isolation distance of at least 30 metres as well as a 15-metre strip of a trap crop of mustard plants between the release sites and other cultivation sites with non-GM potatoes is considered adequate for the purposes of the proposed trial. However, should pollen be transferred to potato plants cultivated to produce table potatoes, no adverse effects are to be expected, since in an agricultural environment potato plants are propagated vegetatively, i.e. not via seeds. As elaborated above, the probability that potentially generated seeds could give rise to plants under the given climatic conditions is very slight. In agricultural areas such plants would be eliminated in the course of conventional soil preparation practices. Even if the tubers of these plants were to be consumed, no health hazards would be expected to result - as stated in the evaluation summarised in III.1.2.1.

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. There is no evidence that the transfer of genetic information from plants or its expression in microorganisms takes place under natural conditions. However, studies on the transformation ability of soil bacteria under natural conditions suggest that the transfer of plant genetic material to soil bacteria is also theoretically possible, although it is assumed that a gene transfer of this type would constitute an extremely rare event.

Insofar as we assume that an exchange of genetic material between organisms which are so distantly related in terms of taxonomy is actually possible, it could 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 cDNA for the gene of zeaxanthin epoxidase from *S. tuberosum*

The inserted cDNAs of the gene for the zeaxanthin epoxidase and their regulatory sequences are derived from *S. tuberosum* and *A. tumefaciens*. These are already commonly found in the environment and therefore there is a far greater probability of horizontal gene transfer from non-GM organisms to microorganisms.

(b) The *npt*II gene

In the genetically modified plants the *npt*II gene is under the control of the *nos* promoter. This gene codes for the enzyme aminoglycoside 3'-phosphotransferase II (APH(3')II), which catalyses the ATP-dependent phosphorylation of certain aminoglycoside antibiotics (kanamycin, neomycin, geneticin), causing their inactivation.

As already elaborated in III.1.2.1. (b), the antibiotics inactivated by the aminoglycoside 3'-phosphotransferase II are of little relevance in human medicine but are widely used in veterinary medicine. It was thus necessary to examine whether the clinical use of the relevant antibiotics would be affected by a potential horizontal gene transfer of the *npt*II gene.

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 the effective transfer between microorganisms by conjugation. Even in the event of a horizontal gene transfer from the genetically modified potatoes to microorganisms, the overall frequency of this resistance mechanism would not be noticeably increased.

The Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) has allocated the *npt*II gene to the group of genes which, in terms of safety, provide no grounds to restrict or ban their usage, either for field trials or for the purpose of placing on the market. In its statement of 6.7.1999 on the biological safety of antibiotic resistance genes in the genome of genetically modified plants, the Central Committee on Biosafety (ZKBS) allocated the *npt*II gene to the group of antibiotic resistant genes which “(a) are already widespread in soil and enterobacteria and (b) whose relevant antibiotics have no, or only little significance in human and veterinary medicine, so that one can assume that the presence - if any - of these antibiotic resistance genes in the genome of transgenic plants will have no effect on the spread of these antibiotic resistance genes in the environment”.

(c) Sequences located outside the T-DNA

As a rule only DNA located within the border regions is integrated into the plant genome in Agrobacterium-mediated transformation events. In the case of lines generated by pPGB121S-mediated transformation events, sequences located outside the border regions can also be integrated into the GM plants. Neither the functional antibiotic-resistance gene *npt*III nor the transposon *IS1,* which was integrated into the non-functional part, were found to be present in the GM potatoes planned for deliberate release.

In the present case the following DNA fragments may have been integrated into the GM potato plants by the transfer of sequences located outside the border regions:

(i) the replication origin *oriV* of the plasmid RK2,

(ii) the *traF* region, which contains the *oriT* of the plasmid RK2,

(iii) the *trfA* locus of the plasmid RK2 (codes for two proteins required for replication of the plasmid),

(iv) a non-functional fragment of the *kilA* gene from the plasmid RK2,

(v) the *tetA* gene of the plasmid RK2 (interrupted by insertion of the T-DNA-region),

(vi) the replication origin of the plasmid pMB1 (similar to *Col* E1).

RK2 belongs to a group of broad host range plasmids (incl. RP1, RP4, R18, R68), which can be replicated in a variety of gram negative bacteria. For DNA fragments deriving from RK2 (i to v), the probability of proliferation via transfer between bacteria is far higher than the probability of proliferation by horizontal gene transfer from genetically modified plants to microorganisms. Some of the DNA fragments are also interrupted (v) or incomplete (iv).

The pMB1 replicon (vi) belongs to the type of ColE1 plasmids, whose host range is restricted to certain gram negative bacteria. Basically, the replicon can replicate in *E. coli* and closely related species of bacteria, such as, for example, *Serratia* or *Salmonella*. Replication does not occur in most gram negative soil bacteria. ColE1 plasmids occur quite frequently in enterobacteria. A gene transfer from enterobacteria to other bacteria is far more likely than a horizontal gene transfer from genetically modified plants to bacteria. The potential presence of the replication origin of pMB1 in the plant chromosome is thus not expected to contribute to an increase in the overall frequency of horizontal gene transfer.