Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0186

Summary of the risk assessment of the genetically modified potato

(*Solanum tuberosum*) EH92-527-1

with altered carbohydrate metabolism

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 25 May 2007

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 fragment of the coding region of a potato starch synthase gene (granule bound starch synthase, GBSS) in antisense orientation.

The fragment of the coding region of a potato starch synthase gene (granule bound starch synthase, GBSS) in antisense orientation is expressed under the control of its own *gbss* promoter primarily in the potato tuber. As a result of the genetic modification of the GM plants, the endogenous transcript of the *gbss* gene is inactivated, thereby inhibiting the production of the GBSS enzyme.

Due to the decreased amount of GBSS protein, a starch with reduced amylose content (amylopectin starch) 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. Within the scope of the proposed release the alteration of the starch composition of the genetically modified potato plants 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 *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'-hydroxyl 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 paramycin 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.

Given the substrate specificity of the neomycin phosphotransferase, it can be assumed that in the absence of substrate under field conditions no new metabolic products can be synthesised in the genetically modified potato plants. No evidence has been recorded to suggest that this enzyme is toxic to plants, animals, microorganisms or humans.

(c) Additional DNA fragments located within the T-DNA

In addition to the expression cassettes of the *npt*II gene and the *gbss* gene fragments, the transformation plasmid pHoxwG contains fragments from the M13mp19 vector with polylinker sequences, as well as fragments of the plasmid pJRD184. These sequences are not functional in plants.

(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, the transfer of DNA fragments outside the borders has been recorded.

The plasmid pHoxwG is a derivative of the vector pBIN19, which contains the following outside its border regions:

- the *aph*AIII (= *npt*III) gene from *Streptococcus faecalis* (= *Enterococcus faecalis*), which is interrupted by the transposon *is*1, but which is functional in procaryotic systems,
- the *tet*A gene of the plasmid pRK2, interrupted by the T-DNA,
- the *trf*A gene of the plasmid pRK2 for replication in *E. coli* and *A. tumefaciens*,
- a fragment of the *kla*C gene from *Klebsiella aerogenes,*
- a *tra*F fragment, which comprises the *ori*T of the plasmid RP4, from *E. coli,*
- the replication origin *ori*V of the plasmid pRK2,
- the replication origin of the pUC vector (ColE1 *ori*) from *E. coli*.

The potato line EH92-527-1 was examined for the presence of vector backbone sequences using PCR and Southern blot analysis. In the course of the examination no vector sequences outside the T-DNA were demonstrated.

(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-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. 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. 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 generally does not 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 in 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 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 analysis or for storage. Surplus tuber material not intended for re-planting will be inactivated by appropriate methods, for example, by 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. If genetically modified potato plants do emerge from seeds or from tubers not detected during the harvest, 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 that would 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 significant change in appearance. Plant growth and yield did not deviate significantly from that of the control lines. In studies on frost tolerance with potatoes of the EH92-527-1 line the genetically modified potatoes and the control varieties exhibited similar survival rates. Even if the genetic modification had brought about a change in the frost sensitivity of the tubers, this would have been 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 strictly bilaterally incompatible; in crossbreeding experiments pollination of the ovule was not achieved. Similarly, the potato does not crossbreed 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. However, 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 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.

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 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 fragment of the coding region of a potato starch synthase gene („granule bound starch synthase“, GBSS)

This gene fragment is derived from the potato, so it is commonly found in the environment. As a result, there is a far greater probability of horizontal gene transfer from non-GM organisms to micro-organisms.

(b) The *npt*II gene

The inactivation of aminoglycoside antibiotics by phosphorylation has been demonstrated as a resistance mechanism in micro-organisms 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 micro-organisms by conjugation.

Even in the improbable event of a horizontal gene transfer from the genetically modified potatoes to micro-organisms, the overall frequency of this resistance mechanism would not be noticeably increased. The presence of the *npt*II gene in the genetically modified potatoes is not expected to have an effect on the therapeutic application of the antibiotics concerned.

(c) Sequences from M13mp19 and pJRD184

The transformation plasmid pHoxwG contains fragments of the M13mp19 vector with polylinker sequences within the T-DNA. DNA fragments of the cloning vector pJRD184 with cloning sequences are also present. The phage M13 belongs to the F-specific *E. coli* phages. In combination with the pJRD184 cloning sequences, the M13mp19 polylinker sequences enabled the integration of nucleotide sequences in the vector. In the case of the procaryotic

DNA fragments, the probability of genetic spreading by transfer between strains of bacteria is far greater than the probability of spreading by horizontal gene transfer from the genetically modified plants to micro-organisms.

(d) Regulation sequences

Transfer of the regulation sequences used in the construct is not likely to lead to an increase in the overall frequency of the respective DNA fragments. These regulation sequences are derived from *A. tumefaciens* and the potato. *A. tumefaciens* is widespread in the environment and the sequences are found in wild-type Agrobacteria on the Ti plasmids, which can be exchanged between different Rhizobiaceae plants.

(e) Sequences located outside the T-DNA

The potato line EH92-527-1 was examined for the presence of vector backbone sequences using PCR and Southern blot analysis. No vector sequences were identified outside the T-DNA.

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

An *Agrobacterium*-mediated binary transformation system was 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 gene is transferred to a cell of another plant via these Agrobacteria, the plant would have to spontaneously regenerate into a whole, fertile plant in order 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.