Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0103

Summary of the risk assessment of genetically modified potato plants (*Solanum tuberosum* L.) (lines *Linien 35S-αUMPS 21, 26, 29, 73; B33-αUMPS 5, 8, 73, 76; 35S-αAdK 4, 8, 20, 24, 28; 35S-StPT2 26, 27, 45; 35S-PL24, 28, 48, 52; 35S-SAT 26, 48*) carried out by the German Competent Authority within the framework of a proposed deliberate release, Berlin, 11 May 1999

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

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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 genes for various enzymes from *Solanum tuberosum, Escherichia coli* and *Lilium longiflorum* in antisense or sense orientation

The coding regions or parts of the coding regions of various enzymes from *S. tuberosum, E. coli* und *L. longiflorum* contained in the genetically modified plants are expressed constitutively – i.e. not tissue-specifically – under the control of the CaMV 35S promoter. Under the control of the B33 promoter, expression occurs specifically in the potato tubers. In addition, the expression activity of the B33 promoter is sucrose-induced.

In the constructs (I.) to (III.) the coding regions were arranged in reverse orientation relative to the respective promoter. In the genetically modified plants, this results in the formation of an antisense RNA which inactivates the endogenous transcript of each respective gene, thus preventing production of the corresponding enzyme. In the case of the constructs (IV.) to (VI.), the expression of the respective DNA fragment leads to the production of the corresponding enzyme in the genetically modified potato plants.

Through the targeted switching off of the expression of an endogenous enzyme and/or through the expression of a heterologous or 'additional' enzyme, the genetic modification altered the metabolism of the genetically modified potato plants with respect to their tuber weight and density, their starch, phosphate and amino acid content, as well as their cell wall composition. The applicant did not observe any change in the habitus of the genetically modified potatoes in the greenhouse.

(I.) (II.) 35S-αUMPS and B33-αUMPS

These constructs contain the cDNA for the uridine monophosphate synthase (UMPS) from *S. tuberosum* in antisense orientation under the control of the B33 promoter from *S. tuberosum* or the 35S promotors of Cauliflower mosaic virus (CaMV), as well as the terminator sequence of the octopine synthase gene from *A. tumefaciens*.

UMPS mediates *de novo* synthesis of uridine monophosphate (UMP). During starch biosynthesis, a component of the phosphorylated glucose (glucose-1 phosphate) is not used to form starch but is converted back to sucrose with the aid of the UDP-glucose pyrophosphorylase (UGPase) and the sucrose synthase. UTP is required for the synthesis of UDP-glucose by the UGPase. The inhibition of UMP synthesis is supposed to reduce the UTP content and prevent the glucose 1-phosphate from being converted back into sucrose.

The cDNA is expressed specifically in the potato tubers under the control of the B33 promoter. In addition, the expression activity of the B33 promoter is sucrose-induced. Under the control of the CaMV 35S promoter, constitutive expression – i.e. not tissue-specific expression – takes place.

The coding regions were arranged in reverse orientation relative to the respective promoter. In the genetically modified plants, this results in the formation of an antisense RNA. Ribonucleases are normal components of every cell. This is not expected to pose a potential hazard.

The genetic modification is supposed to lead to reduced production of the uridine monophosphate synthase and prevent the conversion of glucose 1-phosphate to sucrose.

According to the information provided by the applicant, in the greenhouse, potato plants which have been genetically modified in this way show a 10-20 % increase in tuber weight while maintaining the same density.

(III.) 35S-αAdk

This construct contains the cDNA for the plastidial isoform of the adenylate kinase gene in antisense orientation under the control of the CaMV 35S promoter and the terminator sequence of the octopine synthase gene from *A. tumefaciens*.

Adenylate kinases cleave bound ATP or phosphorylate bound ADP. Consequently, they play a fundamental role in maintaining the energy balance of organisms. The genetic modification is supposed to cause a reduction of the enzyme. This is not expected to pose a potential hazard.

According to information provided by the applicant, potato plants which have been genetically modified in this way exhibit increased specific density in the greenhouse.

