Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0110

Summary of the risk assessment of genetically modified potato plants

(*Solanum tuberosum* L.) (29 independent lines)

carried out by the German Competent Authority within the framework of a

proposed deliberate release, Berlin, 10 June 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

- 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 genes for various enzymes from *E. coli*, *Saccharomyces cerevisiae*, potatoes or spinach in sense and antisense orientation

The expression of the coding regions or parts of the coding regions of various enzymes from *E. coli*, *Saccharomyces cerevisiae*, potatoes or spinach contained in the genetically modified potato plants is controlled by the following promoters:

- Constitutive – i.e. non-tissue-specific – expression takes place under the control of the CaMV 35S promoter.
- Specific expression in the companion cells takes place under the control of the rolC promoter.
- Expression limited to green plant parts takes place under the control of the L700 promoter.

In construct III, the coding region was arranged in antisense orientation relative to the promoter. In the genetically modified plants, this causes the formation of an antisense RNA which inactivates the endogenous transcript of the relevant gene, inhibiting the production of the corresponding enzyme. In the case of constructs, I, II and IV to VIII, the expression of the respective DNA fragment leads to the production of the corresponding enzyme in the genetically modified potato plants.

As a result of the genetic modifications, the metabolism in the genetically modified potato plants was altered by targeted inhibition of the expression of an endogenous enzyme and expression of a heterologous or "additional" enzyme to the effect that the tuber formation was enhanced, the tuber yield was increased, the flowering or germination behaviour was changed or the vegetation period was shortened:

I. L700 FNR PPK:

The construct codes for the polyphosphate kinase (PPK) from *E. coli* and an upstream transit sequence of the ferredoxin:$NADP^+$ oxidoreductase (FNR) from spinach. The transit sequence is to enable the transport of the PPK into the chloroplasts, were the PPK permits part of the chemical energy of the ATP to be stored in the form of polyphosphate at a high energy status of the cell. According to the information provided by the applicant, such transgenic potato plants showed a 10-30% increase in tuber yield under greenhouse conditions due to an increase in the number of tubers per plant.

II. 35S MPP mCS:

The construct codes for the citrate synthase (CS) from *Saccharomyces cerevisiae* and an upstream transit sequence of the matrix processing peptidase (MPP) from potato. The transit sequence of the MPP is to enable the transport of the CS into the mitochondria, where the heterologous CS increases the efficiency of the TCA cycle regarding the introduction of acetyl coenzyme A, thus indirectly enhancing the cellular energy production. According to the information provided by the applicant, such transgenic potato plants showed earlier and enhanced flower formation under greenhouse conditions.

III. 35S αPho2:

The construct codes for a cytosolic starch phosphorylase from potato in antisense orientation. Phosphorylases catalyse the reversible phosphorolysis of terminal glucose units of α-1,4-glucans.

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Depending on the concentration of inorganic phosphate and glucose-1-phosphate, the enzyme can both synthesise and degrade glucans. After having been stored at 20 °C, such transgenic potato plants showed an increased number of mature buds and produced more stems, with the mechanism not being clear. According to the information provided by the applicant, the tuber yield increased by 10-25% under greenhouse conditions.

IV. and V. 35S SoSUT and rolC SoSUT:

The construct codes for a sucrose transporter (SoSUT) from spinach. The heterologous SoSUT enhances the sucrose transport into the phloem, thus increasing the availability of sucrose for tuber formation and development. According to the information provided by the applicant, such transgenic potato plants showed a 10-20% increase in tuber yield under greenhouse conditions due to an increase in the number of tubers per plant.

VI. and VII. rolC ∆PMA1 and rolC ∆PMH2:

The construct codes for a proton ATPase from *Saccharomyces cerevisiae* (∆PMA1) and from *Solanum tuberosum* (∆PMH2). ∆PMA1 and ∆PMH2 cause increased loading of the phloem with photoassimilates. According to the information provided by the applicant, such transgenic potato plants showed a 10-20% increase in tuber yield under greenhouse conditions.

VIII. rolC Susy:

The construct codes for a sucrose synthase (Susy) from *Solanum tuberosum*. The additionally introduced Susy enhances the provision of energy for active loading of the phloem with photoassimilates as well as the development of young tubers and the mobilisation of carbohydrates for the synthesis of reserve starch. According to the information provided by the applicant, such transgenic potato plants showed a 10-20% increase in tuber yield and in the number of tubers per plant under greenhouse conditions.

In contrast to the changes in the transgenic potato plants intended by introducing the constructs IV to VIII, the integration of one of the constructs I to III is likely to induce more complex modes of action in the transgenic potato plants (e.g. changes in secondary metabolism).

