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.) GT73

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 30 July 1999

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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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 gene for glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)

In the genetically modified oilseed rape, the expression of the gene for glyphosate-tolerant EPSPS derived from *Agrobacterium* sp. strain CP4, which is positioned downstream the DNA sequence of the transit peptide of the EPSPS from *Arabidopsis thaliana*, takes place constitutively under the control of the P-CMoVb promoter of the figwort mosaic virus and the E9-3' terminator sequence from *Pisum sativum*. The nucleic acid sequence of the *epsps* gene was optimised for codon usage typical of plants.

Both the EPSPS formed in the genetically modified oilseed rape as a result of transformation and the endogenous EPSPS catalyse the reaction of shikimate-3-phosphate with phosphoenolpyruvate to yield 5-enolpyruvylshikimate-3-phosphate, an intermediate stage in the biosynthesis of aromatic amino acids. In contrast to the endogenous EPSPS, the EPSPS inserted into the genetically modified oilseed rape is not inhibited by glyphosate. The upstream position of the transit peptide of the EPSPS derived from *Arabidopsis thaliana* causes the post-translational import of the chimeric protein into the chloroplasts.

In the genetically modified oilseed rape, the newly formed EPSPS catalyses the same reaction as the equivalent enzymes that occur naturally in oilseed rape and other crop plants. Since no adverse health effects have been attributed to the transit peptide of EPSPS, or to any other currently known signal peptides, whether processed or unprocessed, it can be assumed that the same applies to the transit-peptide-enzyme complex (here EPSPS). There is no evidence to suggest a toxic effect of the newly formed EPSPS.

No risks to human or animal health or to the environment are expected to result from the mode of action of the EPSPS inserted by means of transformation.

(b) The gene for glyphosate oxidoreductase (GOX)

In the genetically modified oilseed rape, the expression of the gene for glyphosate oxidoreductase (GOX), which is positioned downstream the DNA sequence for the transit peptide of the small subunit of ribulose-1,5-bisphospate-carboxylase/oxygenase from *Arabidopsis thaliana*, takes place constitutively under the control of the P-CMoVb promoter of the figwort mosaic virus and E9-3' terminator sequence from *Pisum sativum* statt. The nucleic acid sequence of the used *gox* gene is derived from the sequence of a *gox* gene from *Achromobacter sp*. strain LBAA. The nucleic acid sequence of the *gox* gene used for transformation was optimised for codon usage typical of plants. The gene product was altered at three amino acid positions so that the enzyme exhibits an apparent K_m value for glyphosate that is reduced by a factor of 10.

When treating the transgenic plants with the herbicide Roundup®, the expression of the *gox* gene causes the herbicide's active ingredient glyphosate to be degraded to native plant metabolic products via amino methyl phosphonic acid (AMPA) and glyoxylate. Since no adverse health effects have been attributed to the transit peptide of the small subunit of ribulose-1,5-bisphospate-carboxylase/oxygenase from *Arabidopsis thaliana*, or to any other currently known signal peptides, whether processed or unprocessed, it can be assumed that the same

applies to the transit-peptide-enzyme complex (here GOX). There is no evidence to suggest a toxic effect of the newly formed GOX.

Roundup® containing the active ingredient glyphosate is a non-selective, systemic leaf herbicide, which is distributed to all plant parts with sap flow. Owing to the activity of glyphosate oxidoreductase, the herbicide is degraded to the metabolites glyoxylate and AMPA. Glyoxylate is a metabolite that occurs naturally in plants; by contrast, AMPA is a metabolic product that is formed by the degradation of glyphosate. The available data shows that the GOX enzyme has a narrow substrate spectrum and adverse effects on other plant-specific metabolic paths are therefore not expected. The metabolite AMPA also forms when the herbicide is applied to plants that are not tolerant to the herbicide. This metabolite also forms during the degradation of the herbicide by soil-based microorganisms. The herbicide is approved by the Federal Biological Research Centre for use in a range of agronomic applications, including preharvest application in grain, under the German Plant Protection Act. As part of the licensing process, the herbicide and its metabolites were assessed for toxicity and ecotoxicological impact.

After the end of the trial, the genetically modified oilseed rape plants will be disposed of and are not intended to be used for human consumption or animal feed. Based on the toxicological data, the residues or metabolites of the herbicide glyphosate contained in the genetically modified oilseed rape plants are not expected to pose a risk to human or animal health in the event that parts of the plants are consumed.

Likewise, no adverse effects are expected to result from the consumption of parts of the genetically modified oilseed rape plants containing the GOX protein 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.

(c) Border sequences from Ti plasmids and regulatory sequences

The genetically modified oilseed rape contains sequences of the left and right border region of the binary plasmid pMON17237. The plasmid was originally derived from *Agrobacterium tumefaciens*. Depending on the gene products of the *vir* region of the helper plasmid that is contained in the *Agrobacterium* strain used for transformation and was 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.