(IV.) 35S-StPT2

This construct codes for an endogenous, root-specific phosphate transporter (StPT2) from *S. tuberosum* in sense orientation under the control of the CaMV 35S and the terminator sequence of the octopine synthase gene from *A. tumefaciens*.

The transporter facilitates the uptake of exogenous phosphate. Expression of the endogenous StPT2 gene is induced by phosphate deficiency. This is expected to result in improved uptake of phosphate in the genetically modified plants.

According to information provided by the applicant, in greenhouse experiments the genetic modification affects the phosphate content of the leaves, roots and tubers.

(V.) 35S-PL

This construct codes for the pectate lyase gene from *L. longiflorum* in sense orientation under the control of the CaMV 35S promotor and the terminator sequence of the octopine synthase gene from *A. tumefaciens*.

Pectate lyases destabilise the stability of plant cell walls by cleaving polygalacturonic acid, a component of pectines. They are normal components of potato plants. The additional expression of a heterologous pectate lyase gene is supposed to influence cell wall stability.

According to information provided by the applicant, in the greenhouse, potato plants which have been genetically modified in this way exhibit reduced cell wall stability in the tuber tissue.

(VI.) 35S-SAT

This construct codes for the serine acetyltransferase gene from *E. coli* in sense orientation, fused to the transit peptide of the small sub-unit of ribulose 1,6-bisphosphate carboxylase from *Arabidopsis thaliana* under control of the CaMV 35S promoter and the terminator sequence of the octopine synthase gene from *A. tumefaciens*.

In the chloroplasts, serine acetyltransferases catalyse the production of cysteine and glutathione, which serve as antioxidants in the organism. They are supposed to confer resistance to oxidative

stress. The transit peptide is supposed to transport the heterologous transferase to the chloroplasts where the amino acids are synthesised. Through the expression of the heterologous serine acetyltransferase gene the genetically modified potatoes are expected to develop resistance to oxidative stress and heavy metals.

According to information provided by the applicant, in the greenhouse, potato plants which have been genetically modified in this way exhibit a higher cysteine and glutathione content.

The enzymes expressed by the genetically modified potatoes (pectate lyase, serine acetyltransferase and the phosphate transporter) are ubiquitously present in bacteria and plants. These enzymes are not known to be potentially hazardous. Pectate lyases have long been used in the food industry. All other enzymes cited (uridine monophosphate synthase and adenylate kinase) are native potato enzymes whose expression is inhibited by antisense RNA with the aim of directly blocking certain metabolic steps. This is also not expected to pose a potential hazard.

After the end of the trial, some of the harvested tubers of the genetically modified plants are to be stored for re-planting or used for analytical tests. Surplus potatoes will be inactivated. The potatoes are not intended for human or animal consumption. However, even in the event of unintentional consumption by humans, no adverse health effects would be expected. If tubers were to be consumed by animals, digestive problems could occur due to the altered metabolism of the potatoes. Likewise, effects on pest infestation due to the altered metabolism cannot be ruled out. According to information provided by the applicant, field trials carried out between 1993 and 1998 with other genetically modified potatoes with altered metabolism did not reveal any evidence of an effect on the protection goals specified in § 1 No. 1. Effects on pest infestation might result from the altered cell wall stability. This will be investigated in the context of another deliberate release trial.

The possibility that the expression of one of the constructs in the respective potato lines might have a complex effect on the metabolism of the genetically modified potato plants cannot be ruled out. This does not imply a risk, because such an effect on plant metabolic pathways could also occur at any time in non-genetically modified potato plants from conventional cultivation. For precautionary reasons only, the release site is to be surrounded by a fence (see II.8).

(b) The *nptII* gene

The nptII gene transferred to the genetically modified plants encodes the enzyme neomycin phosphotransferase. It was inserted as a marker gene for selecting transformed plant cells.

The neomycin phosphotransferase is a type II aminoglycoside 3'-phosphotransferase (APH(3')II), which catalyses ATP-dependent phosphorylation of the 3'-OH 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 as well as paromomycin 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. However, kanamycin and neomycin are widely used in veterinary medicine.

