Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0171

Summary of the risk assessment of the genetically modified

oilseed rape (*Brassica napus*)

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 10. May 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

I. Consent [to the application]
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III.2 Appraisal of and reply to objections
IV. Costs
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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.

Dienstsitz Braunschweig
Bundesallee 50, Geb. 247
38116 Braunschweig
Tel: +49 (0)531 21497-0
Fax: +49 (0)531 21497-299

Abt. Pflanzenschutzmittel
Messeweg 11/12
38104 Braunschweig
Tel: +49 (0)531 299-5
Fax: +49 (0)531 299-3002

Dienstsitz Berlin
Mauerstraße 39-42
10117 Berlin
Tel: +49 (0)30 18444-000
Fax: +49 (0)30 18444-89999

Referatsgr. Untersuchungen
Diedersdorfer Weg 1
12277 Berlin
Tel: +49 (0)30 18412-0
Fax: +49 (0)30 18412-2955

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

(a) The gene encoding stilbene synthase VST I from grapevine *(Vitis vinifera)*

In the genetically modified oilseed rape plants, which were transformed using the pPSty5 construct, expression of the gene for stilbene synthase VST I from *Vitis vinifera* is driven by the seed-specific napin promoter from oilseed rape (*Brassica napus*) and the termination signal of the *vstI* gene.

In oilseed rape stilbene synthase catalyses the conversion of p-Cumaroyl-CoA to resveratrol. Resveratrol (3,5,4-trihydroxy-trans-stilbene) belongs to the flavonoid group of compounds and is classed among the phytoalexins. Resveratrol was first identified and isolated from Japanese knotweed plants, but it is also present in grapevine *(Vitis vinifera)*, pine, linseed, sesame seed and peanuts. In grapevine the highest concentration of resveratrol (between 50 and 100 µg/g) is found in the grape skins.

Phytoalexins are a class of antibiotic polyphenol compounds that form part of the plant defence system. Resveratrol is a metabolite produced by the plant in response to stress, for instance, when subjected to high levels of ozone or ultra violet irradiation, or when under attack by fungi or insects. Beneficial effects on the human immune system have also been attributed to resveratrol. This is based mainly on its antioxidant properties. Resveratrol is primarily a scavenger of peroxy radicals. It reduces lipoprotein lipid (LDL) peroxidation and protects cell membranes against the damaging effects of oxidated LDL. Resveratrol is also thought to have anticarcinogenic and cancer-inhibiting properties.

Although oilseed rape plants do not naturally contain resveratrol, resveratrol biosynthesis precursors from the sinapine biosynthesis pathway are present in the plants. The genetically modified oilseed rape plants, which were transformed using the pPSty5 construct, have a resveratrol glucoside content of 258 µg / g seed.

There is no evidence pointing to a toxic effect of the enzyme VST I. Resveratrol and related compounds are present in plants used in the production of foodstuffs. Material harvested from the GM oilseed rape plants within the scope of the proposed deliberate release is not intended for human or animal consumption. Therefore expression of the VST I enzyme in the seeds of the GM oilseed rape plants within the proposed trials is not expected to have any adverse effects on the environment or on human health.

(b) The suppression cassette of the UDP-glucose:sinapate glucosyltransferase (SGT) gene isolated from *Brassica napus*

In the GM oilseed rape plants that were transformed using the construct pLH-BnSGT-GUS the activity of the native oilseed rape enzyme UDP-glucose:sinapate glucosyltransferase (SGT) was reduced with the help of an RNAi construct. As a step in the biosynthesis of sinapine the UDP-glucose:sinapate glucosyltransferase catalyses the conversion of sinapic acid to sinapoyl glucose.

With the continued expansion of oilseed rape cultivation, the potential uses of the protein by-products of rapeseed oil extraction in human and animal nutrition could, theoretically, be limitless. In practice, however, the use of oilseed rape products is severely restricted by a number of plant components including sinapic acid esters. I

n oilseed rape these compounds are present in much higher concentrations than in other oil-rich seeds, which accounts for the bitter taste and astringency of oilseed rape products. The phenolic compounds present in oilseed rape can form complexes with the oilseed rape protein, thus reducing the high nutritional value of the protein. Moreover, these phenolic compounds give rise to an undesirable dark colouration of oilseed rape protein products. Sinapic acid esters, which mainly consist of the bitter substance sinapine (or sinapoyl-choline), normally make up 1-2% of rapeseed meal. Sinapine is the most frequently occurring phenolic acid ester compound, accounting for approximately 80% of the total phenolic content.