These modifications in the genetically modified potato plants are not expected to pose any risk to human or animal health or to the environment.

(b) The *npt*II gene

The *npt*II gene transferred into the genetically modified plants codes for the enzyme neomycin phosphotransferase. It was inserted as a marker gene for the selection of transformed plant cells.

Neomycin phosphotransferase is a type-II aminoglycoside-3'-phosphotransferase (APH(3')II) that 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, and paromomycin belong to the APH(3')II enzyme substrates. The clinically relevant gentamicin and other aminoglycosides and aminocyclitols used in human medicine do not belong to the substrate spectrum of the APH(3')II enzymes. However, kanamycin and neomycin are widely used in veterinary medicine. Given the substrate specificity of neomycin phosphotransferase, it is not expected that new metabolic products will form in the genetically modified potatoes in the absence of substrate under field conditions no. Since the relevant antibiotics are not present in the soil in elevated 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-aminocyclitol antibiotics, such as kanamycin or geneticin, are not metabolised. Hygromycin is not used in human medicine.

Plants in the field are not treated with hygromycin. The presence of hygromycin phosphotransferase is thus not expected to confer a selective advantage to the genetically modified plants under field conditions. Given the enzyme's substrate specificity, the occurrence of physiologically relevant amounts of new metabolic products as a result of the expression of the hygromycin resistance gene in the genetically modified plants is not expected either.

(d) The coding sequence of the α-fragment of the ß-galactosidase, *lac*I sequences

The genetically modified plants were created by using derivatives of the vector pBIN19, the multiple cloning site of which is located within the sequence coding for the α fragment of the ß-galactosidase from *E. coli*.

The native enzyme β-galactosidase splits β-D-galactosides into galactose and the related alcohol compound. The physiologically most important substrate is lactose, which is hydrolysed into galactose and glucose. The first 146 amino-terminal amino acids of the β-galactosidase are referred to as the α fragment. The α fragment by itself is not enzymatically active; however, complementation in suitable hosts is possible.

The sequence coding for the α fragment of the β-galactosidase was interrupted by the insertion of the various expression cassettes into the multiple cloning site, preventing it from coding for an α fragment capable of complementation in *E. coli* bacteria. The interrupted sequence of the α fragment of the β-galactosidase is under the control of a bacterial promoter. This sequence does not code for a functional gene product. The presence of this sequence is not expected to cause any changes in the genetically modified potato plants.

The genetically modified plants additionally contain 5' and 3' sequences of the repressor gene *lacI*. However, these 5' and 3' sequences are separated from each other by the *lacZ* and M13 *ori* sequences. The *lacI* sequences are not expected to be functional in the genetically modified plants.

(e) M13 sequences

The genetically modified plants contain two fragments from M13mp19, namely a 440-bp fragment, which encompasses one part of an open reading frame of a structural protein of M13, and a 433-bp fragment, which contains the origin of replication of phage M13.

If transcription of the fragment of the open reading frame of the structural protein were to occur in the genetically modified potato plants, no functional protein would result, since the fragment only codes for 167 of the total 423 amino acids of the complete phage protein. The presence of this fragment is thus not expected to affect plant metabolism.

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

(f) The fragment of the *ocd* gene

The plants created by transformation using derivatives of the vector pBIN19 contain a fragment of the *ocd* gene (ornithine cyclodeaminase), which is located between the 3’ terminal end of the translated sequence of the *npt*II gene and the NOS terminator sequence. Since this sequence is transcribed as part of the mRNA of the *npt*II gene, but is located downstream of the termination codon of the *npt*II gene, this sequence is not expected to be translated.

(g) Border sequences from Ti plasmids and regulatory sequences

The genetically modified plants contain sequences of the left and right border region of the TL-DNA of the plasmid pTiT37 from *A. tumefaciens*. Depending on the gene products of the *vir* region of the helper plasmid pGV2260 that is contained in the *Agrobacterium* strain GV2260 used for transformation and is not transferred into the plants, these sequences cause the genes located between the border regions to integrate into the 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.

Integrated into the genome, the genetically modified plants contain the following regulatory sequences:

- The promoter region of the ST-LS1 gene (L700) from potato,
- The 35S promoter of the cauliflower mosaic virus (CaMV),
- The promoter of the rolC gene from *Agrobacterium rhizogenes*,
- The promoter and terminator of the nopaline synthase gene from *Agrobacterium tumefaciens*,
- The terminator of the octopine synthase gene and the gene 7 of the T-DNA from *Agrobacterium tumefaciens*.