Due to the substrate specificity of neomycin phosphotransferase, in the absence of substrate under field conditions no new metabolic products are expected to arise in the genetically modified potato plants. Since the relevant antibiotics are not present in the soil in high concentrations, the neomycin phosphotransferase does not confer any selective advantage to the genetically modified plants under

field conditions. There is no evidence to suggest that this enzyme is toxic to plants, animals, microorganisms or humans.

(c) The *hph* gene

The hygromycin resistance gene *hph* was inserted as a marker gene for the selection of transformed plant cells. The hygromycin phosphotransferase encoded by the gene specifically inactivates the antibiotic hygromycin by phosphorylation. Other aminoglycoside-aminocyclitole antibiotics such as kanamycin or geneticin are not converted. Hygromycin is not used in human medicine.

Plants in the field are not treated with hygromycin. Hence, in the field, the genetically modified plants are not expected to have a selective advantage due to the presence of the hygromycin phosphotransferase. Also, due to the substrate specificity of the enzyme, the expression of the hygromycin resistance gene in the genetically modified plants is not expected to give rise to physiologically relevant amounts of new metabolic products.

(d) The coding sequence of the α-fragment of the β-galactosidase, *lacI* sequences

Vectors which derive from the plasmid pBIN19 were used to generate the genetically modified plants. In these vectors, the multiple cloning site is located within the coding sequence of the α-fragment of the β-galactosidase from *E. coli*.

The native enzyme β-galactosidase cleaves β-D-galactoside into galactose and the corresponding alcohol compound. The most physiologically important substrate is lactose, which is hydrolysed into galactose and glucose. The α-fragment refers to the first 146 amino terminal amino acids of β-galactosidase. The α-fragment by itself is not enzymatically active, but complementation may occur in suitable hosts.

Through the insertion of the relevant coding regions into the multiple cloning site, the coding sequence for the α-fragment of the β-galactosidase was interrupted so that in this form, *inter alia* in *E. coli* bacteria, it no longer has the ability to code for an α-fragment that is capable of complementation. The interrupted sequence of the α-fragment of β-galactosidase is expressed under the control of a bacterial promoter. A functional gene product is not encoded by this sequence. The presence of this sequence is not expected to result in any changes in the genetically modified potatoes.

In addition, the genetically modified plants probably also contain 5’ and 3’ sequences of the repressor gene *lacI*. However, these 5’ and 3’ sequences are separated from one another by the *lacZ* and M13 *ori* sequences. The *lacI* sequences are unlikely to have any functional capacity in the genetically modified plants.

(e) M13 sequences

The genetically modified plants, which were created by transformation with a derivative of the vector pBIN19, contain two fragments from M13mp19, namely, a 440-bp fragment comprising part of an open reading frame of a structural protein of M13 as well as a 433-bp fragment containing the origin of replication of the M13 phage.

Should transcription of the fragment of the open reading frame of the structural protein occur in the genetically modified potato plants, a functional protein not would not result because the fragment encodes only 167 amino acids of a total of 423 amino acids of the complete phage protein. Therefore, the presence of this fragment is not expected to affect the metabolism of the plants.

The origin of replication of M13 causes replication of the phage in *E. coli* when *E. coli* is infected with M13, f1 or fd phages. This origin of replication is not expected to be functional in plants.

(f) The fragment of the *ocd* gene

The plants, which were created by transformation with derivatives of the vector pBIN19, contain a fragment of the *ocd* gene (ornithine cyclodeaminase), which is located between the 3' end of the translated sequence of the *nptII* gene and the NOS termination sequence. Because this sequence is transcribed as part of the mRNA of the *nptII* gene, but is located behind the termination codon of the *nptII* gene, the sequence is not expected to be translated.

(g) Border sequences from Ti plasmids and regulatory sequences

The genetically modified plants contain sequences from the left and the right border region of the TL-DNA of the plasmid pTiT37 from *A. tumefaciens*. These sequences, dependent on the gene products of the *vir* regions of the helper plasmid pGV2260 present in the *Agrobacterium* strain used for the transformation, which was not transferred to the plants, cause the genes located between the border regions to integrate into chromosomes of the potato plants. These border regions of the Ti plasmid are non-functional in the genetically modified plants and are not expected to cause any changes in the plants.

