Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

**Notification 6786-01-0162**

**Summary of the risk assessment of the genetically modified**

**Potato (Solanum tuberosum; Seresta, Kuras)**

**within the framework of a proposed deliberate release**

**carried out by the German Competent Authority**

**Berlin, 15 April 2005**

**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.

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 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. In these genetically modified plants, the formation of an antisense RNA causes inactivation of the endogenous transcript of the gene, thus inhibiting the production of the respective enzyme.

The reduction in the GBSS protein content of the genetically modified lines proposed for release was demonstrated by polyacrylamide gel electrophoresis. As a result, a starch with reduced amylose content is synthesised in the tubers. The reduction in amylose content was determined by staining the starch granules with iodine and by spectrophotometry.

The genetically modified potatoes proposed for release are not intended for use in the production of foodstuffs or animal feed. The alteration of the starch composition of the genetically modified potato plants within the scope of the proposed field trials is not expected to pose any threat to human or animal health or to the environment.

(b) The *ahas* gene

A mutant *ahas* gene from *Arabidopsis thaliana* under the control of the *nos* promoter and the *ocs* polyadenylisation sequence from *Agrobacterium tumefaciens* was used to select transformants. The *ahas* gene encodes the enzyme acetohydroxyacid synthase (AHAS), also referred to as acetolactate synthase (ALS), which in plants catalyses the initial steps in the biosynthesis pathways of the amino acids valine, leucine and isoleucine, namely, the reaction of two pyruvate molecules to form 2-acetolactate and the reaction of pyruvat with 2-ketobutyrate to form 2-acetohydroxybutyrate.

AHAS is the target enzyme for various classes of herbicidal agents including sulfonyl urea and imidazolinone. These herbicides act by disrupting the biosynthesis of branched amino acids, causing the plants to die off.

A gene for an AHAS variant was isolated from an *Arabidopsis thaliana* mutant. As a result of its low affinity to the herbicidal agent this gene confers herbicide tolerance to the plants. This variant differs from the wild-type AHAS as a result of an amino acid exchange (S653N, i.e. asparagine instead of serine in position 653).

In the genetically modified plants the herbicide-tolerant AHAS variant catalyses the same reactions as the corresponding endogenous potato enzymes. The formation of new metabolic products in the transgenic potatoes resulting from the expression of the *Arabidopsis thaliana*-derived *ahas* gene is not expected. Within the scope of the proposed trials the transfer of this gene is not expected to pose any threat to the environment or to human health.

(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 transformations. However, the transfer of DNA fragments located outside the border regions has been reported.

These genetically modified potato lines were generated by transformation with the pAP2 plasmid. This plasmid contains the following outside the border regions:

- the *aadA* gene for resistance to the antibiotics streptomycin and spectinomycin,
- the *bom* site from pBR322 for mobilisation of the plasmid from *E. coli* into *Agrobacterium tumefaciens*,
- the origins of replication ColE1 and pVS1 for replication in *E. coli* and *Agrobacterium*.

As demonstrated by real-time PCR, an integration of plasmid sequences from outside either the right or left border did not take place in the lines proposed for release. Therefore, it can assumed that the above-named sequences (and specifically the *aadA* gene) are not contained in these lines.

(d) 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 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-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 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 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 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.

The tubers of the genetically modified test plants are to be mechanically or manually harvested, packed in sealed and labelled containers, and transported to an appropriate facility for subsequent analysis or storage. Surplus tuber material that will not be used for re-sowing will be inactivated using appropriate methods, e.g. steaming, autoclaving, incineration, shredding or fermentation in a biogas facility. The potato haulms (leaves and stalks) will be left to decompose on the release site.

Potato plants can flower and bear fruit. Under Central European climate conditions it is unlikely that potato seeds will overwinter and produce plants. Before harvesting, the applicant plans to apply mechanical or chemical methods to destroy those parts of the potato plants that grow above ground. These measures 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. Crop rotation is designed in such a way that in the following year no potatoes will be cultivated on the individual trial sites. If, in the following year, genetically modified potato plants emerge, these can be detected and inactivated by the usual measures used in farming. Should this occur the release site will be monitored for volunteer plants for a further year.

In previous deliberate release trials carried out by the applicant the genetically modified potato lines did not display any significant changes in habitus. Tuber size, plant growth and yield did not deviate significantly from that of the commercial control lines. One of the aims of the proposed deliberate release is to carry out studies on the overwintering capacity of the genetically modified tubers. For this reason the applicant plans not to harvest parts of the crop in autumn, but to leave some tubers in the soil until the following spring. In April of the following year it will be determined whether and how many of those tubers remained viable.

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 the post-trial monitoring stipulated under provisions II.9 and II.10 (to the present application) and by the planned isolation measures. Throughout the post-trial monitoring period after completion of the release, no plants, or only plants that would not interfere with monitoring, may be planted on the site.

Taking all these factors into account 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, which is unlikely to happen, 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 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 cross-breed 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. The pollen of the potato plant can be transferred by insects or by wind. However, wind dispersal only takes place over short distances. Potatoes are largely self-pollinating; even within a flowering potato field cross-pollination is rare. The likelihood of this occurring is greatest between neighbouring plants.

The genetically modified potato plants proposed for release did not display any significant changes in habitus in comparison to conventional control lines. The proposed minimum isolation distance of 10 m between the release sites and other agricultural areas with non-genetically modified potatoes is considered sufficient. If the transfer of pollen to potato plants intended for the production of table potatoes were to occur despite these measures, no adverse effects are anticipated, since in farming potato plants are propagated vegetatively, i.e. not via seeds.

As already elaborated above, under the given climate conditions the probability of plants emerging from potentially produced seeds is extremely low. In an agricultural setting such plants would be eliminated in the course of common soil preparation practices for crop rotation.

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 possible in theory, 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)

The gene fragments are derived from potato, so they are already widespread in the environment. Therefore horizontal gene transfer in microorganisms is far more likely to occur from non-genetically modified organisms.

(b) The *ahas* gene

Induced and acquired mutations are known to give rise to herbicide-tolerant variants of AHAS enzymes in different plant species. The AHAS enzymes from Enterobacteriaceae often exhibit a sensitivity to sulfonyl urea and imidazolinon herbicides up to twofold lower than corresponding plant enzymes.

The isoenzyme AHAS II from *E. coli* is, for instance, similiarly tolerant of sulfonyl urea substances such as the AHAS variant S653N produced in the genetically modified potato plants.

Therefore the probability that genes encoding herbicide-tolerant AHAS variants will be spread by transfer between bacteria or by horizontal transfer from non-transgenic plants is far greater than the likelihood of spreading by horizontal gene transfer from the GM plants to microorganisms.

(c) Sequences located outside the T-DNA

Real-time PCR demonstrated that no plasmid sequences from outside the left or the right T-DNA border were integrated into the genetically modified potato lines proposed for release as a result of the transformation.