In the genetically modified plants, the promoter and terminator sequences regulate the expression of the DNA sequences located between them in sense and antisense orientation as well as the expression of the *npt*II gene and the *hph* gene. Further information on the effects associated with the expression of these sequences in the plants can be found in III.1.2.1 (a), (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 border regions has been reported and cannot be ruled out based on the information provided in the application.

Based on the information provided in the application, the following functional units may have been transferred into the genetically modified potato plants in this particular case as a result of the integration of DNA fragments located outside the border regions:

(1) The *npt*III gene (codes for a type-III aminoglycoside-3'-phosphotransferase) for resistance to aminoglycoside antibiotics;

(2) The origin of replication *oriV* of the plasmid RK2;

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

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

(5) A non-functional fragment of the *klaC* gene from the plasmid RK2;

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

(7) The insertion element IS1 within the *npt*III gene;

(8) The origin of replication of the plasmid pMB1.

(1): A PCR analysis performed by the applicant demonstrated the presence of the *npt*III gene in 22 of the lines intended for release, whereas it was not detected in the remaining lines. Since the *npt*III gene is under the control of a bacterial promoter, it is not assumed to be expressed in plants. The gene is therefore not expected to affect plant metabolism.

(2) and (3): The origins of replication *ori*V (2) and *ori*T (3) of the plasmid RK2 allow replication of the plasmid in a broad host range of gram-negative bacteria and/or its conjugative transfer, as long as the mobilisation functions are provided by a helper plasmid.

(4), (5), (6), (7) and (8): There is no evidence to suggest that *ori*V or *ori*T of RK2, the origin of replication of pMB1 (8) or the remaining DNA fragments of bacterial origin (4, 5, 6, 7) have a function in higher plants. Moreover, some of the DNA fragments are incomplete (5) or interrupted (6).

(i) Position effects and context changes; allergenicity

Genes 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 neighbouring sequence at the integration site (“position effect”). Under field conditions, the expression level 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 altered to the same degree in the field as under climate-controlled or greenhouse conditions. This does not represent a risk to the environment or to human and animal health.

The insertion of foreign genes may influence the expression or regulation of endogenous plant genes at or near the site of insertion. Such processes can affect plant metabolic pathways. In previous work with the genetically modified plants, however, 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 and were first identified in maize. 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

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influence plant metabolic pathways. In this regard, the genetically modified plants to be deliberately released 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 potential allergenicity of a protein on the basis of its amino acid sequence. In previous experiments with the genetically modified plants as well as in earlier deliberate release trials with genetically modified plants that express the *npt*II gene or the *hph* gene under the control of non-tissue-specific promoters, no evidence was found to suggest an increased allergenic potential of the plants.

The expression of the DNA sequences in antisense orientation leads to the endogenous plant proteins occurring in lower concentrations or not at all. There is no evidence to suggest an allergenic potential of the proteins that are encoded by the heterologous DNA sequences in sense orientation.

Pollen of potato plants is dispersed by wind only to a little extent 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 Europe, the establishment of potatoes in natural ecosystems during this period has not been observed. 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, potatoes do not establish in these areas either. As a result of potato cultivation, "volunteer potatoes" can, depending on winter temperatures, emerge in the subsequent cultivation period from tubers that have overwintered in the soil.

The tubers are planned to be harvested and analysed, processed in a suitable laboratory for starch extraction or stored for replanting in the following year. Any remaining tubers will be inactivated, for example by steaming. The remaining transgenic plant parts will be left on the field to decompose. During the two vegetation periods following the deliberate release, no potatoes will be cultivated on the release sites. Volunteer potato plants will be removed in the year following the deliberate release.

Potato plants of various varieties can flower and produce seeds. It is conceivable that the genetically modified potatoes may be fertilised by the introduction of foreign potato pollen, which is why the formation of seeds cannot be ruled out completely, but is unlikely. Under Central European climate conditions, it is unlikely that potato seeds will overwinter and produce plants.

If tubers or seeds were to remain in the soil, any plants that would emerge from them would be identified within the scope of the post-trial monitoring proposed by the applicant in accordance with the supplementary provision II.9. A possible change in the frost sensitivity of the tubers as a result of the induced metabolic changes in the various lines cannot be ruled out. This possibility is adequately addressed by the proposed two-year cultivation gap and post-trial monitoring. This makes it possible to easily identify any volunteer potatoes.

For the reasons stated above, the genetically modified plants are not expected to persist or 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

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Attempts to crossbreed potatoes with solanaceous plants found in Central Europe were unsuccessful. 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.

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 mainly self-pollinating; cross-pollination is uncommon even within one flowering potato field and is most likely to occur between neighbouring plants.

