Notification 6786-01-00083

Summary of the risk assessment of genetically modified oilseed rape plants (*Brassica napus* L.*)* carried out by the German Competent Authority within the framework of a proposed deliberate release,

Berlin, 29 April 1998

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 oilseed rape plants effected by the transferred nucleic acid sequence

(a) The acyl-[ACP] thioesterase gene (*Cl*FatB3)

Acyl-[ACP] thioesterases hydrolyse the thioester ligation between the ACP (acyl carrier protein) and the synthesised acyl chain in fatty acid biosynthesis. Caprylic- and palmitic-[ACP] are substrates of the *Cuphea lanceolata*-derived enzyme which is encoded by the acyl-[ACP] thioesterase gene (*Cl*Fat*B3*). As a result of the formation of this enzyme the oil produced in the seeds of the genetically modified oilseed rape plants contains capric acid ($C_{10:0}$), which is not normally present in rapeseed oil, and increased levels of palmitic acid ($C_{16:0}$).

The expression of the *Cl*FatB3 gene contained in the genetically modified oilseed rape plants is contolled by its seed-specific promoter and its termination signal. The upstream position of the fourfold enhancer of the CaMV 35S promoter is supposed to amplify the expression in a further construct.

In the genetically modified oilseed rape plants, the newly formed acyl-[ACP] thioesterase catalyses the same reaction as corresponding enzymes which occur naturally in the seeds of other (wild and cultivated) plant species. There is no evidence to indicate that either the enzyme or the new metabolic product would have a toxic effect. Both the new fatty acid (capric acid) and the fatty acid that occurs at a higher concentration (palmitic acid) occur naturally in other vegetable oils used for human consumption (e.g. coconut oil).

(b) The oleate desaturase gene in antisense orientation

As a result of the expression of subgenic domains of an oleate desaturase gene (E12) from *B. napus* in antisense orientation, desaturation of oleic acid ($C_{18:1}$) to linoleic acid ($C_{18:2}$) is inhibited in the genetically modified oilseed rape plants. As a result, the oleic acid content is increased in the seeds of these oilseed rape plants, while the linoleic and linolenic acid content is reduced. Expression of the antisense construct is controlled by the promoter of the *Cl*FatB4 gene from *C. lanceolata* and the corresponding termination signal. Oleic acid is a component of oils used for human consumption. Larger polypeptides are not encoded by the transferred DNA segment in antisense orientation.

(c) The lysophosphatidic acid acyltransferase gene from *Limnanthes douglasii* (*Ld*LPAAT)

Erucic acid, which accounts for approximately 50 % of the oil content of rape seeds, is the characteristic fatty acid of traditional oilseed rape. Since the mid-1970s, conventional plant breeding efforts have enabled the development of erucic acid-free oilseed rape varieties, thus making possible the use of rapeseed oil as a foodstuff (e.g. margarines, cooking oils). Rapeseed oil that contains erucic acid is not suitable for human consumption; however, it is used increasingly in the technology, cosmetics and pharmaceuticals sector. Therefore, in addition to developing erucic acid-free varieties, plant breeding efforts also focus on developing oilseed rape varieties with the highest possible erucic acid content for the non-food sector.

The lysophosphatidic acid acyltransferase gene from *L. douglasii* (*Ld*LPAAT) specifically catalyses the linkage of erucic acid to the sn-2 position of lysophosphatidic acid. The expression of the gene takes place under the control of the seed-specific promoter and the termina-

tion signal of the pNa napin gene from *B. napus*. As a result of the transformation, trierucin is produced in the seeds of the genetically modified oilseed rape plants, which should increase the erucic acid content in the oilseed rape seeds. Due to their basic function in the primary metabolism, lysophosphatidic acid acyltransferases are expected to be widespread in nature and regularly ingested with food. There is no evidence indicating that the enzyme would have a toxic effect.