Suppression of the UDP-glucose:sinapate glucosyltransferase leads to a reduction of sinapine content in the seeds of the GM oilseed rape plants. The GM oilseed rape plants that were transformed using the construct pLH-BnSGT-GUS contain 1,82 mg sinapic acid equivalent per gram of seed (parental variety Drakkar: 7,28 – 10,33 mg sinapic acid equivalent per gram of seed).

The reduction of the antinutritive sinapine content in the seeds of the GM oilseed rape plants is not expected to have any harmful effects on the environment or on human health within the scope of the proposed deliberate release.

(c) The gene for the stilbene synthase VST I from *Vitis vinifera* in combination with the suppression cassette of the gene for the UDP-glucose:sinapate glucosyltransferase (SGT) gene from *Brassica napus*

The aim is to reduce the sinapine content and at the same time to synthesise resveratrol in the GM plants by combining the suppression cassette for the UDP-glucose:sinapate glucosyltransferase (SGT) gene with the gene that encodes the stilbene synthase VST I. In order to achieve this, the oilseed rape plants were co-transformed using the constructs pPSty5 and pLH-BnSGT-GUS.

The suppression of sinapic acid synthesis leads to increased availability of p-coumaric acid - a substrate required for the production of resveratrol. The seeds of the oilseed rape plants co-transformed with the constructs pPSty5 and pLH-BnSGT-GUS contain 424 μg resveratrol glucoside per gram of seed and 1,30 mg sinapic acid equivalents per gram of seed (parental variety Drakkar: 7,28 – 10,33 mg sinapic acid equivalents per gram of seed).

There is no evidence pointing to a toxic effect of the enzyme VST I. Resveratrol and related compounds are present in plants used in the production of foodstuffs. The material harvested from the GM oilseed rape plants within the proposed trials is not intended for release into the human/animal food/feed chain. Thus, within the scope of the proposed trials, the expression of the VST I enzyme in the seeds of the GM oilseed rape plants in combination with the reduced antinutritive sinapine content is not expected to result in any adverse effects on the environment or on human health.

(d) The suppression cassette for the UDP-glucose:sinapate glucosyltransferase (SGT) gene and the sinapoylglucose:choline sinapoyltransferase (SCT) gene from *Brassica napus*

In the GM oilseed rape plants that were transformed using the construct pLH7000-SGT/SCT, the activity of the native oilseed rape enzymes UDP-glucose:sinapate glucosyltransferase (SGT) and sinapoylglucose:choline sinapoyltransferase (SCT) was reduced with the help of an RNAi construct. In oilseed rape plants UDP-glucose:sinapate glucosyltransferase catalyses the conversion of sinapic acid to sinapoylglucose. Sinapoylglucose:choline sinapoyltransferase (SCT) catalyses the conversion of sinapoylglucose to sinapoyl-choline (sinapine).

By suppressing UDP-glucose:sinapate glucosyltransferase and sinapoylglucose:choline sinapoyltransferase the sinapine content in the seeds of the GM oilseed rape plants is further reduced. The GM oilseed rape plants that were transformed using the pLH7000-SGT/SCT construct contain 4,97 mg sinapine per gram of seed (parental variety Lisora: 7,54 – 12,58 mg sinapine per gram of seed).

The reduction of the antinutritive sinapine content in the GM oilseed rape plants is not expected to result in any adverse effects on the environment or on human health within the scope of the proposed trials.

(e) The *npt*II gene

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

The neomycin phosphotransferase gene is a type II aminoglycoside 3'-phosphotransferase (APH(3')II) that catalyses the ATP-dependent phosphorylation of the 3'-hydroxyl group of the aminohexose ring of certain 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 paramycin belong to the APH(3')II enzyme substrates. Clinically relevant gentamicins and other aminoglycosides and aminocyclitoles used in human medicine do not belong to the substrate spectrum of APH(3')II enzymes. Kanamycin and neomycin are, however, widely used in veterinary medicine.

Given the substrate specificity of the neomycin phosphotransferase, it can be assumed that in the absence of substrate under field conditions no new metabolic products can be synthesised in the GM oilseed rape plants. Since the relevant antibiotics are not present in the soil in higher concentrations, neomycin transferase does not confer a selective advantage to the GM plants under field conditions. No evidence has been recorded to suggest that this enzyme is toxic to plants, animals, microorganisms or humans.

