Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

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Summary of the risk assessment of the genetically modified oilseed rape

(*Brassica napus* L. ssp. *oleifera*. (Metzg.) Sinsk.) „Falcon GS 40/90“ and „Liberator 8/92-01“ within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 17 August 2001

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

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 effected by the transferred nucleic acid sequences

(a) The synthetic *pat* gene

In the genetically modified oilseed rape, the synthetic *pat* gene codes for a phosphinothricin acetyltransferase (PAT). The *pat* gene is originally derived from *Streptomyces viridochromogenes*.

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

Initially, the tripeptide L-phosphinothricyl-L-alanyl-L-alanine (bialaphos) is formed in *S. viridochromogenes*. After bialaphos is taken up by bacterial cells, L-phosphinothricin is released by hydrolysis. L-phosphinothricyl-L-alanyl-L-alanine and L-phosphinothricin have an antibiotic effect, with the tripeptide being considerably more effective than L-phosphinothricin. Neither substance has any significance in human and veterinary medicine.

Unlike in non-genetically modified plants treated with Basta®, the use of Basta® in genetically modified plants causes L-phosphinothricin to be acetylated by phosphinothricin acetyltransferase (PAT), thereby creating N-acetyl-L-phosphinothricin, which has no herbicidal effect. This makes the genetically modified plants tolerant to the herbicide Basta®. The substrate specificity of phosphinothricin acetyltransferase is high. Even the phosphinothricin analogue glutamate is hardly converted. D-phosphinothricin is not metabolised by phosphinothricin acetyltransferase.

Due to its excellent 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 under the German Plant Protection Act. As part of the licensing process, the herbicide and its metabolites were assessed for toxicity and ecological impact. 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 are not expected to pose a risk to human and animal health or the environment.

The coding region of the *pat* gene was derived from the amino acid sequence of the PAT enzyme of the soil bacterium *Streptomyces viridochromogenes* Tü494 and chemically synthesised. The original gene exhibits a high GC content (70%) typical of this group of bacteria. In order to ensure effective expression of the gene in plants, codons that are typical of plant genes were selected for gene synthesis. This alteration of the nucleic acid sequence is not

expected to pose any risk, since the amino acid sequence of the gene product, the PAT enzyme, is not altered.

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.

(b) Border sequences from the Ti plasmid pHoe6/Ac and regulatory sequences

The genetically modified oilseed rape contains sequences of the left and right border region of the binary Ti plasmid pTiT37 and the right border region of the Ti plasmid pTiAch5. The plasmids were originally derived from *Agrobacterium tumefaciens*. Depending on the gene products of the *vir* region of the helper plasmids that are contained in the *Agrobacterium* strains used for transformation and were not transferred into the plants, these sequences caused the genes located between the border regions to integrate into the chromosomes of oilseed rape. These border regions of the Ti plasmids are non-functional in the genetically modified oilseed rape and are not expected to cause any changes in the plants.

The 35S promoter and the termination signal of CaMV were transferred into the oilseed rape transformants “Falcon GS 40/90” and “Liberator 8/92-01” as regulatory elements. In the genetically modified oilseed rape plants, the promoter and terminator sequences regulate the expression of the coding sequences located between them. Further information on the effects associated with the formation of these enzymes in the genetically modified oilseed rape plants can be found in III.1.2.1 (a) to (c).

(c) Sequences located outside the T-DNA

As a general rule, only sequences of the binary transformation vector located within the border regions are integrated into the plant genome in *Agrobacterium*-mediated transformation events. However, the transfer of sequences outside the border regions has been reported in isolated cases.

In 1996 and 1998, the company AgrEvo GmbH in Germany submitted an application requesting approval for placing the two oilseed rape lines “Falcon GS40/90” and “Liberator 8/92-01” as well as the lines derived from them by conventional breeding methods on the market (applications C/DE/96/05 and C/DE/98/06). In its statements, the RKI endorsed placing these plants on the market. A final decision on the applications has yet to be made in an EU procedure.

The above-mentioned applications presented detailed results on the characterisation of the DNA fragments introduced into the oilseed rape lines “Falcon GS40/90” and “Liberator 8/92-01” as well as data on the expression of the transferred genes. The transformation events were not found to involve the transfer of sequences outside the vectors’ border regions.

(d) 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 level of expression may be influenced by environmental factors, for instance, by temperature. In this particular case, this could mean that the genetically modified plants do not tolerate glufosinate ammonium to the same degree in the field as under climate-controlled or greenhouse conditions. The application of glufosinate ammonium could result in damage to the genetically modified plants. 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 native plant genes at or near the site of insertion. Such processes can affect plant metabolic pathways. However, during the propagation of genetically modified plants in the greenhouse and in previous work with the genetically modified plants within a number of deliberate release trials in Germany and abroad, 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.

Given the current state of knowledge, it is not possible to make reliable predictions about the potential allergenicity of a protein on the basis of its amino acid sequence. In previous experiments with genetically modified oilseed rape in the greenhouse, and also in earlier deliberate release trials in Germany and abroad, no evidence was found to suggest an increased allergenic potential of the pollen of these plants.

Since 1994, a great number of deliberate release trials have been conducted in Germany with the genetically modified plants derived from “Falcon GS40/90” and “Liberator 8/92-01”. Previous deliberate release trials with the above-mentioned plants provided no indications of increased allergenicity or other adverse effects of the plants on human health or the environment.

Refined oil made from genetically modified oilseed rape derived from the two transformation events “Falcon GS40/90” and “Liberator 8/92-01” has been registered with the European Commission as novel food and novel food additive since October 1999.

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, rape seeds can persist in the ground for over 10 years. The persistence of seeds from the genetically

modified oilseed rape plants can be minimised by bringing any seeds released to germination during the same vegetation period. The resulting plants can be easily destroyed.

