Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

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Summary of the risk assessment of the genetically modified potato

(*Solanum tuberosum*) AM04-1020

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, April 06, 2010

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.1. Requirements for approval according to section 16 GenTG [German Gene Technology Act]
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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.

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

(a) Fragments of the coding region of a 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 *gbss* in sense and antisense orientation (as an inverted repeat) is expressed under the control of the potato's own gbss promoter primarily in the potato tubers. In the genetically modified (GM) plants this causes the formation of a double-stranded RNA and as a result the endogenous transcript of the corresponding gene is inactivated, thus inhibiting production of the GBSS enzyme.

As a consequence of the decrease in GBSS protein, a starch with a lower level of amylose 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 GM potatoes resulting from 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 within the scope of the planned field trial 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 *ahas* gene

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

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 wild-type AHAS by a single amino acid exchange (S653N, i.e. asparagine in place of serine in position 653).

The herbicide-tolerant AHAS variant catalyses the same reactions in the GM potato plants as the corresponding endogenous enzymes in potato. No new metabolic products are expected to result from the expression of the *ahas* gene derived from *A. thaliana* in the GM potato. The transfer of this gene within the scope of the planned field trials is not expected to have any adverse effects on the environment or on 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 transformation events. However, transfers of DNA fragments outside the border regions have been reported in the literature.

The GM potato line was obtained by transformation with the plasmid pAP4. 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 *Escherichia coli* to *A. tumefaciens*,
- the origins of replication ColE1 and pVS1-repA for replication in *E. coli* and *A. tumefaciens*, as well as the sta (stability) region from pVS1.

Using a primer/probe set at both the right and the left border regions, real-time PCR showed that no integration of plasmid sequences occurred outside either the right or the left T-DNA border in the potato lines intended for release. The primer/probe set at the right border region is directed against an internal sequence of the *aadA* gene. It can be assumed that the above-listed sequences, in particular the *aadA* gene, are not contained in the GM lines.

(d) Position effects and context changes; allergenicity

The level at which genes which have been integrated into the plant genome by genetic engineering methods are expressed depends on the chromosomal site of integration and on the sequences neighbouring the integration site ("position effect"). Under field conditions the level of expression may also be influenced by environmental factors, for instance, by temperature. In the present case this could mean that the characteristics of the GM potato plants are not altered 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 native plant genes at or near the site of insertion. Such processes may alter plant metabolic pathways. However, in the course of the work carried out to date on these GM plants, no evidence of such an event has been observed.

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 commonly used in plant breeding. Such events can therefore influence plant metabolic pathways at any time, even in non-GM plants. To that extent the GM plants do not differ fundamentally from non-GM plants in relation to these characteristics.

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. Acetohydroxyacid synthase has not been described as a protein with allergenic potential. The GM potatoes are not intended for use as food or feed within the scope of the proposed deliberate release. Pollen from 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 GM plants to persist or establish in the environment

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

The tubers of the GM trial plants are to be harvested mechanically, packed in closed and marked containers, and transferred to the appropriate S1 facilities for storage, analysis or disposal. Surplus tuber material not intended for re-planting will be inactivated by the appropriate methods, e.g. by steaming, autoclaving, incineration or fermentation in a biogas plant. The leaves and stalks of the potato plants are to 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 are to be mechanically or chemically destroyed. This serves to counteract seed maturation. In order to minimise the emergence of volunteers shallow soil cultivation is to be carried out after harvesting and subsequent cultivation of the area with a disc harrow is planned so that most potato tubers lying on the surface will be shredded, thus promoting rotting. Should tubers or seeds remain in the soil, the emerging plants would be detected during post-trial monitoring. The crop rotation is designed in such a way that potatoes will not be cultivated on the individual release sites in the following year. During the post-trial monitoring period, no plants or only plants which do not interfere with monitoring are to be cultivated on the release sites. If GM potato plants do emerge from seeds or from tubers not detected during the harvest, these can be identified and inactivated by conventional agricultural practices. Concerning this, the applicant - in consultation with the surveillance authority - intends to apply the appropriate herbicides. If volunteers emerge, the post-trial monitoring period will be extended and the release site controlled for volunteers for a further year.