(f) The *bar* gene

The GM oilseed rape plants that were transformed using the pLH-BnSGT-GUS and pLH7000-SGT/SCT constructs contain the *bar* gene from *Streptomyces hygroscopicus,* which serves as a selectable marker and is driven by the 35S promoter and the 35S terminator of the cauliflower mosaic virus (CaMV) in the GM plants. This gene was used to select transformed plant cells. The *bar* gene codes for an acetyltransferase (PAT) that selectively catalyses the acetylation of L-phosphinothricin. L-phosphinothricin is the active component of the herbicidal agent glufosinate ammonium (= ammonium-d,l-phosphinothricin). L-phosphinothricin is an analogue of glutaminic acid and it blocks glutamine synthetase by competitive inhibition. In non-genetically modified tissues the application of glufosinate ammonium results in a build-up of ammonium, causing the cells to die off. In the transformed plant cells acetylation results in the conversion of L-phosphinothricin to its derivative N-acetyl-phosphinothricin, which has no herbicidal effect.

Expression of the *bar* gene is unlikely to give rise to the formation of any additional metabolic products, since only amino acids that are structurally related to phosphinothricin (e.g. glutamate) can provide the necessary substrate for the PAT enzyme. However, corresponding studies have shown that even in the case of glutamate and other structurally related amino acids conversion is barely detectable. Within the framework of this experimental release the GM oilseed rape plants are not to be treated with glufosinate ammonium.

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

In addition to the sequences described above, the plasmid used to transform the oilseed rape plants with the pPSty5 construct also contains nucleotides of the *lac*Z gene from *E. coli*. These are non-functional in plants.

(h) Sequences located outside the T-DNA

The construct pPSty5:

The plasmid used to transform the oilseed rape plants with the pPSty5 construct is derived from the binary vector pPZP111 (Hajdukiewicz *et al.*, 1994) and contains the following genetic elements outside the border regions:

- a bacterial chloramphenicol acetyltransferase gene (Cm^R gene, *cat*-Gen), which confers resistance to the antibiotic chloramphenicol;
- the *bom* sequence from pBR322 for mobilisation of the plasmid from *E. coli* in *Agrobacterium tumefaciens*;
- the origins of replication from ColE1 and pVS1 for replication in *E. coli* or *Agrobacterium*.

As a rule, in *Agrobacterium*-mediated transformations only DNA fragments located between the border regions are integrated into the plant genome. However, in isolated cases the transfer of DNA fragments located outside the border regions has been reported and - based the information contained in the present application - such a transfer cannot be ruled out. However, given that the chloramphenicol acetyltransferase gene is controlled by a prokaryotic promoter, it can be assumed that this gene would not be expressed in plants. Therefore, effects on the plant metabolism are not anticipated. No evidence exists to suggest that the ColE1 and pVS1 replication regions have a function in higher plants.

The constructs pLH-BnSGT-GUS and pLH7000-SGT/SCT:

The plasmids used to transform the oilseed rape plants with the constructs pLH-BnSGT-GUS and pLH7000-SGT/SCT are derived from the binary vector pLH7000 (Hausmann and Töpfer, 1999) and contain the following genetic elements outside the border regions:

- the *aadA* gene for resistance to the antibiotics streptomycin and spectinomycin;
- the *bom* sequence and the *nic* sequence from pBR322 for mobilisation of the plasmid from *E. coli* in *Agrobacterium tumefaciens*;
- the origins of replication from ColE1 and pVS1 for replication in *E. coli* or *Agrobacterium*.

As a rule, in *Agrobacterium*-mediated transformations only DNA fragments located between the border regions are integrated into the plant genome. However, in isolated cases the transfer of DNA fragments located outside the border regions has been reported and - based the information contained in the present application - such a transfer cannot be ruled out. However, given that the *aadA* gene is controlled by a prokaryotic promoter, it can be assumed that this gene would not be expressed in plants. Therefore, effects on the plant metabolism are not anticipated. No evidence exists to suggest that the ColE1 and pVS1 replication regions have a function in higher plants.