Integrated into the genome, the genetically modified oilseed rape contains the P-CMoVb promoter of the figwort mosaic virus and the E9-3’ terminator sequence derived from *Pisum sativum*. In the genetically modified oilseed rape plants, the promoter and terminator sequences regulate the expression of the coding sequences for the chimeric genes 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 (b).

(d) 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; based on the study result presented by the applicant, the transfer of sequences of the binary vector outside the border regions is not assumed in this particular case.

If, contrary to all expectations, sequences outside the T-DNA border sequences should have been transferred into the plant genome, this would be assessed as follows: The *aadA* gene that codes for a streptomycin/spectinomycin resistance gene is under the control of a prokaryotic promoter. Therefore, this gene is not expected to be expressed in plants. Adverse effects on plant metabolism as well as on humans or animals following consumption of the genetically modified plants or parts thereof are not expected. In respect of the origins of replication of the binary transformation vector (ori-322 for replication in *E. coli* and ori-V for replication of the binary vector in *Agrobacterium tumefaciens*), there is no evidence to suggest that these replication regions have any function in higher plants.

(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 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 glyphosate to the same degree in the field as under climate-controlled or greenhouse conditions. The application of Roundup® 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.

In this connection, it should be mentioned that oilseed rape cultivated from the transformant GT73 has already been approved for unlimited commercial production and use in Canada and the USA following inspection by the corresponding authorities.

Refined oil made from genetically modified oilseed rape derived from the transformation event GT73 has been registered with the European Commission as novel food and novel food additive since November 1997.

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. Unless they are proposed by the applicant, the corresponding measures are described in the supplementary provision II.9.

Any seeds of oilseed rape or oilseed rape hybrids that remain in the soil after completion of these measures can re-emerge and germinate when the soil is being prepared in the course of the planned common agricultural cultivation. The resulting plants will be identified and destroyed during the planned post-trial monitoring period. The proposed duration of the post-trial monitoring period of two years prescribed in the supplementary provision II.10 is regarded as sufficient. If genetically modified oilseed rape plants or hybrids re-emerge in the last year of the post-trial monitoring period, the post-trial monitoring period will be extended for a further year. In order to ensure the identification or re-emerging oilseed rape plants, no oilseed rape will be cultivated on the release site during the post-trial monitoring period. This cultivation gap ensures that any re-emerging oilseed rape plants and hybrids will be identified and destroyed.

The potential emergence of genetically modified oilseed rape seedlings on the release site after the end of the post-trial monitoring 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.

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 glyphosate is used as an herbicide. Trials in Great Britain with genetically modified, 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 supplementary provision II.7 prescribes a minimum separation distance of 50 km in combination with a 3 m wide strip of conventional oilseed rape plants, or, without such border strips, 100 m to neighbouring oilseed rape and turnip rape stocks (*Brassica napus* and *B. campestris*), including their ruderal and wild types. Border strips of conventional oilseed rape and separation distances are suitable to considerably reduce the dispersal of pollen from the release site. However, it can be assumed that small amounts of oilseed rape pollen can be dispersed by insects also beyond the separation distance of 50 m or 100 m.

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 distributed 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 glyphosate-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 GOX protein and the EPSPS protein would be separated from the oil, along with all other proteins. The proteins 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 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.

The genetically modified plants contain the *epsps* gene from *Agrobacterium* sp. CP4 and the das *gox* gene, whereby the coding region of this gene is fused to plant “leader peptide” sequences at its N terminus. Such “leader peptide” sequences would be non-functional in bacteria; *epsps* genes are ubiquitously present in soil microorganisms. The *gox* gene is also derived from a gene of a bacterium naturally occurring in the environment. Studies on the breakdown of glyphosate in soil have demonstrated that metabolic activities of microbes which cause the decomposition and inactivation of glyphosate are widespread. Glyphosate is rapidly degraded in soil; the scientific literature describes two ways of degradation for the herbicide. The application of glyphosate as an herbicide is not expected to change the composition of soil microflora. Even if herbicide application were to lead to the selection of a group of glyphosate-degrading bacteria, the origin and distribution of the metabolic activity would be accounted for by the bacteria themselves and would not be traced back to the transfer of genes from the genetically modified plants to microorganisms. The potential horizontal transfer of genes would not contribute to any noteworthy increase in the overall frequency of glyphosate-degrading metabolic activities in bacteria.

Located outside the T-DNA borders, the binary vector pMON17237 used to produce the genetically modified oilseed rape plants contains the *aadA* gene, which confers resistance to streptomycin und spectinomycin as well as the bacterial origins of replication ori-322 and oriV. Based on the results of the studies submitted, the presence of these sequences in the genetically modified plants is not assumed. Since they frequently occur in bacteria and the exchange of nucleic acids between microorganisms is possible by effective transfer mechanisms, it can be assumed that even if the sequences were present in the genetically modified plants, horizontal gene transfer between genetically modified plants and bacteria would not significantly increase the overall frequency of these sequences in the environment.

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 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.