Notification 6786-01-0175

Summary of the risk assessment of genetically modified potato plants (*Solanum tuberosum L.*) (transformation events BG1BA-24, -31 and -32) carried out by the German Competent Authority within the framework of a proposed deliberate release, Berlin, 11 Max 2006

Explanatory note to this document:

The following text reflects the summary of the risk assessment of (a) genetically modified organism(s) to be used for experimental field trials (deliberate releases) in Germany. The text forms part of the official authorisation regarding applications for the permit of deliberate releases (field trials) of genetically modified organisms in Germany under the legal framework of Directive 2001/18/EC and the German Gene Technology Act (Gentechnikgesetz, GenTG). The authorisation is issued by the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL [*Federal Office of Consumer Protection and Food Safety*], as the German Competent Authority. It comprises the chapters

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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 sequence

(a) The genes for the plastid metabolite translocators *gpt* and *ntt*

The purpose of the deliberate release is to corroborate the results of greenhouse trials which showed that a transformation with the genes that code for two plastid metabolite translocators (glucose-6-phosphate/phosphate and nucleotide translocator) resulted in an increase in the yield and starch content of tubers of the genetically modified potato plants relative to the non-genetically modified parental variety under field conditions. In particular, the influence of the photoassimilate distribution between the sites of synthesis (sources) and the sites of consumption (sinks) is to be examined under field conditions to test whether the findings from the greenhouse trials showing that the expression of the two plastid translocators in the tubers of the genetically modified potatoes have a positive effect on the sink starch of the plants can be confirmed. Should that be the case, further experiments aimed at a higher tuber yield by simultaneously increasing the *source* capacity in the plants could be carried out. Furthermore, the planned trial is designed to examine whether the increased tuber yield of the genetically modified plants is attributable to the artificial greenhouse conditions and whether, under field conditions, the genetically modified potatoes no longer demonstrate a growth advantage with regard to their tubers or whether growing under field conditions might, on the contrary, lead to an even higher fresh tuber weight relative to the parental variety.

The coding region of the *gpt* gene contained in the pBinB33(Hyg)::GPT, which codes for a plastid glucose-6-phosphate/phosphate translocator, originates from pea (*Pisum sativum*); its expression is controlled by the B33 promoter of the patatin class I gene from *Solanum tuberosum*. Selection of the promoter allows for tissue-specific expression of the gene since the B33 promoter is active mainly in the tubers of the potato plants. Expression in the shoot region can also be induced by sucrose. The glucose-6-phosphate/phosphate translocator is an antiporter whose main physiological function is to import glucose-6-phosphate, in exchange for anorganic phosphate, into the plastids of heterotrophic tissue. Here glucose-6-phosphate functions as a precursor for the energy-dependent synthesis of starch, whereby anorganic phosphate is released. Besides glucose-6-phosphate and anorganic phosphate, other substrates of the GPT are triose-phosphate and 3-phosphoglycerate, but not other hexose-phosphates such as glucose-1-phosphate or fructose-6-phosphate.

The coding region of the *ntt* gene contained in the pBinB33(Kan)::NTT construct, which codes for a plastid nucleotide translocator, originates from *Arabidopsis thaliana*; in this case, too, the expression is controlled by the B33 promoter of the patatin class I gene from *Solanum tuberosum*. The nucleotide translocator is also an antiporter which catalyses the plastid uptake of ATP in exchange for ADP and is consequently also referred to as the ATP/ADP translocator. Its main function is to supply non-green storage plastids with ATP, which is needed there for starch synthesis.

For the control of transcription termination, both constructs contain the terminator of the *ocs* (octopine synthase) gene from *Agrobacterium tumefaciens* at the 3'-end of the coding region of the respective metabolite translocator.

Three lines (BG1BA-24, -31 and -32) in which the expression of the inserted metabolite translocators was demonstrated by northern blot analysis were selected for the deliberate release. Under greenhouse conditions, expression of the two genes *gpt* and *ntt*, homologues of which exist in non-genetically modified potato tubers, led to an increase of the transport ac-

tivities for glucose-6-phosphate and ATP in the genetically modified potato plants, which in turn resulted in an increased tuber yield as well as an increase in the starch content in the tubers of the genetically modified potato plants relative to the non-genetically modified parental variety. Apart from that, in the greenhouse, no phenotypic differences between the genetically modified and the non-genetically modified plants were observed with respect to growth, size, leaf morphology, leaf colour or root formation. In addition, biochemical analyses showed no further effects on photosynthesis or the content of soluble sugars.