(d) The β-ketoacyl-[ACP] synthase gene from *B. napus* (KAS)

In the fatty acid biosynthesis ketoacyl synthases (KAS), which are active in the plastids, condense acyl residues with a malonyl-[ACP] residue while giving off CO_2 thus leading to elongation of the fatty acid chain. At the membrane of the endoplasmic reticulum, other ketoacyl synthases (elongases) catalyse the formation of longer fatty acids by elongating the oleic acid exported from the plastids. KAS are key enzymes of fatty acid biosynthesis. They are ubiquitous in the environment and are ingested regularly with food. There is no evidence to indicate that the enzyme would have a toxic effect.

(e) The lysophosphatidic acid acyltransferase gene from *B. napus* (*Bn*LPAAT)

The LPAAT gene from *B. napus* also encodes a lysophosphatidic acid acyltransferase (*Bn*LPAAT) which, however, unlike the *Ld*LPAAT mentioned above, does not link any specific fatty acids to the sn-2 position of lysophosphatidic acid. Transformation of the oilseed rape plants with an antisense construct of the *Bn*LPAAT gene is supposed to lower the expression of the endogenous LPA-acyltransferase and thereby reduce the linkage of unspecific fatty acids to the sn-2 position of lysophosphatidic acid. The simultaneous expression of *Ld*LPA acyltransferase is instead supposed to promote production of trierucin in this genetically modified oilseed rape through the specific binding of erucic acid at this position.

(f) 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 gene 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 high substrate specificity. The antibiotics kanamycin, neomycin, geneticin, butirosin, gentamicin A and B, and paromomycin belong to the APH(3')II enzyme substrates. Therapeutically important gentamicins and other aminoglycosides and aminocyclitoles used in human medicine do not belong to the substrate spectrum of the APH(3')II enzyme. Kanamycin and neomycin are, however, widely used in veterinary medicine. Due to the substrate specificity of neomycin phosphotransferase, no new metabolic products are expected to arise in the genetically modified plants in the absence of substrate under field conditions. Since high concentrations of the relevant antibiotics are not present in the soil, 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.

(g) Border sequences from Ti-plasmids and regulatory sequences

The genetically modified plants contain sequences from the left and right border regions of the T_L-DNA of the plasmid pTiB6S3 from *Agrobacterium tumefaciens*. Depending on the gene products of the *vir* region of the helper plasmid pMP90RK present in the *Agrobacterium* strain used for the transformation, which was not transferred to the plants, these sequences effected the integration of the genes located between the border region into chromosomes of the oilseed rape plants. These border regions of the Ti plasmids have no function in the genetically modified oilseed rape plants and are not expected to cause any changes in the plants.

In addition to the 35S promoter and terminator of cauliflower mosaic virus (CaMV), the following were also transferred to the genetically modified oilseed rape plants as regulatory sequences: the promoters and the terminator signals of the *Cl*FatB3 gene, the *Cl*FatB4 gene (both from *C. lanceolata*), the pNA napin gene from *B. napus* or the Dc3 promoter from *Daucus carota* in conjunction with the Ω-leader of tobacco mosaic virus (TMV). The promoter and termination sequences regulate the expression of the coding sequences of the transferred genes located between them in the genetically modified oilseed rape plants. Statements on the effects of the formation of these proteins in the plants can be found under point III.1.2.1.(a) to (f).

(h) Sequences located outside the T-DNA

As a rule, only DNA sequences located within the border regions are integrated into the plant genome in *Agrobacterium*-mediated transformations. However, the transfer of DNA sequences from outside the border regions has been reported in individual cases and, based on the information contained in the application, this possibility cannot be ruled out. As a result, the risk assessment also considers those regions on the vector used for transformation of the oilseed rape which are located beyond the T-DNA border regions. These include, in particular, the following DNA fragments:

- The *aadA* gene

The *aadA* gene encodes the enzyme streptomycin adenyltransferase. As a marker gene it is a component of the vector pRE1, from which the individual vectors used for the transformation of the oilseed rape were developed.

