Notification 6786-01-0090 / 42010.0090

Summary of the risk assessment of the genetically modified oilseed rape

(*Brassica napus* L. ssp. *oleifera* (Metzg.) Sinsk.) MS8 and RF3

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 22 July 1998

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 sequences

(a) The *barnase* gene; the *barstar* gene

The *barnase* gene in the oilseed rape line MS8 is derived from *Bacillus amyloliquefaciens* and codes for the RNase barnase. In the genetically modified plants, the *barnase* gene is under the control of the tapetum-specific promoter PTA29 from *Nicotiana tabacum*. The tapetum is essential for nutrition and wall formation in growing pollen grains. The barnase activity in the tapetum cells causes this cell layer to die off. The flowers of plants of the oilseed rape line “MS8” are characterised by a lack of anther development. They do not produce any fertile pollen grains and are thus male sterile.

The *barstar* gene in the oilseed rape line RF3 is also derived from *Bacillus amyloliquefaciens* and codes for the barnase inhibitor barstar. Just like the *barnase* gene, the *barstar* gene is expressed specifically in the tapetum tissue during anther development under the control of the promoter PTA29. The barstar protein specifically inhibits the barnase and allows the development of anthers and pollen grains in hybrid plants produced by hybridising the progeny of the MS8 and RF3 lines. Due to the high specificity of the inhibitor barstar for the barnase, it is not expected to also inhibit RNases in plants or animals.

In the genetically modified oilseed rape plants, the expression of the *barnase* gene and the *barstar* gene is closely limited both spatially (tapetum tissue of anthers) and temporally (anther development). Both genes are derived from a ubiquitously occurring bacterium. RNases with an activity comparable to that of barnase are naturally found in plants.

Due to the tissue specificity of the promoter PTA29 used and the properties of the proteins formed (barnase and barstar), effects on plant metabolism other than those described are not expected.

(b) The *bar* gene

In the genetically modified oilseed rape plants, the *bar* gene is expressed in green plant tissue under the control of the PSsuAra promoter. The gene codes for the enzyme phosphinothricin acetyltransferase (PAT).

L-phosphinothricin is a glutamic acid analogue and inhibits glutamine synthetase in plants. The inhibition of glutamine synthetase leads to apoptosis resulting from accumulated ammonium. This is why phosphinothricin (glufosinate) is used as the active ingredient in the non-selective herbicide Basta® (Liberty®). Basta® contains the enantiomers D- and L-phosphinothricin in a 1:1 ratio. D-phosphinothricin does not act as a glutamine synthetase inhibitor.

Unlike in non-genetically modified plants treated with Basta® (Liberty®), the use of Basta® in genetically modified plants causes L-phosphinothricin to be acetylated by the phosphinothricin acetyltransferase (PAT), thereby creating N-acetyl-L-phosphinothricin, which has no herbicidal effect. This makes the genetically modified oilseed rape plants tolerant to the herbicide Basta®. The substrate specificity of phosphinothricin acetyltransferase is high. Even the phosphinothricin analogue glutamate is hardly acetylated. Due to its high substrate specificity, the PAT enzyme is not expected to acetylate substrates other than L-phosphinothricin and thereby affect plant metabolism in the genetically modified plants. D-phosphinothricin is not metabolised by phosphinothricin acetyltransferase.

Due to its good water solubility, N-acetyl-L-phosphinothricin formed in the genetically modified plants after treatment with Basta® is distributed in the plants during further plant growth, while its concentration is reduced with increasing biomass. There are no indications of N-acetyl-phosphinothricin being further metabolised in the genetically modified plants.

Any N-acetyl-phosphinothricin still present in those parts of the genetically modified plants that remain on the field enters the soil during decomposition, where it is converted back into L-phosphinothricin by microorganisms. D/L-phosphinothricin is degraded in the soil, also by microorganisms.

According to the available data, N-acetyl-L-phosphinothricin has a significantly lower toxicity than phosphinothricin (= active ingredient in the herbicide Basta®). Basta® is approved by the Federal Biological Research Centre (Biologische Bundesanstalt) under the German Plant Protection Act (Pflanzenschutzgesetz). As part of the authorisation process, the herbicide and its metabolites were assessed for toxicity and ecotoxicity. Based on the toxicological and ecotoxicological data on phosphinothricin and N-acetyl-L-phosphinothricin, the residues or metabolites of the herbicide Basta® contained in the genetically modified oilseed rape plants are not expected to pose a risk to human and animal health or the environment.