The proposed isolation distance of 10 m between the release site and the nearest field with non-transgenic potatoes is considered adequate. However, should pollen be transferred to potato plants cultivated to produce table potatoes, 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. Even if the tubers of these plants were to be consumed, no health hazards would be expected to result – as stated in the evaluation summarised in III.1.2.1.

III.1.2.4 Assessment of the possibility of horizontal gene transfer of the inserted foreign genes from the genetically modified plants to microorganisms

The inserted sequences are stably integrated in the chromosomes of the recipient organisms. No evidence exists to suggest 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 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 that are as distantly related in terms of taxonomy as plants and bacteria is actually possible, it can 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 any heterologous genetic material, including all forms of plant DNA.

The inserted genes, including the regulatory sequences, are derived from potato, spinach, *E. coli, Agrobacterium tumefaciens* and *A. rhizogenes,* i.e. are already widespread in the environment. Horizontal gene transfer from non-genetically modified organisms to microorganisms in the environment is thus far more likely to occur.

As already elaborated in 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. It was thus necessary to examine whether the clinical use of the relevant antibiotics would be affected by a potential horizontal gene transfer of the *npt*II gene.

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In soil microorganisms, the inactivation of aminoglycoside antibiotics by phosphorylation is a naturally occurring resistance mechanism. 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 effective transfer by conjugation. Even in the event of horizontal gene transfer from the genetically modified potatoes to microorganisms, the overall frequency of this resistance mechanism in the environment would not be noticeably increased.

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

Hygromycin-resistant bacteria have been found in animal and human-derived specimens (faeces, urine, blood). As part of a study, the hygromycin phosphotransferase gene *hph* was also detected in the bacteria. The prevalence of hygromycin resistance genes is attributed to the use of hygromycin in veterinary medicine. Since hygromycin-resistant bacteria are released to the environment by the affected humans and animals and the *hph* genes – in the investigated cases – were located on plasmids, the spread of the trait of hygromycin resistance among the microorganisms present in the environment is possible. A gene for a hygromycin phosphotransferase is also found 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, preventing the formation of a functional gene product. This would also be the case in bacteria receiving the gene by horizontal gene transfer. The same applies to the 3' and 5' sequences of the *lac*I gene.

The situation is similar with 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, as elaborated in III.1.2.1 (f), the fragment of the *ocd* gene is not likely to be translated.

The genetically modified potatoes contain the origin of replication of M13. M13 belongs to the F-specific *E. coli* phages. In the case of this origin of replication, the probability of genetic spread by transfer between bacteria is thus far higher than the probability of horizontal gene transfer from the genetically modified plants to microorganisms.

In this particular case, the following DNA fragments may have been integrated into the genetically modified plants by the transfer of sequences located outside the border regions:

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

(ii) The origin of replication *ori*V of the plasmid RK2;

(iii) The *tra*F region, containing the *ori*T of the plasmid RK2;

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

(v) A non-functional fragment of the *kla*C gene from the plasmid RK2;

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

(vii) The transposon IS1 within the *npt*III gene;

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

A PCR analysis performed by the applicant demonstrated the presence of the *npt*III gene (i) in 22 of the lines intended for release, whereas it was not detected in the remaining lines. According to literature references, the *npt*III gene, which is under the control of 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 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 plants to microorganisms and the absence of selection 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 – vi), the probability of genetic spread by transfer between bacteria is far higher than the probability of 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) occurs naturally in various species of *Enterobacteriaceae*. It has been found, for example, in species of the genera *Escherichia*, *Shigella*, *Klebsiella*, *Serratia* and *Salmonella*. In the case of IS1, the number of copies per bacterial genome can be up to > 40. IS1 copies can have either a chromosomal or a plasmid location and have also been detected in prophages. It can be assumed that this insertion element would be easily spread by horizontal gene transfer between bacteria. In comparison, the probability of spread by horizontal gene transfer from the genetically modified plants to microorganisms, although theoretically conceivable, would be negligibly low.

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 be replicated 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

In order to generate the genetically modified plants, sterile potato leaves were incubated with agrobacteria which harbour 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 for the presence of agrobacteria by incubating plant homogenates in suitable growth media. Only potato plants that were free of agrobacteria were used further.

In contrast to the common wild types of *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 were transferred to a cell of another plant by these agrobacteria, this cell 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 plant progeny. 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 vector 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 event of infection and subsequent transformation by wild-type *A. tumefaciens* or *A. rhizogenes*, a crown gall or hairy root would develop from the transformed plant cell. Under natural conditions, such a tumour would not be expected to give rise to a plant.

Furthermore, the transfer of the inserted genes from agrobacteria to other soil bacteria would have to be considered. The potential effects of such a transfer were already addressed in III.1.2.4.