Streptomycin adenyltransferase is an aminoglycoside-3-adenyltransferase (AAD(3´´)(9)) which catalyses the adenylation of the 3´´-OH-group of the N-methyl-L-glucosamine ring of streptomycin and the 9-hydroxyl group of spectinomycin causing these to become inactivated. The enzyme is characterised by high substrate specificity. Substrates of the AAD(3´´)(9) enzyme include streptomycin and spectinomycin. Today, streptomycin is used only for specific indications in human medicine (e.g. tuberculosis).

The *aadA* gene is controlled by a prokaryotic promoter. As a result, this gene is not expected to be expressed in plants. Hence effects on plant metabolism are just as unlikely to occur as effects on humans or animals as a result of the potential consumption of the genetically modified plants or parts thereof.

- The replication origins of the pRE1 plasmid

The plasmids used to generate the genetically modified oilseed rape plants are derived from the pRE1 plasmid. In addition to the ColE1 origin of replication for replication in *E. coli,* out-

side the T-DNA the replication region they also contain the replication region of the pVS1 plasmid from *Pseudomonas aeruginosa*, strain PAT, with the genetic information for replication and stability. The pVS1 plasmid has a narrow host range (e.g. *Pseudomonas* species, *Aeromonas formicans, Agrobacterium tumefaciens, Rhizobium leguminosarum*). The plasmid is not stable in *E. coli*. In this particular case the replication region of the pVS1 plasmid serves to replicate the binary vector in *A. tumefaciens*.

There is no evidence that the replication regions of ColE1 and pVS1 have any function in higher plants.

(i) Position effects and context changes; allergenicity

The level of expression of genes that have been integrated into the plant genome by genetic engineering methods is dependent on the site of insertion on the chromosome and/or on the environment around the insertion site (position effect). Under field conditions the levels of expression may be additionally influenced by environmental factors, for instance, by temperature. In the present case this could mean that the genetically modified oilseed rape plants might not produce the same levels of the expected fatty acids in the field as under 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 native plant genes at or near the site of insertion. Such processes may alter plant metabolic pathways. However, during propagation of the genetically modified plants in the greenhouse and in previous deliberate releases of some of the genetically modified oilseed rape lines with genetically engineered modifications of the storage lipid compositions, 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. 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 influence plant metabolic pathways. With regard to these properties the genetically modified plants proposed for release here do not differ fundamentally from non-genetically modified plants.

With the current state of knowledge it is not possible to predict the potential allergenic effect of a protein on the basis of its amino acid sequence. However, through the use of seed-specific promoters, the expression of the transferred genes and gene fragments of fatty acid metabolism is limited to the stages of seed development and seed maturation.

Expression of the *nptII* gene is regulated by the constitutive 35S promoter of CaMV. In previous greenhouse trials with these genetically modified plants as well as in deliberate releases of other genetically modified plants that express the *nptII* gene under the control of the 35S promoter, no evidence of increased allergenicity of the plants was found.

Expression of the *aad*A gene is controlled by prokaryotic regulatory elements. Therefore, its expression in plant tissues is not anticipated.

III.1.2.2. Evaluation of the ability of the genetically modified oilseed rape plants to persist or establish in the environment

Due to the proposed measures for minimising dispersal through pollen or seed, the spread of the genetically modified oilseed rape plants beyond the trial area is not to be expected (in this regard, see the description of the trial in the application, provisions II.5. to II.7. as well as the statements under point III.1.2.3.).

Summer oilseed rape is an annual, winter oilseed rape a perennial plant. Following the generative phase the plant dies off; new plants can only emerge from the seeds produced. If they become buried deep in the soil and enter secondary dormancy, rape seeds can persist in the ground for over 20 years. More recent studies show that alterations in the fatty acid composition of the storage lipids of the seeds can affect seed persistence in oilseed rape. However, whether the capric acid and the higher levels of palmitic, oleic and/or erucic acid or the lower levels of linoleic and linolenic acid produced in these genetically modified rape-seeds do in fact influence their capacity to persist is not known at the present time.