No adverse effects are expected to result from the consumption of parts of the genetically modified plants containing phosphinothricin acetyltransferase by animals or humans. In the event of oral intake, it can be assumed that this enzyme would be fully degraded in the digestive tract, as is generally the case with proteins. The PAT protein does not possess any properties typical of allergenic proteins in food (heat stability, stability in the digestive tract) and no sequence homology with known allergens.

(c) Border sequences from Ti plasmids and regulatory sequences

The genetically modified plants contain sequences of the left and right border region of the TL-DNA of the plasmid pTiB6S3 from *Agrobacterium tumefaciens*. Depending on the gene products of the *vir* region of the helper plasmid pGV4000 that is contained in the *Agrobacterium* strain used for transformation and is not transferred into the plants, these sequences cause the genes located between the border regions to integrate into the chromosomes of the oilseed rape plants. These border regions of the Ti plasmid are non-functional in the genetically modified oilseed rape plants and are not expected to cause any changes in the plants.

The tapetum-specific promoter fragment PTA29 from *Nicotiana tabacum*, the termination signal of the nopaline synthase gene from *Agrobacterium tumefaciens*, the promoter of the small subunit of ribulose-1,5-bisphosphate carboxylase from *Arabidopsis thaliana* (PSsuAra) as well as the termination signal of gene 7 of the TL-DNA from *Agrobacterium tumefaciens* were transferred into the genetically modified oilseed rape plants as regulatory elements. In the genetically modified plants, the promoter and terminator sequences regulate the expression of the coding sequences of the transferred genes (*barnase*, *barstar*, *bar*) located between them. Further information on the effects associated with the expression of these genes in the plants can be found in III.1.2.1 (a) and (b).

(d) Sequences located outside the T-DNA

Based on the results of PCR analyses, the transformation events were not found to involve the transfer of sequences outside the border regions of the vectors used.

(e) Position effects and context changes; allergenicity

Genes integrated into the plant genome by genetic engineering methods are expressed at different levels, depending on the site of integration on the chromosome and on the neighbouring sequence at the integration site ("position effect"). Under field conditions, the expression level may be influenced by environmental factors, for instance, by temperature. In this particular case, this could mean that plants of the MS8 line are not completely male sterile, that plants of the RF3 line are not fully able to restore male fertility through hybridisation with plants of the MS8 line or that the genetically modified plants exhibit reduced tolerance to phosphinothricin (glufosinate). This does not represent a risk to the environment or to human and animal health.

The insertion of foreign genes may influence the expression or regulation of endogenous plant genes at or near the site of insertion. Such processes can affect plant metabolic pathways. During cultivation of the genetically modified plants within a number of deliberate release trials, 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-genetically modified plants, such events can always influence plant metabolic pathways. In this regard, the genetically modified plants to be deliberately released do not differ fundamentally from non-genetically modified plants.

Since 1990, a great number of deliberate release trials have been performed with the genetically modified plants of the MS8 and RF3 lines and their progeny. Based on the data obtained, an application was submitted in 1996 requesting approval for placing these plants on the EU market. In 1996, the company PGS was granted a qualified approval for placing two comparable oilseed rape lines (MS1 and RF1) on the EU market (cultivation for seed generation), into which the *bar*, *barnase* and/or *barstar* genes have also been inserted. Previous deliberate release trials with the plants of the MS8 and RF3 lines as well as the cultivation of plants of the MS1 and RF1 lines and their progeny provided no indications of increased allergenic potential or other adverse effects of the plants on human health or the environment. Due to the tissue specificity of the promoters used, the proteins barnase, barstar or PAT are not assumed to be contained in pollen of the genetically modified plants.

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

Summer oilseed rape is an annual plant; winter oilseed rape is a plant that overwinters. 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, seeds of oilseed rape can persist in the soil for over 20 years. The persistence of seeds from the genetically modified oilseed rape plants can be minimised by using appropriate measures to

bring any seeds released to germination during the same vegetation period. The resulting plants can be easily destroyed. Corresponding measures are proposed by the applicant.