The genetically modified plants contain the following regulatory sequences integrated into the genome:

- B33 promoter from *S. tuberosum*;
- 35S promoter of Cauliflower mosaic virus (CaMV);
- the promoter and terminator of the nopaline synthase gene from *A. tumefaciens*;
- the terminator of the octopine synthase gene and T-DNA gene 7 of *A. tumefaciens*.

The promoter and termination sequences regulate the expression of the DNA sequences located between them in antisense or in sense orientation as well as the expression of the *nptII* gene or the *hph* gene in the genetically modified plants. Further information on the effects of the expression of these sequences in the plants are found in section III.1.2.1. (b) and (c).

(h) Sequences located outside the T-DNA

As a general rule, only DNA located within the border regions is integrated into the plant genome in *Agrobacterium*-mediated transformation events. However, the transfer of sequences outside the borders have been reported and, on the basis of the information contained in the notification, this possibility cannot be ruled out.

According to the information gleaned from the notification, in the present case, the following functional units might have been transferred to the genetically modified potato plants through integration of DNA fragments from outside the border regions:

(i) the *nptIII* gene (codes for a type III aminoglycoside-3'-phosphotransferase) for resistance to aminoglycoside antibiotics;

(ii) the origin of replication *oriV* of the plasmid RK2;

(iii) the *traF* region, containing the *oriT* of the plasmid RK2;

(iv) the *trfA* locus of the plasmid RK2 (codes for two proteins which are required for replication of the plasmid);

(v) a non-functional fragment of the *klaC* gene from the plasmid RK2;

(vi) the *tetA* gene of the plasmid RK2 (interrupted by insertion of the T-DNA region);

(vii) the transposon IS1 within the *nptIII* gene;

(viii) the origin of replication of the plasmid pMB1.

Because the *nptIII* gene (i) is under the control of a bacterial promoter, it is not expected to be expressed in plants. Even in the event of its expression, the gene would not be expected to affect the metabolism of the plant.

The origins of replication *oriV* (ii) and *oriT* (iii) of the RK2 plasmid facilitate the replication of the plasmid in a broad host range of gram-negative bacteria and its transfer by conjugation, respectively, provided the mobilisation functions are supplied by a helper plasmid. There is no evidence to suggest that the origins of replication of RK2, the origin of replication of pMB1 (viii) or the other DNA fragments of bacterial origin (iv, v, vi, vii) would have a function in higher plants. Moreover, some of the DNA fragments are incomplete (v) or interrupted (vi).

(i) 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 insertion on the chromosome, and on the environment at the site of insertion (position effect). Under field conditions the level of expression may be additionally influenced by environmental factors, for instance by temperature. In the present case this means that the properties of the genetically modified potato plants might not be altered to the same extent under field conditions as under climate-chamber or greenhouse conditions. This does not imply any risks 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 with the genetically modified plants, no observations were made that would indicate 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 and were first detected in maize. The inactivation of genes or alterations in gene regulation also take place in a range of other naturally occurring processes, e.g. point mutations, deletions or translocations, and are traditionally used in plant breeding. Therefore, even in non-genetically modified plants, such events can always have an effect on plant metabolic pathways. In this respect the genetically modified plants proposed for release here do not differ fundamentally in those characteristics 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 its amino acid sequence. However, in previous experiments with the genetically modified plants, as well as in numerous deliberate releases of plants which

express the *nptII* gene and the *hph* gene under the control of non-tissue-specific promoters, no evidence of increased allergenicity of the plants was found.

As a result of the expression of the cDNA for the uridine monophosphate synthase and the adenylate synthase in antisense orientation, the native plant protein is present in low concentrations or not at all. The expression of the cDNA of the phosphate transporter as well as the expression of the cDNA for the pectate lyase and the serine acetyltransferase lead to the production of the enzymes which can catalyse metabolic processes of the genetically modified potatoes. There is no evidence for allergenicity of these proteins.

In any event, the pollen of potato plants is only spread to a small extent by wind and, in general, does not play a noteworthy role as a trigger for pollen allergies.