(i) Position effects and context changes; allergenicity

Genes that have been integrated into the plant genome by genetic engineering methods are expressed at varying levels, depending on the site of integration on the chromosome and on the nucleotide sequences neighbouring the integration site ("position effect"). Under field conditions the level of expression may be influenced by environmental factors, for instance, by temperature. In this particular case this could mean that the characteristics of the GM oilseed rape plants are not modified to the same degree in the field as under climate chamber or greenhouse conditions. This is not expected to pose a risk to the environment or to human or animal health.

The insertion of foreign genes may influence the expression or regulation of the 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 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. 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-GM plants such events can always influence plant metabolic pathways. With regard to these properties the GM plants do not differ fundamentally from non-GM plants.

Given the current state of knowledge, it is impossible to make reliable predictions about the possible allergenic action of a protein on the basis of its amino acid sequence. In numerous previous trials with plants that express the *npt* II gene or the *bar* gene under the control of non-tissue-specific promoters, the plants were not found to display any increased allergenic potential. In the GM oilseed rape plants the VST I (stilbene synthase) expression cassette, the SGT suppression cassette and the SGT/SCT suppression cassette are all controlled by seed-selective promoters. Thus they are not expected to be expressed in the pollen of these plants. Moreover, these expression cassettes do not cause the production of additional proteins in the GM oilseed rape plants; rather they cause a lowering in level of concentration of one, or as the case may be, two native oilseed rape proteins.

III.1.2.2. <u>Evaluation of the ability of the genetically modified plants to persist or establish in the environment</u>

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.

The persistence of seeds from the GM oilseed rape and from potentially occurring oilseed rape hybrids can be minimized by taking appropriate measures after every harvest to ensure that any seeds released are brought to germination during the same vegetation period and any plants emerging from these seeds are subsequently destroyed. These measures are planned for the proposed experimental release. On completion of the trials the surface of the release site will be lightly tilled (repeatedly and without turning the soil) in order to encourage germination of any seeds dropped in the field and to facilitate their eradication.

Subsequent experimental releases are planned to take place on the same release site. As a result, any seeds from oilseed rape or oilseed rape hybrids that remain in the soil after completion of the above post-trial measures can re-emerge and germinate on the surface when the soil is being prepared for future trials. Within the planned five-year cultivation gap that applies to cruciferous plants, any emerging plants will be identified and destroyed by the applicant during the course of crop rotation monitoring. If during the final post-monitoring year the average number of GM oilseed rape plants or oilseed rape hybrids per 150 m^2 of former release site exceeds 5, the monitoring period will be extended by a further year (provision II.10.).

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

Outside cultivated sites oilseed rape is only found as a weed in or near areas where the crop is grown, e.g. on verges and other ruderal sites. Oilseed rape is not capable of establishing in natural, intact plant communities. These GM oilseed rape plants are not expected to develop altered plant sociological traits as a result of the introduction of the novel genes nor are they expected to populate other biotopes.

Therefore, even in the event that individual GM oilseed rape seedlings emerge and the transfer of pollen to non-GM plants is possible, no long-term, sustainable spread of the GM oilseed rape is expected. The temporal and spatial limitation of the release is thus guaranteed.

III.1.2.3. Invasiveness

Oilseed rape is a crop plant which has no known wild form. It is only found as a weed in the vicinity of cultivated areas, on verges and other ruderal sites. Oilseed rape is not known to establish in natural, intact plant communities.

No differences were found between the GM oilseed rape and conventional oilseed rape in terms of growth, onset of flowering, flowering time and seed formation. Attached to the application are the results of comparative analyses of plant height at the onset and at the end of flowering, seed weight per plant, and thousand-grain weight for plants transformed with the construct pLH-BnSGT-GUS as well as with the construct combination pPSty5 / pLH-BnSGT-GUS. Comparative values for oil and protein content and the content of various unsaturated fatty acids were also recorded. When compared to control lines, both the plants transformed with the construct pLH-BnSGT-GUS and those transformed with the construct combination pPSty5 / pLH-BnSGT-GUS were found to have a shorter flowering time; the construct combination transformants additionally displayed greater height at the onset of flowering and increased seed biomass production. However, each of these significant differences only occurred in one of two generations studied.

Based on previous experience and the findings of earlier field trials with similarly modified oilseed rape plants, the resveratrol glucoside produced in the seeds is not expected to have an impact on pest infestation.

