Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0191

Summary of the risk assessment carried out by the German competent authority on the genetically modified potato (*Solanum tuberosum*) with altered carbohydrate metabolism within the framework of a proposed deliberate release

Berlin, 31 March 2008

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) Fragments of the coding region of the potato starch synthase gene (granule-bound starch synthase, GBSS) in sense and antisense orientation (as an inverted repeat)

The fragment of the coding region of the potato starch synthase gene (granule-bound starch synthase, GBSS) in sense and antisense orientation (as an inverted repeat, plasmid pAP4) is expressed under the control of the potato's own *gbss* promoter primarily in the potato tuber. In these genetically modified plants, the formation of a double-stranded RNA causes inactivation of the endogenous transcript of the respective gene, thus preventing production of the GBSS enzyme.

Due to the decreased amount of GBSS protein, a starch with reduced amylose content is synthesised in the tubers. This reduced amylose content was determined by the applicant by staining the starch granules with iodine and by spectrophotometry.

The genetically modified potatoes harvested in the field trials are not intended for use in the production of foodstuffs or animal feed. Alteration of the starch composition of the genetically modified potato plants within the scope of the proposed experiments is not expected to pose any threat to human or animal health, or to the environment. No new proteins will be generated in the plant as a consequence of the genetic modification.

(b) The resistance genes *Rpi-blb1* and *Rpi-blb2* from *Solanum bulbocastanum* for improved resistance to *Phytophthora infestans*

Two resistance genes, *Rpi-blb1* and *Rpi-blb2,* were transferred from the wild potato *Solanum bulbocastanum* into the genetically modified potato lines. Expression of *Rpi-blb1* and *Rpi-blb2* in the genetically modified potatoes is regulated by their native promotors and terminators from *S. bulbocastanum*.

In contrast to conventional potatoes, the expression of the *Rpi-blb1* and *Rpi-blb2* genes in the genetically modified potatoes should confer increased resistance to *Phytophthora infestans*. *Rpi-blb1* and *Rpi-blb2* code for proteins of the NBS-LRR (nucleotide binding site-leucine rich repeat) type. NBS-LRR proteins are known to be present in food crops. No toxic or allergenic characteristics have been reported for these proteins.

Expression of *Rpi-blb1* and *Rpi-blb2* in the genetically modified potato lines was analysed by the applicant on the basis of RNA expression using real-time RT-PCR. Both genes exhibited very weak expression in the leaves, stems, flowers, tubers and roots, whereby the expression of both genes differed in the flowers, to the extent that *Rpi-blb1* expression was hardly detectable.

The target organism of the genetically modified potato lines is *P. infestans*, the potato blight pathogen. Expression of the resistance genes *Rpi-blb1* and *Rpi-blb2* is expected to significantly reduce the ability of *P. infestans* to infect the genetically modified potatoes. In previous experiments the applicant observed increased resistance to *P. infestans* in the fungus-resistant potato lines, whereby individual lines exhibited different levels of resistance. Disease resistance genes code for proteins which, through direct or indirect interaction, identify pathogen-specific avirulence factors and elicit a defence response in the plant via a signal cascade. No additional effects on non-target organisms are expected, other than those which would be expected to result from the interaction of non-genetically modified potatoes with non-target organisms.

The genetically modified potatoes are not intended for use in the production of foodstuffs or animal feed in the proposed field trials. Within the scope of the proposed experiments neither the expression of *Rpi-blb1* and *Rpi-blb2* from the wild potato *S. bulbocastanum* nor the altered resistance of the genetically modified potato plants to *P. infestans* are expected to pose risks to human or animal health or to the environment.

(c) The *ahas* gene

An *ahas* gene from an *Arabidopsis thaliana* mutant controlled by the *nos* promotor and the *nos* terminator from *Agrobacterium tumefaciens* was used for the selection of transformants. The *ahas* gene codes for the enzyme acetohydroxy acid synthase (AHAS), also known as acetolactate synthase (ALS), which catalyses the first steps in the biosynthesis pathways of the amino acids valine, leucine and isoleucine, i.e. the reaction of two pyruvate molecules to form 2-acetolactate and the reaction of pyruvate with 2-ketobutyrate to form 2-acetohdroxybutyrate.

AHAS is the target enzyme for various classes of herbicide agents, including sulfonylurea derivatives and imidazolinones. The effect of the herbicides is to disturb the biosynthesis of the branched-chain amino acids, causing the plant to die off.

A gene for an AHAS variant, which confers herbicide tolerance to the plants because of its reduced affinity for the herbicide agents, was isolated from an *A. thaliana* mutant. This variant differs from the wild-type AHAS by a single amino acid exchange (S653N, i.e. asparagine in place of serine in position 653).