Any seeds of oilseed rape that remain in the soil after completion of the proposed measures can re-emerge and germinate when the soil is being prepared in the course of the planned agricultural cultivation. The applicant has proposed to subject the release site to a one-year post-trial monitoring period, which will be extended for a further year if genetically modified oilseed rape plants are detected on the release site.

The potential emergence of genetically modified oilseed rape seedlings on the release site after the end of the release period does not pose a risk concerning pollen transfer to other plants (cf. 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 waysides and other ruderal sites. Oilseed rape is not capable of establishing in natural, intact plant communities. In this respect, the genetically modified oilseed rape plants are not expected to differ from non-genetically modified oilseed rape as a result of the introduction of the genes, nor are they expected to populate other biotopes. These plants only have a selective advantage over other plants in areas where glufosinate is used as herbicide. The plants can be destroyed using mechanical methods or other non-glufosinate herbicides.

Therefore, even in the event that genetically modified oilseed rape seedlings emerge after the end of the release period and pollen is transferred to non-genetically modified plants, the genetically modified oilseed rape is not expected to spread permanently and in the long term, whereby the spatial and temporal limitation of the release project is sufficiently ensured.

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

About two-thirds of oilseed rape stocks are self-pollinating and about one-third of them are cross-pollinating. Oilseed rape pollen is dispersed mainly by insects, in particular bees, and over smaller distances also by wind.

Oilseed rape plants near the release site may be pollinated by the genetically modified oilseed rape plants. The pollination of non-genetically modified oilseed rape and the possible saving of this oilseed rape as intercrop for green manuring or green fodder production would result in the temporary emergence of individual genetically modified oilseed rape plants in the surroundings of the release site. Since without the application of glufosinate the inserted genes do not confer any selective advantage to the plants, this is not associated with any risks for the environment or agriculture.

Seeds resulting from the pollination of non-genetically modified oilseed rape plants by the genetically modified oilseed rape plants could be used to produce rapeseed oil. Due to the tissue specificity of the promoters used, the proteins barnase, barstar or PAT are not expected to be contained in pollen or mature seeds of the genetically modified plants. Should they nevertheless be present in them, they would be separated from the oil along with all other proteins as part of the usual processing steps of rapeseed oil production. The proteins

would remain in the expressed residue, referred to as “oilcake”, which is used as fodder. The evaluation of changes in the genetically modified oilseed rape plants effected by the transferred nucleic acid sequences (cf. III.1.2.1) has shown that this is not expected to pose any risk.

Swede (*B. napus* var. *napobrassica*) belongs to the same species as oilseed rape. It can be assumed that oilseed rape and swede are hybridisable.

Swede is a biennial plant that develops a hypocotyl bulb in the first year, however, only flowers in the second year. When cultivated for sale and consumption, the plants are harvested in the first year. Pollination by genetically modified oilseed rape would be possible if swede were allowed to reach the flowering stage for the purpose of seed production (e.g. for personal requirements). Although they belong to the same species, swede and oilseed rape differ considerably in terms of morphology (oilseed rape does not develop a hypocotyl bulb). It can be assumed that hybrids resulting from the pollination of swede by oilseed rape would differ considerably from swede in terms of appearance. Since atypical plants would not be used to propagate swede, genetically modified hybrids are not expected to be consumed or used for further seed production.

There are several *Brassicaceae* species that are closely related to oilseed rape; these are potential crossing partners. Oilseed rape (*B. napus*) is a hybrid of turnip (*B. rapa*) and cabbage (*B. oleracea*) and is therefore theoretically hybridisable with these species – subject to the limitations described below.

It was possible to produce hybrids of *B. napus* and *B. oleracea* by laboratory means by extracting embryos from the ovules and regenerating them to plants on culture mediums (“embryo rescue”). However, spontaneous emergence of such hybrids under field conditions has so far not been observed.