The soil cultivation measures described in the present application were selected on the basis of common agricultural practice in order to be able to examine the persistence of the genetically modified oilseed rape seeds under the influence of practical soil cultivation regimes. For this purpose, transgenic oilseed rape seeds are also planned to be sown or left on the release site in quantities that considerably exceed the common amount of seed sown, and the soil is to be cultivated in various ways directly after sowing. Deep ploughing for succeeding crop is also being envisaged. Larger amounts of oilseed rape seeds are therefore expected to be incorporated in deeper soil layers in individual trial phases, where they can enter secondary dormancy and persist. As a result of the soil cultivation measures to be taken in the following years, oilseed rape seeds in deeper soil layers are expected to gradually rise to the surface, where they can germinate.

For the second trial (V2), oilseed rape seeds sewn into nylon bags are planned to be buried on the release site in summer and removed from the soil in the following spring. If the nylon bags are not damaged (e.g. by mice), the oilseed rape seeds are expected to be removed from the soil without any loss.

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 (trial) cultivation. The resulting plants will be identified and destroyed during the post-trial monitoring period. The supplementary provision II.10 prescribes a five-year cultivation gap for oilseed rape on the release site after the end of the trial. During this period, the release site will be monitored for re-emergence of genetically modified oilseed rape. If genetically modified oilseed rape plants re-emerge on the monitored site in the last year of the post-trial monitoring period, the cultivation gap and the post-trial monitoring period will each be extended for a further year.

Considering the inserted genes, the potential emergence of individual genetically modified oilseed rape seedlings outside the release site or on the release after the end of the post-trial monitoring period does not pose a risk concerning pollen transfer to other plants or long-term establishment within the meaning of the German Genetic Engineering Act (GenTG) (cf. III.1.2.3). The possible consumption of rapeseed oil gained from the seeds of plants pollinated by the genetically modified plants does not pose a risk either within the meaning of the GenTG. Refined oil made from the two genetically modified oilseed rape lines has been registered with the European Commission as novel food and novel food additive since October 1999.

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.

The genetically modified oilseed rape is not expected to develop altered plant sociological traits as a result of the introduction of the genes, nor is it expected to populate other biotopes. This oilseed rape only has a selective advantage over other plants in areas where glufosinate ammonium is used as an herbicide. Trials in Great Britain with genetically modi-

fied, herbicide-tolerant oilseed rape confirmed that neither the genetically modified plants nor the non-genetically modified control plants can establish at natural locations.

Therefore, even in the event that individual genetically modified oilseed rape seedlings emerge and pollen is transferred to non-genetically modified plants, the genetically modified oilseed rape is not expected to spread permanently; adverse effects on ecosystems are not expected either.

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 transported mainly by insects and over smaller distances by wind.

The location of the release site on the experimental station Ihinger Hof was selected by the applicant in such a way that a separation distance of 200 m is kept to neighbouring oilseed rape stocks. The separation distance of 200 m to oilseed rape used for human consumption prescribed by the supplementary provision II.8 for this location is to be extended to also include oilseed rape used as animal feed. In addition, the supplementary provision II.7 prescribes that any *Brassica napus* and *Brassica campestris* plants, including their ruderal and wild types, be removed or destroyed before seed maturation within a radius of 100 m around release sites where genetically modified oilseed rape reaches the flowering stage.

At the location of Wendhausen, the applicant plans to cultivate an area of 50 m width around the actual release site with non-genetically modified oilseed rape and to destroy this oilseed rape by mulching following the flowering stage. Further isolation measures are not planned by the applicant.

Border strips of conventional oilseed rape and separation distances to other oilseed rape stocks are suitable to considerably reduce the dispersal of pollen from the release site. Oilseed rape pollen is assumed to be dispersed by wind or insects beyond the surrounding oilseed rape stocks of 50 m and beyond a distance of 200 m to potential other oilseed rape stocks only to a limited extent.

It cannot be excluded that self-harvested oilseed rape seeds are saved in the surroundings of the release site as intercrop for green manuring or greed fodder production. With this type of one-time seed-saving, the plants usually do not reach the flowering stage. Genetically modified seeds can thus not be produced or spread this way.

The pollination of individual flowers of non-genetically modified oilseed rape and the one-time saving of these oilseed rape seeds would result in the temporary emergence of individual glufosinate-tolerant 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. When producing rapeseed oil (e.g. also for human consumption) from seeds that may have resulted from the pollination of individual oilseed rape flowers by genetically modified oilseed rape plants,

the PAT protein would be separated from the oil, along with all other proteins. The protein would remain in the expressed residue, referred to as “oilcake”, which is used as fodder.

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 basically 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. 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 (*S. arvensis*), radish (*Raphanus sativus*), wild radish (*R. 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 pants, is only slightly limited. Even if isolated cases of hybridisation 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 gene is 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 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 to microorganisms

The inserted sequences were integrated into the chromosomes of the recipient organisms in the course of the transformation. 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 and 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 all forms of heterologous genetic material, including all forms of plant DNA.

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 *pat* 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 constructs were to be transferred, there is no reason to fear that the overall frequencies of the respective DNA sequences will increase. These regulatory sequences are derived from cauliflower mosaic virus, a plant-infecting, double-stranded DNA virus.

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

In order to generate the genetically modified oilseed rape plants, agrobacteria containing the genes to be transferred between the border regions of the binary vector plasmid pHoe6/Ac were used. 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. Following transformation, antibiotic treatment is usually carried out to eliminate the agrobacteria.

The seeds intended for release were produced by generative propagation over several generations. As a result of these generative phases, any agrobacteria that survived the antibiotic treatment were removed from the genetically modified oilseed rape lines.