In the genetically modified potato plants the herbicide-tolerant AHAS variant catalyses the same reactions as the corresponding endogenous enzymes in the potato. No new metabolic products are expected to result from the expression of the *ahas* gene derived from *A. thaliana* in the genetically modified potato. The transfer of this gene within the framework of the

planned field trials is not expected to have any adverse effects on the environment or on human health.

(d) Sequences located outside the T-DNA

As a general rule, only DNA located within the border regions is integrated into the plant genome in Agrobacteria-mediated transformation events. However, transfers of DNA fragments outside the border regions have been reported in the literature.

The genetically modified potato lines were obtained by transformation with the plasmids pAP4, VCPMA16 and VCPMA19. These plasmids contain the following outside the border regions:

- the *aadA* gene, which confers resistance to the antibiotics streptomycin and spectinomycin,
- the *bom* site from pBR322 for mobilisation of the plasmid from *Escherichia. coli* to *A. tumefaciens*,
- the origins of replication ColE1 and pVS1-repA for replication in *E. coli* or in *A. tumefaciens*, as well as the *sta* (stability) region from pVS1.

Using a primer/probe set at both the right and left border regions, real-time PCR showed that no plasmid sequences had been integrated outside either of the border regions in the genetically modified lines intended for release. The primer/probe set at the right border region is directed at an internal sequence of the *aadA* gene. It can be assumed that the above-mentioned sequences, in particular the *aadA* gene, are not contained in the genetically modified lines.

(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 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-controlled 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 these genetically modified 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 alterations 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. In this regard 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. The genetically modified potatoes are not intended for use as food or feed within the framework of the proposed release. The pollen of potato plants is only dispersed over short distances by wind and does not normally play a noteworthy role in triggering pollen allergies.

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

The cultivation of potatoes in Central Europe goes back several hundred years. In areas where potatoes have been cultivated, tubers or seeds may remain in the soil after harvesting. Depending on temperatures in the winter following cultivation, these may give rise to volunteer potato plants in the following year. In Europe the establishment of potatoes in natural ecosystems has not been observed, since potatoes compete poorly against wild plants and they are not frost resistant. From time to time potato plants are found beyond the cultivated areas, but only on non-natural sites such as verges and other ruderal areas. Owing to the lack of frost hardiness the cultivated potato does not establish in these areas either.

Tubers of the genetically modified trial plants will be mechanically or manually harvested, packed in sealed and marked containers, and transferred to the appropriate S1 laboratories for subsequent analyses or for storage. Surplus tuber material not intended for re-planting will be inactivated using appropriate methods, for example, steaming, autoclaving, incineration, shredding or fermentation in a biogas facility. The leaves and stalks of the potato plants will be left to decompose on the release site.

Potato plants can blossom and bear fruit. However, under Central European climate conditions there is little likelihood that potato seeds will overwinter and produce plants. Prior to harvesting, the parts of the potato plants growing above ground will be mechanically or chemically destroyed. This serves to counteract seed maturation.

In the event that tubers or seeds remain in the soil, the resulting plant growth would be detected during post-trial monitoring. The crop rotation is designed in such a way that potatoes are not cultivated on the individual release sites in the following year. In overwintering experiments post-trial monitoring will be conducted from May to December of the harvest year. If genetically modified potato plants do emerge from seeds or tubers not detected during harvesting, these can be identified and inactivated by conventional agricultural practices. In such cases post-trial monitoring is extended and the release site is controlled for volunteers for a further year. No plants, or only plants which do not interfere with monitoring, may be cultivated on the release sites during the post-trial monitoring period.

In previous experiments carried out by the applicant the genetically modified potato lines did not display any change in appearance. Apart from the intended aim of the respective genetic modification, no evidence of any significant differences between the genetically modified lines and their parent varieties emerged. Furthermore, a comparison with other conventional varieties did not reveal any differences beyond the known range of natural variability. One of the objectives of the proposed release is to carry out studies on the overwintering capability of the genetically modified potato tubers. For this purpose, selected parts of the experimental release crop will not be harvested in autumn; these tubers are to remain in the soil until the following spring.

Even if the genetic modification were to bring about a change in the frost sensitivity of the tubers, this would be adequately addressed by the designated cultivation gap for potatoes, by post-trial monitoring and by the planned isolation measures.