A change in the genetically modified potato plants in terms of constituents that may be toxic or harmful to health due to unintended effects of the genetic modification on the plant metabolism is, in principle, conceivable. Investigations in this regard were not carried out. However, the genetically modified potatoes are not intended for human or animal consumption, and the release will be carried out on a fenced and marked trial area, so that the execution of the proposed experimental trials is not expected to result in any risks to animal or human health.

(b) The *hph* gene

Plants transformed with the construct pBinB33(Hyg)::GPT contain a *hph* gene from *Streptomyces hygroscopicus* as a selection marker. The hygromycin-phosphotransferase (HPT) encoded by the *hph* gene specifically inactivates the antibiotic hygromycin by phosphorylation. This substrate specificity justifies the expectation that in the absence of substrate no new metabolic products can be synthesised in the genetically modified plants under field conditions. Moreover, this gene does not confer any selective advantage to the genetically modified plants under field conditions, since hygromycin is not present in the soil in higher concentrations.

(c) The *npt* II gene

The *npt* II gene transferred to the genetically modified plants encodes the enzyme neomycin phosphotransferase. It was introduced as a marker gene for selecting transformed plant cells.

The neomycin phosphotransferase is a type II aminoglycoside 3'-phosphotransferase (APH(3')II), which catalyses the 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. Substrates for the APH(3')II enzyme include the antibiotics kanamycin, neomycin, geneticin, butirosin, gentamicin A and B, and paromomycin. Clinically relevant gentamicins and other aminoglycosides and aminocyclitoles used in human medicine do not belong to the substrate spectrum of the APH(3')II enzymes. Kanamycin and neomycin are however widely used in veterinary medicine.

Given the substrate specificity of neomycin phosphotransferase, in the absence of substrate under field conditions, no new metabolic products are expected to be synthesised 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 on the genetically modified plants. There is no evidence to suggest that this enzyme is toxic to plants, animals, micro-organisms or humans.

(d) Additional DNA fragments located within the T-DNA

In addition to the sequences already described above, the plasmids used in the transformation of the potato plants also contain nucleotides of the *lac*I gene and the *lac*Z gene from *E. coli*, the origin of replication and the gene III of the *E. coli* phage M13, as well as parts of the *ocd* gene from *A. tumefaciens*. These are not functional in plants.

(e) Sequences located outside the T-DNA

As a general rule, only the DNA located within the border regions is integrated into the plant genome during *Agrobacterium*-mediated transformation. However, the transfer of DNA fragments outside the border regions has been reported.

The transformation plasmids pBinB33(Hyg)::GPT and pBinB33(Kan)::NTT used here are both derived from the binary vector pBin19 and contain the following genetic elements outside the border regions:

- the *ori*T of the RP4 plasmid from *E. coli*;
- nucleotides of the *kla*C gene from *Klebsiella aerogenes*;
- the transposable insertion sequence IS1 from *E. coli*;
- nucleotides of the *tra*F gene of the RP4 plasmid from *E. coli*;
- the origin of replication *ori*V of the RK2 plasmid from *E. coli*;
- the *trf*A gene of the RK2 plasmid from *E. coli*;
- the *tet*A gene of the RK2 plasmid from *E. coli* (interrupted by the T-DNA);
- the origin of replication of the ColE1 plasmid (ColE1 *ori*) from *E. coli*;
- the *npt* III gene from *Streptococcus faecalis.*

A PCR analysis carried out by the applicant demonstrated the presence of the *npt* III gene in two (lines 24 and 32) of the three transformants proposed for release, leading to the assumption that all of the genes contained in the plasmids pBinB33(Hyg)::GPT and pBinB33(Kan)::NTT may have been integrated into the plant DNA. Because the presence of the *npt* III gene was only tested by PCR analysis and not by Southern blot analysis, it also cannot be completely ruled out for line 31 that a part of the *npt* III gene, whose product might yet show activity, is integrated into the plant genome. As a result, the risk assessment of all three lines (24, 31 and 32) is carried out under the assumption that all genes located outside the border regions of the two transformed vectors might be integrated into the plant DNA.

However, these sequences would not be expected to produce functional gene products because they are not under the control of plant-specific promoters.

(f) Position effects and context changes; allergenicity

Genes which have been integrated into the plant genome by genetic engineering are expressed at different levels, depending on the integration site on the chromosome or rather 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 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-controlled 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 these genetically modified plants, no observations indicating the occurrence of such an event were made.