III.1.2.2. Evaluation of the ability of the genetically modified plants to persist or become established in the environment

The cultivation of potatoes in Central Europe goes back several hundred years. During this time, establishment of potato in natural ecosystems in Europe has not been observed. Potato plants are occasionally found outside cultivated areas, but only on non-natural sites such as waysides and other ruderal areas. Because potatoes are not frost resistant, no permanent establishment occurs in in those areas either. On agricultural land which has been used for potato cultivation, "volunteer potatoes" can emerge in the following growing season from tubers left in the soil after the harvest if winter temperatures are mild enough.

The plan is that the above-ground parts of the potato plants will be mechanically or chemically destroyed before reaching maturity to prevent maturation of the seeds. The tubers will be analysed after harvest or stored for replanting in the following year. Surplus tubers are to be inactivated, e.g. by steaming. The remaining transgenic plant parts are to be left on the field to decompose. Potatoes will not be cultivated during the two-year post-trial monitoring period. During this time, volunteer potatoes can be identified and destroyed. The probability of persistence of the genetically modified plants due to tubers potentially remaining in the ground after harvest will be minimised by the measures stipulated in supplementary provision II.9. To remove any tubers remaining in the ground, the soil on the release site will be loosened to a depth of approx. 15 cm after harvesting the tubers as well as in the spring of the following year. Any tubers found will be inactivated.

Potato plants of the variety “Désirée” can flower and produce seeds. The genetically modified potatoes could conceivably be fertilised by foreign potato pollen, so that seed formation, although very unlikely, cannot be completely ruled out. Under Central European climate conditions, potato seeds are unlikely to overwinter and give rise to plants.

Should tubers or seeds remain in the soil, any plants that might emerge from them would be detected during the post-trial monitoring period proposed by the applicant. A possible change in the frost sensitivity of the tubers as a result of the genetic modifications cannot be ruled out. The proposed cultivation gap of two years and the performance of post-trial monitoring adequately take this possibility into account. During the post-trial monitoring period following the deliberate release, no plants or only plants which do not interfere with the monitoring may be cultivated on the control sites. This makes it possible to easily identify volunteer potatoes.

For the reasons stated above, the genetically modified plants are not expected to establish or persist 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 unsuccessful. Under field conditions, genetically modified potatoes did not hybridise with *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 which, 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 ovules was not achieved. Potato is also not cross-compatible with 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 potato plants can be transferred by insects or by wind. However, wind dispersal takes place over short distances only. Potatoes are primarily self-pollinating; foreign pollination is rare, even within a field of flowering potato plants. If at all, it is most likely to occur between neighbouring plants.

Since the 1996 growing season, genetically modified potatoes with altered carbohydrate and phosphate metabolism are already released on the trial area at the Golm site within the scope of various deliberate release trials of the Max Planck Institute for Molecular Plant Physiology, Golm. The deliberate release proposed here will be incorporated into the system crop rotation of genetically modified potatoes which have already been approved for release on the trial area. Thereafter follows a two-year period of monitoring and post-trial treatment of the respective deliberate release area. This means that potatoes will only be planted out or put down again on this area in the third year.

Besides, the applicant plans to maintain a distance of 20 m between the deliberate release site and nearby potato crops and this is considered adequate. However, should pollen be transferred to potato plants cultivated to produce table potatoes despite this measure, no adverse effects are to be expected. Planting material for the cultivation of potatoes is propagated vegetatively, i.e. not via seeds. As already explained above, the probability that potentially generated seeds could give rise to plants under the given climatic conditions is very slight. Such plants would be eliminated in the course of conventional soil preparation practices during crop rotation. Even if the tubers of such plants were to be consumed, no health hazards would be expected to result, as follows from the evaluation undertaken in section III.1.2.1.

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 to indicate that a transfer of genetic information from plants and 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 might, in principle, be possible, although it is assumed that a gene transfer of this nature would constitute an extremely rare event.

Insofar as we assume that an exchange of genetic material between organisms which are as distantly related in terms of taxonomy as plants and bacteria actually occurs, it follows 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.