There are no grounds to assume that the genetically modified potato plants have different ecological traits compared to conventionally cultivated potatoes, nor are they expected to have the ability to colonise natural ecosystems. Therefore, even if the fruit, seeds or tubers of the genetically modified plants were to be dispersed by animals, the GM potato plants would not be expected to 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 cross-breed potatoes with solanaceous plants found in Central Europe were not successful. 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 bilaterally incompatible; in crossbreeding experi-

ments pollination of the ovule was not achieved. Similarly, the potato does not cross-breed with the tomato (*Lycopersicon esculentum*).

The following passage, therefore, deals only with a possible pollen transfer from the genetically modified potato plants to other potatoes. In agricultural practice, potatoes are propagated vegetatively via tubers. The pollen of the potato plant can be transferred by insects or by wind. However, wind dispersal only takes place over short distances.

In previous trials the genetically modified potato plants intended for release showed no significant changes in appearance when compared with conventional control lines. The minimum isolation distance of 10 m between the release sites and other agricultural areas with non-GM potatoes is considered sufficient for the purposes of the proposed trial. Should pollen be transferred to other potato plants in spite of these measures, no adverse effects are to be expected, since in an agricultural environment potato plants are propagated vegetatively, i.e. not by seed. Pollen transfer, therefore, would have no effect on the results of cultivation.

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.

III.1.2.4. Assessment of the possibility of transfer of the inserted foreign genes from the genetically modified plants to micro-organisms 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 micro-organisms 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 theoretically possible, although it is assumed that a gene transfer of this type would constitute an extremely rare event.

Assuming that an exchange of genetic material between organisms as distantly related in terms of taxonomy as plants and bacteria 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) Fragments of the coding region of a potato starch synthase gene (granule-bound starch synthase, GBSS)

The nucleotide sequences are derived from the potato, so they are already commonly found in the environment. Therefore, the likelihood of horizontal gene transfer of these gene fragments to microorganisms would be no greater as a result of the release.

(b) The resistance genes *Rpi-blb1* and *Rpi-blb2* from *Solanum bulbocastanum*

The transferred genes *Rpi-blb1* and *Rpi-blb2* are derived from the wild potato *S. bulbocastanum*, which is native to South America, so these genes occur naturally in the environment. Furthermore, homologous genes are known to exist in a range of other plant species. As a result, horizontal gene transfer to microorganisms is far more likely to occur from the donor organism or from plants which contain homologous genes. Therefore, even in the unlikely event of horizontal gene transfer, no selective advantage would be conferred upon the receiver organism.

(c) The *ahas* gene

Herbicide-tolerant variants of the AHAS enzyme resulting from induced or acquired mutations are known to exist in many plant species. The AHAS enzymes of Enterobacteriaceae naturally exhibit a level of sensitivity to sulfonylurea and imidazolinone herbicides which is often up to two times lower than the corresponding plant enzymes. The isoenzyme AHAS II from *E. coli*, for example, is as tolerant of sulfonylurea derivatives as the AHAS variant S653N synthesised in the genetically modified potato plants.

The genetic spread of herbicide-tolerant AHAS variants is far more likely to result from transfer between bacteria or by horizontal transfer from non-genetically modified plants.

(d) Sequences located outside the T-DNA

In the genetically modified potato lines intended for release real-time PCR showed that no integration of plasmid sequences had taken place outside the right or the left T-DNA borders.

III.1.2.5. Agrobacteria used to generate the genetically modified plants

Agrobacterium-mediated binary transformation systems were used to generate the genetically modified plants. It was shown that the lines intended for release do not contain any backbone sequences from the vector used for transformation. It can therefore be assumed that the plants are free of all Agrobacteria used in the transformation.

In contrast to the common wild-type *A. tumefaciens*, the *Agrobacterium* strains used are “disarmed”, i.e. they no longer have the capacity to induce tumours. In the unlikely, but theoretically conceivable, event that the inserted foreign genes are transferred to a cell of another plant via these Agrobacteria, the plant would have to spontaneously regenerate into a whole, fertile plant for the foreign genes to enter the germ cells. This is the only way that these genes could be passed on to the plant offspring. Such an event is not expected to occur under natural conditions.

Assuming that the presence of small amounts of recombinant Agrobacteria in the genetically modified plants cannot be ruled out, the potential transfer by conjugation of the binary plasmids contained in the Agrobacteria to wild-type Agrobacteria (*A. tumefaciens* or *A. rhizogenes*) present in the environment would also have to be considered, since these could, in turn, pass on the foreign genes to individual cells of other plants. In the case of infection and subsequent transformation via wild-type *A. tumefaciens* or *A. rhizogenes* a crown gall or hairy root tumour would develop from the transformed plant cell. Under natural conditions such a tumour would not be expected to give rise to a plant.