Mobile genetic elements (transposable elements), which when transposed within the genome can exert effects on existing plant genes at the target site, occur naturally in plants. The inactivation of genes or alterations in gene regulation also take place in a range of other naturally occurring processes such as point mutations, deletions or translocations and are traditionally used in plant breeding. Therefore, even in non-genetically modified plants 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 their 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 the amino acid sequence. However, in numerous releases of plants that express the *hph* gene or the *npt*II gene under the control of non-tissue-specific promoters no evidence has been found to indicate an increased allergenicity of the plants. In any case, pollen of potato plants is only dispersed to a small extent by wind and does not generally play a noteworthy role in triggering 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. 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. However, 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. Potato plants are occasionally found beyond cultivated areas, but only on non-natural sites such as verges and other ruderal areas. Because potatoes are not frost hardy they do not form persistent populations in these areas either.

The tubers of the experimental plants will be harvested, examined, weighed and brought to the S1 laboratories of the University of Cologne (genetic engineering facility 64-K-1.90/02) for further analyses. Surplus potato tubers will be inactivated by autoclaving. The leaves of the potato plants will be left on the trial site to decompose.

After harvesting, the release site will be tilled in order to force any residual tubers to the surface and to level the area. Crop rotation on the experimental site is designed in such a way that potatoes will not be grown on the respective areas for a minimum of three years following the release of the genetically modified potatoes. Within this two-year cultivation gap, after completion of the deliberate release, the site be monitored for volunteer potato plants for a period of one year as required by provision II.11 (to the present application). If genetically modified volunteer potato plants are detected during that period of observation, the monitoring period is to be extended for a further year.

Potato plants can flower and bear fruit. Under Central European climate conditions it is unlikely that potato seeds will overwinter and produce plants. Should tubers or seeds remain in the soil, the resulting plant growth would be detected during post-trial monitoring.

The increased tuber yield of the genetically modified potato plants observed under greenhouse conditions could result in increased post-emergence following mild winters due to the higher number of tubers produced in comparison to the non-transformed parental variety. But even when this factor is taken into account, the genetically modified potato plants are not expected to exhibit altered plant-physiological characteristics in comparison to conventional crop potatoes nor are they likely to establish in natural ecosystems. The possibility of increased emergence of volunteer plants from tubers will be sufficiently controlled within the framework of the deliberate release trial through the planned post-trial monitoring measures.

III.1.2.3. Assessment of the possibility of a 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 no outcrossing 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. Potato and *Solanum dulcamara* (bittersweet or woody nightshade) proved to be strictly bilaterally incompatible species; in crossbreeding experiments pollination of the ovule was not achieved. Similarly, potato does not cross-breed with tomato (*Lycopersicon esculentum*). In agricultural practice, potatoes are propagated vegetatively via tubers.

Hence the following passage deals solely with a possible pollen transfer from the genetically modified potato plants to other potato plants. Pollen of potato plants can be transferred by insects or by wind. However, wind dispersal takes place only over short distances. Potatoes are both self- and cross-pollinating. In a field of flowering potato plants, mainly self-pollination takes place; cross-pollination is rare and is most likely to occur between neighbouring plants.

The separation distance of at least 20 metres to neighbouring potato plantings proposed in the application is considered sufficient. Should pollen nevertheless be transferred to potato plants being cultivated for the production of table potatoes, no adverse effects would be expected since planting material for the agricultural cultivation of potatoes is propagated vegetatively, i.e. not via seeds.

As already elaborated above, the probability that potentially generated seeds could give rise to plants under the given climate conditions is very slight. On areas used for agriculture, such plants would be eliminated by conventional soil preparation practices during the course of crop rotations.

III.1.2.4. Assessment of the possibility of a transfer of the inserted foreign genes from the genetically modified plants to micro-organisms by horizontal gene transfer

The inserted sequences are stably integrated into the chromosomes of the recipient organisms. There is no evidence that a 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 ge-

netic material to soil bacteria is also theoretically possible, although it is assumed that a gene transfer of this type would constitute an extremely rare event.

If 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 genes for the plastid metabolite translocators *gpt* and *ntt*

The *gpt* (glucose-6-phosphate/phosphate translocator) gene present in the pBinB33(Hyg)::GPT construct is derived from the pea (*Pisum sativum)* and the ntt (nucleotide translocator) gene contained in the pBinB33(Kan)::NTT construct is derived from *Arabidopsis thaliana*; i.e. both genes are already commonly found in the environment. Therefore a horizontal gene transfer in micro-organisms is much more likely to occur via non-genetically modified organisms.