The genes for uridine monophosphate synthase, adenylate kinase and the phosphate transporter StPT2 and the patatin promoter (B33) derive from potato and are thus already widespread in the environment. This also applies to the gene of the pectate lyase from *L. longiflorum* and the gene of the serine acetyltransferase from *E. coli* as well as the transport peptide from *A. thaliana*. Therefore, they could also – with a far higher probability – enter microorganisms in the environment by horizontal gene transfer from non-genetically modified organisms.

As already elaborated under III.1.2.1.(b), the antibiotics inactivated by the neomycin-phosphotransferase are of little relevance in human medicine but are widely used in veterinary medicine. Therefore, it was necessary to examine whether a potential horizontal gene transfer of the *nptII* gene would affect the therapeutic use of the relevant antibiotics.

The inactivation of aminoglycoside antibiotics by phosphorylation is a naturally occurring resistance mechanism in soil microorganisms. In addition, APH(3')II enzymes have 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 their effective transfer between microorganisms by conjugation. Even in the event of a horizontal gene transfer from the genetically modified potatoes to microorganisms, the overall frequency of this resistance mechanism would not noticeably increase.

The antibiotic hygromycin is used for specific applications in veterinary medicine, but not in human medicine. It was thus necessary to examine whether the therapeutic use of hygromycin in veterinary medicine would be compromised by a potential horizontal gene transfer.

Hygromycin-resistant bacteria have been found in human- and animal-derived specimens (faeces, urine, blood). In one study, the hygromycin phosphotransferase gene *hph* was also found in the bacteria. The spread of hygromycin resistance genes is attributed to the use of hygromycin in veterinary medicine. Because hygromycin-resistant bacteria are released into the environment by the affected humans and animals, and the *hph* genes – in the cases studied – were localised on plasmids, a spread of the hygromycin resistance trait among microorganisms present in the environment is possible. A gene for a hygromycin phophotransferase is also present in the soil bacterium *Streptomyces hygroscopicus*, which synthesises hygromycin. Since effective genetic exchange by conjugation is possible between various bacterial species – even under natural conditions – the release of the genetically modified potato plants would not increase the likelihood of hygromycin resistance being spread among soil microorganisms in the environment.

The gene for the α-fragment of the β-galactosidase is interrupted so that no functional gene product can be produced. This would also be the case in bacteria that receive the gene through horizontal gene transfer. The same applies to the 3' and 5' sequences of the *lacI* gene.

A similar situation applies to the fragment of the gene for a structural protein of the phage M13 and the fragment of the *ocd* gene. These fragments are not expected to be functional in bacteria. In addition, the fragment of the *ocd* gene is unlikely to be translated, as explained under III.1.2.1.(f).

The genetically modified potatoes contain the origin of replication of M13. M13 belongs to the F-specific *E. coli* phages. Therefore, the likelihood of this origin of replication being spread by transfer between bacteria is far greater than the likelihood of being spread by horizontal gene transfer from the genetically modified plants to microorganisms.

The sequences inserted into the potatoes to regulate the transferred genes derive from *A. tumefaciens* and CaMV. Regarding a horizontal gene transfer of these sequences to microorganisms, it should be noted that *A. tumefaciens* is widespread in the soil and that a transfer of the relevant sequences from *Agrobacterium* is far more likely than their transfer from the genetically modified plants. The theoretical possibility of a transfer of the CaMV sequences from the genetically modified plants would not constitute a new situation compared to that found in nature because CaMV, as a double-stranded plant-infecting DNA virus, is already present in plants.

As a general rule, only sequences located within the borders are integrated into the plant genome in *Agrobacterium*-mediated transformation events. However, on the basis of the information contained in the notification, a transfer of sequences outside the borders cannot be ruled out. In the present case, the following DNA fragments could have been integrated into the genetically modified plants through the integration of sequences located outside the border regions:

(i) the *nptIII* gene from *Streptococcus faecalis* (codes for a type III aminoglycoside-3'-phosphotransferase) for resistance to aminoglycoside antibiotics;

(ii) the origin of replication *oriV* of the plasmid RK2;

(iii) the *traF* region, containing the *oriT* of the plasmid RK2;

(iv) the *trfA* locus of the plasmid RK2 (codes for two proteins required for replication of the plasmid);

(v) a non-functional fragment of the *klaC* gene from the plasmid RK2;

(vi) the *tetA* gene of the plasmid RK2 (interrupted by insertion of the T-DNA region);

(vii) the transposon IS1 within the *nptIII* gene;

(viii) the origin of replication of the plasmid pMB1.