In the absence of the herbicide glufosinate ammonium, the *bar* gene contained in the constructs pLH-BnSGT-GUS and pLH7000-SGT/SCT as a selectable marker does not confer any selective advantage to the GM oilseed rape plants or to potential hybrids.

Therefore, there is no reason to assume that the GM oilseed rape plants differ from non-GM oilseed rape plants in their ability to establish and persist in the environment as a result of the transformation. If such variations were to occur, they would be adequately addressed by the proposed isolation and post-trial monitoring measures.

III.1.2.4. Assessment of the possibility of pollen-mediated transfer of the inserted genes from the genetically modified plants to other plants

Oilseed rape pollen is mainly transported by insects (particularly bees) and to a small extent by wind. Oilseed rape stocks are about two thirds self-pollinating and one third cross-pollinating. In accordance with seed legislation, the agricultural production of seeds calls for isolation distances of 100 m for certified seed and 200m for basic seed.

The applicant plans to sow a 30 m wide strip of conventional oilseed rape of the parental variety around the 16 release plots, each of which measure 3 x 10m. In addition, each of the eight trial fields will be surrounded by a 6m wide border row of conventional male sterile oilseed rape.

No flowering cruciferous plants (incl. oilseed rape, cabbage, turnip rape) will be cultivated within a radius of 1,5 km of the release site (from the outer perimeter of the border rows).

Outside the isolation zone *Brassica* species, particularly winter oilseed rape, may be grown for agricultural purposes. In order to avoid simultaneous or overlapping flowering with winter oilseed rape, the release site will not be planted before the end of April. Summer oilseed rape is not normally grown in this region, but was put down as a preceding crop on the release site and may therefore emerge during the trial as volunteer oilseed rape.

The isolation measures proposed by the applicant with regard to field stocks of potential crossing partners outside the release zone are deemed sufficient.

Summer oilseed rape was planted as a preceding crop on the trial site and may therefore appear on the site as volunteer oilseed rape during the trial.

Ruderal populations of oilseed rape can occur in the vicinity of cultivated areas, on waysides and other ruderal sites. Throughout the vegetation period these plants may flower. Many species of *Brassicacae* are closely related to oilseed rape; these can be considered potential crossing partners. Oilseed rape (*B. napus*) is a hybrid of turnip rape (*B. rapa*; syn. *B. campestris*) and cabbage (*B. oleracea*) and is therefore capable of back-crossing with these species. As a result, gene transfer of oilseed rape to its parental species cannot be ruled out. In view of the planned isolation measures the likelihood of such an event is, however, very small.

Other potential crossing partners for oilseed rape found among the *Brassicacae* include, for example, leaf mustard (*B. juncea*), black mustard (*B. nigra*), white mustard (*Sinapis alba*), wild mustard (*S. arvensis*), wild radish (*Raphanus raphanistrum*), shortpod mustard (*Hirschfeldia incana*), annual wall-rocket (*Diplotaxis muralis*), perennial wall-rocket (*Diplotaxis tenuifolia*) and common dogmustard (*Erucastrum gallicum*). These species may appear as arable weeds on cultivated areas and on ruderal sites. Owing to the low level of chromosome homology between these plant species and oilseed rape, the likelihood that pollination of these plants with oilseed rape pollen would result in fertile progeny is extremely small. As a result of meiotic dysfunction, the primary hybrids are anueploid and are characterised by pronounced functional deficiencies. The progeny of these hybrids are also aneuploid, displaying stunted growth and very limited fertility.

Isolated hybrid events between the GM plants and wild plants would be very unlikely to lead to the spread of the transferred foreign genes to wild plant populations, since this would require subsequent back-crossing of the hybrid with the wild plant species.

None of the potential hybrids of the GM plants and non-GM crop plants or wild plants are expected to develop altered sociological traits as a result of the newly introduced genes nor are they expected to populate other biotopes.

To minimize the emergence of volunteer oilseed rape plants from previous stocks of conventional summer oilseed rape or other potential crossing partners, the applicant plans to treat the release site with a herbicide before sowing the GM oilseed rape.

Due to the preceding cultivation of oilseed rape on plot section 11/3, provision II.8 states that during the GM oilseed rape flowering period all simultaneously flowering volunteer oilseed rape and wild-growing oilseed rape plants are to be removed from plot section 11/3 and from the area within a 50 m radius of the release plots before their seeds reach maturity. This measure ensures the spatial limitation of the release.