Turnip rape (*B. rapa* ssp. *oleifera*) is cultivated as a crop plant for oil production and as intercrop and is found in wild form outside cultivated sites at locations influenced by humans (ruderal sites, waysides, field edges). Hybrids of *B. napus* and *B. rapa* occur sporadically in oilseed rape fields if pollination with *B. rapa* has taken place during the propagation of oilseed rape seeds.

The above statements on oilseed rape apply accordingly to the possible consequences of pollination of individual flowers of non-genetically modified turnip rape or other crop plants of the Brassicaceae family. In addition, the fertility of primary hybrids of *B. rapa* and *B. napus* is usually limited. They are anorthoploid and are characterised by pronounced functional deficiency of the gametes as a result of irregular meiotic chromosomal distribution. The progeny of such gametes are aneuploid, usually of weak growth and also exhibit limited fertility.

Other potential crossing partners of oilseed rape found among the *Brassicaceae* include, for example, leaf mustard (*Brassica juncea*), black mustard (*Brassica nigra*), white mustard (*Sinapis alba*), wild mustard (*Sinapis arvensis*), radish (*Raphanus sativus*), wild radish (*Raphanus raphanistrum*) and shortpod mustard (*Hirschfeldia incana*). Owing to the low level of chromosome homology between these plant species and oilseed rape, the above statements concerning *B. rapa* and *B. oleracea* apply to hybrids of these plants with oilseed rape

to an even greater extent. The only exceptions are amphidiploid hybrids produced by experimental crossing of oilseed rape with related *Brassicaceae*. The pollen fertility of these hybrids, which probably originate from unreduced gametes of the parent plants, is only slightly limited. Even if isolated cases of cross-breeding between the genetically modified oilseed rape plants and these *Brassicaceae* were to occur, spread of genetically transferred genetic material in wild plant populations is very unlikely.

The inserted genes are only expected to confer a selective advantage to any potential hybrids of the genetically modified plants and non-genetically modified crop plants or wild plants if the herbicide Basta® or other glufosinate-containing herbicides are used. However, unintentional spread of such plants is not expected.

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

The inserted sequences are integrated into the chromosomes of the recipient organisms. Studies on the transformation ability of soil bacteria under natural conditions suggest that the transfer of plant genetic material to soil bacteria is theoretically possible, although it is assumed that a gene transfer of this type would constitute an extremely rare event.

Insofar as we assume that an exchange of genetic material between organisms that are as distantly related in terms of taxonomy as seed plants and bacteria is actually possible, it can be concluded that the occurrence of an exchange of heterologous genetic material does not in itself represent a safety criterion, since such an exchange could always result in the uptake of any heterologous genetic material, including all forms of plant DNA.

Microorganisms receiving the *barnase* gene by horizontal gene transfer would have a substantial selective disadvantage. In addition, genes for RNases are ubiquitously present. The donor organism *Bacillus amyloliquefaciens* is also a ubiquitously occurring bacterium.

The *barstar* gene, which codes for a specific inhibitor of the barnase, is also derived from *Bacillus amyloliquefaciens*. The gene can thus also be spread by horizontal gene transfer from non-genetically modified microorganisms.

In soil microorganisms, the inactivation of phosphinothricin by acetylation is a naturally occurring process. Bacteria with a corresponding resistance are commonly found in the environment. This resistance can therefore also be spread by horizontal gene transfer from non-genetically modified microorganisms. Even if the *bar* gene were to be transferred from the genetically modified plants to microorganisms, the overall frequency of this resistance in the environment would not be significantly increased.

Even if regulatory sequences used in the construct were to be transferred, there is no reason to fear that the overall frequency of the respective DNA sequences will increase. These regulatory sequences are derived from *Arabidopsis thaliana* (thale cress) and from tobacco. Both plants species occur naturally in the environment.

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

The genetically modified oilseed rape plants were generated by transformation with agrobacteria containing the genes to be transferred between the border regions of binary vector plasmids (pTHW107 and pTHW118). In contrast to the common wild types of *A. tumefaciens*, the agrobacteria used are disarmed, i.e. they no longer have the capacity to induce tumours.

The seed of the genetically modified oilseed rape lines intended for release is produced by hybridisation or self-pollination. As a result of these generative phases, any agrobacteria that survived after transformation were removed from the genetically modified oilseed rape lines.