In any case, the persistence of seeds from the genetically modified oilseed rape and from any potentially occurring oilseed rape hybrids can be minimised by taking appropriate measures after every harvest to ensure that any seeds released are brought to germination during the same vegetation period. The plants that emerge from these seeds can easily be destroyed. The applicant plans to implement corresponding measures.

After the above measures have been implemented, while the soil is being prepared for future conventional agricultural use, as planned, any seeds from oilseed rape or oilseed rape hybrids that remain in the soil will be brought close to the soil surface where they can germinate. The resulting plants will be identified and destroyed, either during the inspections planned by the applicant within the crop rotations or during the multi-year cultivation pause for oilseed rape and/or by the measures required by provision II.7. and the post-trial monitoring period. If genetically modified oilseed rape plants or oilseed rape hybrids continue to emerge during the third post-trial monitoring year, the monitoring period is to be extended by a further year. To ensure that re-emerging plants can be detected, no oilseed rape plants will be cultivated on the trial plot during the monitoring period after the experiment has been completed. This cultivation pause and the monitoring measures carried out during the trial ensure that any potentially re-emerging oilseed rape plants and oilseed rape hybrids can be identified.

The potential emergence of individual genetically modified oilseed rape seedlings on the release site after the end of the post-trial monitoring period does not pose a risk with regard to pollen transfer to other plants (see III.1.2.3.) or long-term establishment.

Outside cultivated sites oilseed rape is found only as a weed in or near areas where the crop is grown, e.g. on waysides and other ruderal sites. Oilseed rape is not capable of establishing in natural, intact plant communities.

The genetically modified oilseed rape plants are not expected to develop altered plant sociological traits as a result of the inserted genetic modifications nor are they expected to populate other biotopes. They do not have any apparent selective advantage over other plants. Therefore, even in the event of the emergence of individual genetically modified oilseed rape seedlings and the possible transfer of pollen to non-genetically modified plants, no long-term,

sustainable spread of the genetically modified oilseed rape is expected. The temporal and spatial limitation of the release is thus sufficiently guaranteed.

III.1.2.3. Assessment of the possibility of pollen-mediated transfer of the inserted genes from the genetically modified oilseed rape plants to other plants

Oilseed rape stocks are about two thirds self-pollinating and one third cross-pollinating. The oilseed rape pollen is dispersed primarily by insects (particularly bees) and, over shorter distances, by wind. To minimise undesirable foreign pollination in agricultural seed production, seed legislation calls for isolation distances of 100 m for certified seed and 200 m for basic seed.

In this field trial, the applicant plans to maintain a minimum isolation distance of 50 m from the genetically modified plants to neighbouring oilseed rape fields in conjunction with a 2 m wide border row consisting of non-genetically modified oilseed rape plants or a minimum isolation distance of 100 m without a border row. In accordance with the requirements of provision II.6, in the case of an isolation distance of 50 m, a 6 m wide border row should be created. However, it must be assumed that to a limited extent oilseed rape pollen can be carried beyond the proposed isolation distance.

The release site is used by the applicant for the deliberate release of other genetically modified oilseed rape plants. Cross-pollination events between the different genetically modified plants cannot be ruled out. However, the measures proposed by the applicant in conjunction with the requirements set out in the provisions adequately ensure that any potentially emerging plants with unintended combinations of the different genetic modifications will be controlled and removed.

There are no other crop nurseries in the immediate vicinity of the release site. However, the possibility of oilseed rape from self-harvested seed being grown in the vicinity of the release site as a catch crop for green manure or for the production of green fodder cannot be ruled out. In this type of one-time reproduction, the plants do not normally reach the flowering stage. Therefore, genetically modified seed cannot be produced and spread by this means.

Pollination of individual flowers of non-genetically modified oilseed rape and a one-time reproduction of this oilseed rape would result in the temporary appearance of isolated oilseed rape plants in the vicinity of the release site, the seeds of which would exhibit an altered fatty acid profile. Since the transferred characteristics do not confer any apparent selective advantage, this is not expected to pose a risk to the environment or to agriculture.