According to the literature references, the *nptIII* gene (i), which may be contained in the genetically modified plants along with its own promoter, confers resistance not only to kanamycin and neomycin, but also to the antibiotic amikacin. In Germany, amikacin is not authorised for use as a veterinary medicinal product, but it may be employed in human medicine where it serves as a so-called reserve antibiotic. Because of its status as a reserve antibiotic and its attendant infrequent use, amikacin resistance is so far not widespread. Given the low probability of a horizontal gene transfer from plant DNA to microorganisms and the absence of selective pressure on the release sites, it can also be assumed that the presence of this gene in the genetically modified potato plants would not lead to a significant increase in the overall frequency of this resistance mechanism in microorganisms.

RK2 belongs to a group of broad host-range plasmids (incl. RP1, RP4, R18, R68), which are replicable in numerous gram-negative bacteria. Hence, in the case of the RK2-derived DNA fragments (ii to vi), the probability of a spread by transfer between bacteria is far higher than the probability of a spread via horizontal gene transfer from the genetically modified plants to microorganisms. Moreover, some of the DNA fragments are incomplete (v) or interrupted (vi).

The insertion element IS1 (vii) is naturally present in various species of *Enterobacteriaceae*. For example, it has been found in species of the genera *Escherichia*, *Shigella*, *Klebsiella*, *Serratia* and *Salmonella*. In the case of IS1, the number of copies per bacterial genome can amount to > 40 copies. Copies of IS1 may be localised on chromosomes or on plasmids and they have also been detected in prophages. It is assumed that the spread of this insertion element via horizontal gene transfer is easily possible. By comparison, the risk of spreading from the genetically modified plants to microorganisms via horizontal gene transfer, although theoretically conceivable, is negligible.

The pMB1 replicon (viii) belongs to the ColE1-type plasmids whose host range is limited to a number of gram-negative bacteria. Basically, this replicon can replicate in *E. coli* and closely related species of bacteria such as *Serratia* or *Salmonella*. In most gram-negative soil bacteria, replication does not take place. ColE1 plasmids occur frequently in enterobacteria. Gene transfer from enterobacteria to other bacteria is considered far more likely than a horizontal gene transfer from the genetically modified plants to bacteria. Therefore, the potential presence of the origin of replication of pMB1 in the plant chromosome is not expected to contribute to an increase in the overall frequency of horizontal gene transfer.

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

To generate the genetically modified plants, sterile potato leaves were inoculated with agrobacteria which contained the genes to be transferred between the border regions of the binary vector plasmid. Following transformation, antibiotic treatment was carried out to eliminate the agrobacteria. The regenerated plants were examined to determine whether they were free of agrobacteria by incubating plant homogenates in the appropriate culture media. The agrobacteria used for the transformation were not detected. Only potato plants that were free of agrobacteria were used further.

In contrast to the ubiquitous wild forms of *A. tumefaciens*, the *Agrobacterium* strain used for the transformation is disarmed, i.e. it no longer has the capacity to induce tumours. In the unlikely but theoretically conceivable event of an *Agrobacterium*-mediated transfer of the inserted foreign genes to a cell of another plant, that cell would have to spontaneously regenerate into a whole, fertile plant for the foreign genes to enter the germ cells and thereby be passed on the progeny of the plant. This is the only way that these genes could be passed on to the progeny 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 genetically modified plants cannot be ruled out, a potential transfer by conjugation of the binary vector plasmids contained in the agrobacteria to wild-type agrobacteria present in the environment (*A. tumefaciens* or *A. rhizogenes*) must also 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 by wild-type *A. tumefaciens* or *A. rhizogenes,* a crown gall or hairy root tumour would develop from the transformed plant cell. A tumour of this type would not be expected to give rise to a plant under natural conditions.

A possible transfer of the inserted genes from agrobacteria to other soil bacteria must also be considered. The potential impact of such a transfer has already been addressed under III.1.2.4..