In experiments carried out to date by the applicant, the GM potato line did not exhibit any significant change in appearance. No evidence emerged to suggest the existence of significant differences between the GM line and conventional varieties of potato that go beyond the goal of the genetic modification. In the trials carried out so far, the overwintering capacity of the GM potatoes has also been studied. Even if the genetic modification had brought about a change in the frost sensitivity of the tubers, this would be adequately addressed by the designated cultivation gap for potatoes, by the implementation of post-trial monitoring and by the planned isolation measures.

The GM potato plants are not expected to exhibit altered 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 GM plants were to be dispersed by animals, the GM potato plants would not be expected to establish in the environment.

III.1.2.3. <u>Evaluation of the possibility of a pollen-mediated transfer of the inserted genes from the GM plants to other plants</u>

Attempts to cross-breed potatoes with solanaceous plants found in Central Europe were not successful. Under field conditions no incrossing took place from GM potatoes to *Solanum nigrum* (black nightshade). 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. Potato and *Solanum dulcamara* (bittersweet or woody nightshade) proved to be strictly bilaterally incompatible; in crossbreeding experiments fertilisation of the ovule was not achieved. Similarly, potato can not be crossbred with tomato (*Lycopersicon esculentum*).

The following passage, therefore, deals only with a possible pollen transfer from the GM 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. In agricultural practice, potatoes are vegetatively propagated from tubers, i.e. not from seed.

In trials carried out to date the GM potato plants intended for release showed no significant changes in appearance when compared with conventional control lines. Should a pollen transfer to other potato plants occur, no adverse effects are to be expected because the seed potatoes used for the cultivation of potatoes are propagated vegetatively. Thus, pollen transfer would have no effect on the cultivation result. Also, only potato tubers are consumed. The minimum distance of 10 m between the release sites and other agricultural areas with non-GM potatoes is considered sufficient.

As elaborated above, the likelihood of potentially generated seeds giving rise to plants under the given climatic conditions is low. However, should plants emerge from seed on land used for agriculture they would be eliminated in the course of crop rotation by conventional soil cultivation measures.

III.1.2.4. Assessment of the possibility of horizontal gene transfer of the inserted foreign genes from the GM plants to micro-organisms

The inserted sequences are stably integrated into the chromosomes of the recipient organisms. There is no evidence to suggest 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 may theoretically be 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 must 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 fragments of the coding region of a potato starch synthase gene (granule bound starch synthase, GBSS)

These nucleotide sequences are derived from potato and are therefore already widespread in the environment. A horizontal gene transfer to micro-organisms could therefore just as well take place from non-GM organisms. A transfer of these nucleotide sequences to micro-organisms is not expected to have any adverse effects.

(b) 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 GM potato plants.

The spread of genes for herbicide-tolerant AHAS variants is much more likely to occur via transfer between bacteria or via horizontal transfer from non-GM plants.

(c) Regulatory sequences

There is no reason to fear that a transfer of the regulatory sequences used in the construct would lead to an increase in the overall frequency of the respective DNA fragments. These regulatory sequences originate from *A. tumefaciens* and potato. *A. tumefaciens* is widespread in the environment and the sequences mentioned are found in wild-type agrobacteria on Ti plasmids, which can be exchanged by conjugation between different *Rhizobiaceae*.

(d) Sequences located outside the T-DNA

Real-time PCR has shown that no integration of plasmid sequences took place outside of the right and left T-DNA borders in the GM potato lines intended for release.

III.1.2.5. Agrobacteria used to create the GM plants

An *Agrobacterium*-mediated binary transformation system was used to create these GM plants. The lines intended for release were shown not to contain any backbone sequences from the transformation vector. It can be assumed that these plants are free of all agrobacteria used for the transformation.

In contrast to the common wild-type *A. tumefaciens*, the *Agrobacterium* strain used is “disarmed”, i.e. it no longer has 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 offspring of the plant. Such an event is not expected to occur under natural conditions.

Assuming that the presence of small amounts of recombinant agrobacteria in the GM 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 nature 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.