(b) The antibiotic resistance genes *hph* and *npt* II

The *hph* gene, which codes for the enzyme hygromycin phosphotransferase (HPT), was isolated from *Streptomyces hygroscopicus*. Due to its high toxicity for eukaryote organisms, hygromycin is not used in human medicine and only for specific indications in veterinary medicine. Hygromycin-resistant *Enterobacteriaceae* containing a gene for a hygromycin phosphotransferase have been found in sample material (faeces, urine, blood) of animal and human origin and are released into the environment by these animals/humans.

In the genetically modified plants, the *npt* II gene, like the *hph* gene, is under the control of the *nos* promoter. The gene codes for the enzyme aminoglycosid-3'-phosphotransferase II (APH(3')II), which catalyses the ATP-dependent phosphorylation of certain aminoglycoside antibiotics (kanamycin, neomycin, geneticin), causing them to become inactive.

As already outlined in point III.1.2.1 (c), the antibiotics inactivated by the neomycin-phosphotransferase are of little relevance in human medicine, but they are widely used in veterinary medicine. It was therefore necessary to examine whether the clinical use of the relevant antibiotics would be compromised by a potential horizontal gene transfer of the *npt*II gene.

The inactivation of aminoglycoside antibiotics by phosphorylation is a naturally occurring resistance mechanism in soil micro-organisms. 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 use of these antibiotics, and by the fact that these genes are often located on plasmids, enabling their effective transfer between micro-organisms by conjugation. Even if a horizontal gene transfer from the genetically modified potatoes to micro-organisms were to occur, the overall frequency of this resistance mechanism would not be noticeably increased.

The Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) has assigned the genes *hph* and *npt* II to the group of genes which, with regard to safety, provide no grounds to restrict or ban their usage, either for field trials or for the purpose of placing on the market. In its statement of 6 July 1999 on the biological

safety of antibiotic resistance genes in the genome of genetically modified plants, the ZKBS (Central Committee on Biological Safety) allocated the *hph* gene and the *nptII* gene to the group of antibiotic resistant genes which "(a) are already widespread in soil bacteria and enterobacteria and (b) whose relevant antibiotics have no, or only little significance in human and veterinary medicine, so that one can assume that the presence – if any - of these antibiotic resistance genes in the genome of transgenic plants will have no effect on the spread of these antibiotic resistance genes in the environment".

(c) The antibiotic resistance gene *npt* III

The *aphA* III (= *npt* III) gene, which codes for the enzyme 3´-5´-aminoglycoside phosphotransferase type III, was isolated from *Streptococcus faecalis*. According to the literature, this gene, which has been shown to be present along with its native promoter in two (lines 24 and 32) of the three transformants proposed for release, confers resistance not only to kanamycin and neomycin, but also to the antibiotic amikacin. Amikacin is not approved for use as a veterinary drug in Germany, but it may be used in human therapy where it is an important reserve antibiotic. Resistance to amikacin is so far not widespread and a further spread is not desirable.

In its opinion of 2 April 2004, the Scientific Panel of the EFSA concluded that, for precautionary reasons, antibiotic resistance genes of group III, to which the *npt* III gene was also allocated, should no longer be contained in plants intended for placing on the market and for deliberate release trials in view of their current importance in clinical human medicine.

The EFSA opinion was drawn up in connection with the implementation of Article 4(2) of Directive 2001/18/EC. According to that Directive, GMOs containing genes which confer resistance to antibiotics used for medical or veterinary treatment must be taken into particular consideration when carrying out an environmental risk assessment with a view to identifying and phasing out antibiotic resistance markers in GMOs which may have adverse effects on human health and the environment. This phasing out shall take place by the 31 December 2004 in the case of GMOs placed on the market according to part C and by 31 December 2008 in the case of GMOs authorised under part B.

Concerning the present application, the ZKBS found that the placing on the market of genetically modified organisms which contain the *aphA* III (= *npt* III) gene is no longer acceptable in view of the current state of science and technology. However, it also pointed out that the use of such genetically modified organisms is currently still tolerated within the scope of deliberate release experiments.

The genetically modified potato plants are to be released on a limited area only for two vegetation periods (2006 and 2007). The plants may not be used for animal or human consumption. Due to the very low likelihood of a horizontal gene transfer from plant DNA to micro-organisms and the absence of selection pressure on the trial areas, the presence of the *npt*III gene in the genetically modified potato plants is unlikely to lead to a significant increase in the overall frequency of this resistance mechanism in micro-organisms.