During extraction of the rapeseed oil (e.g. also as a foodstuff) from seeds that may have developed as a result of pollination of individual oilseed rape flowers with pollen from the genetically modified oilseed rape plants, the produced enzymes would be separated from the oil along with the rest of the proteins. The proteins would remain in the pressing residue, the so-called “press cake”, which is used as animal fodder. Oil used for human consumption is subject to strict quality controls which, among other things, determine the erucic acid content. Capric acid and oleic acid are also components of oils and fats of other origins which are used for human consumption. Therefore, the potential pollination of individual flowers with pollen from the genetically modified oilseed rape plants is not expected to have adverse effects on human health.

Swede (*B. napus* var. *napobrassica*) belongs to the same species as oilseed rape. Oilseed rape and swede are assumed to be cross-compatible.

Swede is a biennial plant which develops a tuberous hypocotyl in the first year, but flowers only in the second year. When cultivated for sale and consumption the plants are harvested in the first year. The possibility of fertilisation with pollen from genetically modified oilseed rape is given when swede is brought to flowering for the purpose of harvesting seeds (e.g. for the cultivator's own requirements). Although they belong to the same species, swede and oilseed rape differ significantly in terms of morphology (oilseed rape does not develop a tuberous hypocotyl). It can be assumed that hybrids resulting from the pollination of swede by oilseed rape pollen would be markedly different in appearance from swede. Since untypical plants would not be cultivated for the further propagation of swede, genetically modified hybrids are not expected to be consumed or used for further seed production.

Several species in the *Brassicaceae* family are closely related to oilseed rape; these are potential crossing partners. Oilseed rape (*B. napus*) is a hybrid of wild turnip (*B. rapa*) and wild cabbage (*B. oleracea*) and is therefore, in principle, cross-compatible with these species – with the following limitations.

Hybrids of *B. napus* and *B. oleracea* have been generated experimentally by extracting embryos from the ovules and regenerating them to plants on culture media (embryo rescue). However, the spontaneous development of such hybrids under field conditions has not been observed to date.

Winter turnip rape (*B. rapa* ssp. *oleifera*) is cultivated as a crop plant for oil production and as a catch crop. Outside of cultivated areas it is also found growing wild on sites influenced by human activity (ruderal sites, waysides, field edges). Hybrids of *B. napus* x *B. rapa* appear sporadically in oilseed rape fields if fertilisation with pollen from *B. rapa* took place when the oilseed rape seeds were propagated.

With regard to the possible consequences of pollination of individual flowers of non-genetically modified winter turnip rape plants, the above statements on oilseed rape apply correspondingly. In addition, the fertility of primary hybrids of *B. rapa* and *B. napus* is generally limited. They are anorthoploid and are characterised by a marked reduction in the function of the gametes resulting from irregular meiotic chromosome distribution. The progeny of such gametes are aneuploid; they are generally of low vigour and in turn exhibit low fertility.

Other *Brassicacae*, such as leaf mustard (*B. juncea*), black mustard (*B. nigra*), white mustard (*Sinapis alba*), wild mustard (*S. arvensis*), species of common radish (*Raphanus sativus*), wild radish (*R. raphanistrum*), shortpod mustard (*Hirschfeldia incana*) and sea kale (*Crambe maritima*) are potential crossing partners for oilseed rape. Because of the low chromosomal homology between these plant species and oilseed rape, the above statements on *B. rapa* and *B. oleracea* apply to an even greater extent to hybrids of these plants and oilseed rape. Amphidiploid hybrids obtained by experimental crossing of oilseed rape with related *Brassicacae* species represent the only exception. These hybrids, which probably arise from unreduced gametes of the parent plants, exhibit only slightly reduced pollen fertility. Even if isolated cases of hybridisation between the genetically modified plants and these species of *Brassicacae* were to occur, it is highly unlikely that the genetic material transferred to the genetically modified plants would spread to into wild plant populations.

The following holds for all theoretically possible hybrids between the genetically modified plants and non-genetically modified crop plants or wild plants: the altered fatty acid profile in the seeds of the genetically modified plants does not confer a recognizable selective advantage. There are no grounds for concern about the unintended spread of these plants.

