



Notification 6786-01-0170

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

Berlin, 8 September 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]
- 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 fragment of the *gbss* (granule-bound starch synthase) gene from *Solanum tuberosum* (potatoes)

The fragment of the *gbss* gene in antisense orientation is expressed under the control of the potato *gbss* promoter primarily in the potato tuber. This induces the formation of an antisense RNA in the GM plant tubers, which inactivates the endogenous transcript of the *gbss* gene, thereby inhibiting the production of the GBSS enzyme. As a result, the production of the amylose fraction of the starch is largely suppressed in the tubers and so-called amylopectin starch is synthesised. Amylopectin starch, which is chemically extracted from potato starch, and naturally-occurring amylopectin starch from maize are used as binders or stabilisers in the food processing industry and have various technical applications in the paper, textiles and construction sectors. New proteins are not synthesised in the GM plants as a result of the expression of fragments of the *gbss* gene in antisense orientation.

PCR analysis was used to investigate the integration of the DNA fragment into potato line #1332. This showed that the T-DNA fragment designated for transfer had been integrated into the potato genome together with three copies. No further information on the integration sites is currently available.

The GM potatoes in the proposed deliberate release are not intended for use in the production of food or feed. The alteration of the starch composition of the GM potato plants is not expected to pose any risks to human or animal health or to the environment within the scope of the proposed trial.

A selectable marker for the identification of genetically modified plant tissue was not used.

- (b) 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.

The transformation plasmid pMF1p9-1 is a derivative of plasmid pLH9000 (from pUC19) and contains the following sequences outside the border regions:

- the *aadA* gene from *E. coli* for bacterial streptomycin/spectinomycin resistance,
- the replication origin of the plasmid pVS1 for replication of the plasmid in *Agrobacterium tumefaciens*,

- the replication origin of the plasmid pUC (ColE1 *ori*) from *E. coli*.

The potato line #1332 was tested by Southern blot analysis for the presence of vector backbone sequences, but no vector sequences were detected outside the T-DNA.

(c) Position effects and context changes; allergenicity

Genes that 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 the present case, this could result in the characteristics of the genetically modified potato plants not being 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 the foreign genes may influence the expression or regulation of native plant genes at or near the integration site. Such processes may alter plant metabolic pathways. However, during the trials 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 there is a possibility that such events may influence plant metabolic pathways. The genetically modified plants planned for release do not differ fundamentally from non-genetically modified plants with respect to these specific characteristics.

The genetically engineered modification of these GM plants does not lead to the formation of a protein. Therefore, a change in the allergenic potential of these potatoes is not expected. The GM potatoes cultivated within the scope of the proposed deliberate release will not be used as food or feed. Pollen from potato plants is only marginally dispersed 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 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 are not frost resistant. From time to time potato plants are found beyond cultivated areas, but only on non-natural sites such as roadsides and other ruderal areas. Owing to the lack of frost hardiness the cultivated potato does not establish in these areas either.

The tubers of the GM trial plants will be mechanically or manually harvested, packed in marked, sealed containers and transferred to a genetic engineering facility for further analysis or for storage. Surplus tubers which are not intended for replanting will be inactivated by suitable methods, e.g. steam sterilisation, autoclaving, incineration, shredding or fermentation in a biogas plant. The leaves and stalks of the potato plants will be left to decompose on the release sites.

Potato plants can flower and bear fruit. However, under Central European climate conditions there is little likelihood 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 the designated 4-year post-trial monitoring period. Crop rotation is designed in such a way that no potatoes will be cultivated on the individual trial sites during the subsequent post-trial monitoring period. If genetically modified volunteer plants emerge during this period from tubers that were not detected during harvesting or from the seeds of GM potato plants, they can be identified and inactivated by the usual agronomic practices. If volunteers emerge in the last year of post-trial monitoring, the release site will be monitored for one more year.

Given several years of previous experience with these and comparable transgenic potato plants, this genetic modification is not expected to influence the form and rate of plant reproduction, the likelihood of spreading, or the ability to persist in the environment. Further observations of these characteristics in the trial transformants will be made within the scope of the proposed deliberate release.

Even if the genetic modification produced a change in the frost sensitivity of the tubers, this would be adequately taken into account by the planned cultivation gap for potatoes, the post-trial monitoring conducted in accordance with provisions 11.10 and 11.11 [of the decision on this application], and the specified isolation measures. During the post-trial period following the deliberate release, no plants or at least only plants which will not obstruct the post-trial monitoring will be cultivated on the control sites.

Even taking these factors into account, it is not to be assumed that, compared to conventionally cultivated potatoes, the GM potato plants feature altered ecological characteristics and can colonise natural ecosystems.

Even if berries, seeds or tubers of the GM plants are dispersed, e.g. by animals, the establishment of the genetically modified potatoes in the environment is not expected.

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 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*). 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 potato plants. Pollen from potato plants 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 habitus when compared with conventional control lines. The minimum isolation distance of 20 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 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 micro-organisms take 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 as plants and microorganisms 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 *gbss* (granule bound starch synthase) gene from *Solanum tuberosum* (potatoes)

The introduced fragment of the *gbss* gene and the promoter inserted for its expression are derived from the potato (*S. tuberosum*), so they are commonly found in the environment. The spread of these genetic elements in the environment is therefore far more likely to occur as a result of horizontal gene transfer from non-GM organisms to microorganisms. The same applies for the 35S termination signal of the cauliflower mosaic virus.

No other genes were transferred.

- (b) Sequences located out the T-DNA

The potato line EH92-527-1 was tested using PCR and Southern blot analysis for the presence of vector “backbone” sequences, but no vector sequences were detected outside the T-DNA.