(d) Regulatory sequences

Even in the event of a transfer of the regulatory sequences used in the two constructs, an increase in the overall frequency of the respective DNA fragments is not expected to occur. These regulatory sequences originate from *A. tumefaciens* and the potato (*Solanum tu-*

berosum) itself. *A. tumefaciens* is widespread in the environment, and the sequences in question are found in wild-type Agrobacteria on Ti-plasmids which can be exchanged between different Rhizobiaceae by conjugation.

(e) Sequences of the *lac*I gene and the *lac*Z gene from *E. coli*

The genes *lac*I and *lac*Z originate from *E. coli* and are therefore widespread in the environment. The presence of parts of these genes in the genetically modified potato plants is therefore not expected to pose any hazard.

(f) M13 sequences

pBin19 and its derivatives contain two fragments from the M13 phage within the T-DNA, namely, a 440-bp fragment comprising a component of an open reading frame of a structural protein from M13 and a 433-bp fragment containing the origin of replication of the M13 phage. Because the M13 phage belongs to the F-specific *E. coli* phages, the likelihood of a spread of the above-mentioned DNA fragments by transfer between bacteria is far greater than the likelihood of a spread by horizontal gene transfer from the genetically modified plants to micro-organisms. Should expression of the gene fragment for the structural protein take place, a functional protein would not result because the fragment encodes only 167 of a total of 423 amino acids of the complete phage protein. This part of the structural protein is not expected to be functional in bacteria.

(g) Additional sequences located outside the T-DNA

The genetically modified potatoes may contain the following genetic elements, which are located on the pBin19 derivatives outside the border regions:

- the *ori*T of the RP4 plasmid from *E. coli*;
- nucleotides of the *kla*C gene from *Klebsiella aerogenes*;
- the transposable insertion sequence IS1 from *E. coli*;
- nucleotides of the *tra*F gene of the RP4 plasmid from *E. coli*;
- the origin of replication *ori*V of the RK2 plasmid from *E. coli*;
- the *trf*A gene of the RK2 plasmid from *E. coli*;
- the *tet*A gene of the RK2 plasmid from *E. coli* (interrupted by the T-DNA);
- the origin of replication of the ColE1 plasmid (ColE1 *ori*) from *E. coli*.

RK2 and RP4 belong to a group of broad-host-range plasmids (including RP1, R18 and R68) which are capable of replication in numerous gram-negative bacteria. Therefore, for the DNA fragments derived from RK2 and RP4, the likelihood of spreading by transfer between bacteria is far greater than the likelihood of spreading by horizontal gene transfer from the genetically modified plants to micro-organisms. Moreover, some of the DNA fragments are interrupted or incomplete (*kla*C, *tra*F, *tet*A).

Plasmids of the ColE1 type have a host range that is limited to a small number of gram-negative bacteria. Essentially, the replicon has the ability to replicate in *E. coli* and other closely related species of bacteria. In most gram-negative soil bacteria, replication does not occur. ColE1 plasmids occur quite commonly in enterobacteria. The likelihood of gene transfer from enterobacteria to other bacteria is considered to be far greater than the likelihood of a horizontal gene transfer from the genetically modified plants to bacteria. The potential presence of the replication origin of ColE1 in the plant chromosome is therefore not expected to contribute to an increase in the overall frequency of horizontal gene transfer.

The insertion element IS1 occurs naturally in different species of Enterobacteriaceae. It has, for example, been found in species of the genera *Escherichia, Shigella, Klebsiella, Serratia* and *Salmonella*. In IS1 the number of copies per bacterial genome can amount to more than 40. Copies of IS1 may be localised on the chromosome or the plasmid and were also found in prophages. These insertion elements are likely to be easily spread by horizontal gene transfer between bacteria. Therefore, in comparison, the theoretically conceivable risk of a spread by horizontal gene transfer from the genetically modified plants to micro-organisms is negligible.

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

An *Agrobacterium*-mediated binary transformation system was used to generate the genetically modified plants. The transformants were then tested for the absence of Agrobacteria. Subsequently, only plants that were free of Agrobacteria were used.

In contrast to the ubiquitous wild-type *A. tumefaciens*, the *Agrobacterium* strain GV2260 used here 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 by these Agrobacteria, that 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 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 genetically modified plants cannot be ruled out, a potential transfer by conjugation of the binary plasmids contained in the Agrobacteria to wild-type Agrobacteria (*A. tumefaciens* or *A. rhizogenes*) present in the environment 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 via 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.

Furthermore, a possible transfer of the inserted genes from Agrobacteria to other soil bacteria must be considered. The potential impact of such a transfer has already been addressed in III.1.2.4.