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 integrated into chromosomes of the recipient organisms. There is no evidence to suggest that a transfer of genetic information from plants and its expression in microorganisms takes place under natural conditions. Studies on the transformation capacity of soil bacteria under natural conditions suggest that a transfer of plant genetic material to soil microorganisms is also theoretically possible, although it is assumed that such a gene transfer would constitute an extremely rare event.

Insofar as we assume that a genetic exchange between organisms that are so distantly related in terms of taxonomy as seed plants and bacteria actually does take place, it would have to 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.

All of the transferred genes and gene fragments of the fatty acid metabolism are expressed under the control of seed-specific regulatory sequences. In addition, genes which encode enzymes with the same function occur naturally in a number of plant species.

The acyl-[ACP] thioesterase gene contains multiple introns. Even in the event of a transfer of this gene to microorganisms, formation of the corresponding enzyme in the microorganisms is not anticipated.

The oleate desaturase gene fragments and the *Bn*LPAAT gene were transferred in antisense orientation and do not confer any apparent selective advantage on microorganisms following a horizontal gene transfer from the genetically modified plants.

As already described in III.1.2.1.(f), antibiotics that are 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 therapeutic use of the relevant antibiotics would be compromised by a potential horizontal gene transfer of the *nptII* gene.

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

The same applies to the *aadA* gene located outside the T-DNA border regions for which, according to the information provided in the application dossier, a possible transfer into the

plant genome cannot be ruled out. The *aadA* gene codes for the enzyme streptomycin adenyltransferase.

The sequences inserted to regulate the *nptII* gene originate from CaMV; the omega-leader used to amplify expression in one construct originates from TMV. The theoretical possibility of a transfer of the CaMV or the TMV sequences from these plants would not represent a new situation compared to the naturally occurring one because, as plant-infecting DNA viruses, both are found in plants anyway.

In addition to the ColE1 origin of replication for replication in *E. coli*, the plasmids pNBM99-ClFatB3, pNBM99-EnClFatB3, pHS124, pHS126, pHS127, pRESS, pNKAT55, pRST55, pALM1 and pALM2 used to generate the genetically modified oilseed rape plants contain the region of replication of the pVS1 plasmid from *Pseudomonas aeruginosa*, strain PAT, with the genetic information for replication and stability outside the T-DNA. These replication origins may have been chromosomally integrated into the genetically modified oilseed rape plants (see III.1.2.1.(h)). These nucleic acid sequences are non-functional in the genetically modified plants; by contrast, they are expected to function as a replication origin in certain bacteria.

The host range of the ColE1 replicon is limited to certain gram-negative bacteria. Essentially, the replicon can replicate in *E. coli* and closely-related bacterial species, such as *Serratia* and *Salmonella*. Replication does not occur in the majority of gram-negative soil bacteria. ColE1 plasmids are quite frequently present 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 there is no reason to expect that the potential presence of the origin of replication of ColE1 in the plant chromosome would contribute to an increase in the overall frequency of horizontal gene transfer.

In this particular case the replication region of the pVS1 plasmid is used for replication of the binary vector in *A. tumefaciens*. The vectors used for the transformation are mobilisable but do not have their own transfer system. Since the conjugative exchange of nucleic acids between microorganisms is widespread, the pVS1-derived replication region from the genetically modified plants 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 oilseed rape plants, wounded explants were inoculated with Agrobacteria containing the genes intended for transfer between the border regions of the binary vector plasmid. In contrast to the common wild-type *A. tumefaciens*, the *Agrobacterium* strains used are “disarmed”, i.e. they no longer have the capacity to induce tumours. After transformation had occurred, antibiotic treatment was carried out to eliminate the Agrobacteria.

The seeds of the genetically modified oilseed rape lines proposed for experimental release are obtained by cross-breeding or self-pollination. Due to these generative phases, any agrobacteria that might possibly remain after the antibiotic treatment are removed from the genetically modified oilseed rape lines.