



Notification 6786-01-00075

Summary of the risk assessment of genetically modified oilseed rape plants

(*Brassica napus* L.) (progeny of the F1-hybrid 23-198)

carried out by the German Competent Authority within

the framework of a proposed deliberate release,

Berlin, 25 March 1997

Explanatory note to this document:

The following text reflects the summary of the risk assessment of (a) genetically modified organism(s) to be used for experimental field trials (deliberate releases) in Germany. The text forms part of the official authorisation regarding applications for the permit of deliberate releases (field trials) of genetically modified organisms in Germany under the legal framework of Directive 2001/18/EC and the German Gene Technology Act (Gentechnikgesetz, GenTG). The authorisation is issued by the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL [*Federal Office of Consumer Protection and Food Safety*], as the German Competent Authority. It comprises the chapters

- I. Consent [to the application]
- II. Provisions [to be respected in execution of the field trials]
- III. Justification
 - III.1. Requirements for approval according to section 16 GenTG [German Gene Technology Act]
 - III.1.1. Requirements for approval according to section 16 (1) Nr. 1 GenTG
 - III.1.2. Requirements for approval according to section 16 (1) Nr. 3 GenTG
 - III.1.3. Requirements for approval according to section 16 (1) Nr. 2 GenTG
 - III.1.4. Formal requirements according to section 16 (4, 5) GenTG
 - III.2 Appraisal of and reply to objections
- IV. Costs
- V. Legal instruction

Only the original German document is legally binding. The following passage is a courtesy translation of the chapter III.1.2. and was prepared for the Biosafety Clearing-House.

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

(a) The gene for a C 12:0 acyl carrier protein thioesterase (ACP-TE)

The expression of the ACP-TE gene from *Umbellularia californica* contained in the genetically modified oilseed rape plants takes place under the control of the seed-specific napin promoter from *Brassica napus* and the terminator of the napin gene.

The napin promoter causes expression of the ACP-TE gene in the developing seeds. The produced thioesterase cleaves lauryl-ACP so that lauric acid (C12:0) accounts for up to 40% of the total fatty acid content of the seed. The proportion of lauric acid in conventional plants is less than 1%. The thioesterase from *U. californica* shows homology to naturally occurring thioesterases in oilseed rape, which have different substrate specificities. Thioesterases have been described in various different plants. There are no indications that the newly formed thioesterase would have a toxic effect. Lauric acid is a fatty acid that occurs in many plant storage oils, e.g. palm oil and coconut oil.

The mode of action of the inserted gene is not expected to pose a hazard to human health or the environment.

(b) The *nptII* gene from the transposon Tn5

The *nptII* gene for the neomycin-phosphotransferase II from the Tn5 transposon is under the control of the 35S cauliflower mosaic virus (CaMV) promoter and the tml 3' terminator sequence from the *Agrobacterium tumefaciens* pTiA6 plasmid. The *nptII* gene served as a marker gene for selecting transformed plant cells.

The neomycin phosphotransferase gene is a type II aminoglycoside 3'-phosphotransferase (APH(3')II) which catalyses the ATP-dependent phosphorylation of the 3'-OH group of the aminohexose ring of specific aminoglycoside antibiotics, causing these to become inactivated. The enzyme is characterised by high substrate specificity. The antibiotics kanamycin, neomycin, geneticin, butirosin, gentamicin A and B, and paromomycin belong to the APH(3')II enzyme substrates. The therapeutically important agent gentamicin and other aminoglycosides and aminocyclitols used in human medicine do not belong to the substrate spectrum of the APH(3')II enzyme. Kanamycin and neomycin are, however, widely used in veterinary medicine. Due to the substrate specificity of neomycin phosphotransferase, no new metabolic products are expected to arise in the genetically modified plants in the absence of substrate under field conditions. Since high concentrations of the relevant antibiotics are not present in the soil, the neomycin phosphotransferase does not confer any selective advantage on the genetically modified plants under field conditions. There is no evidence to suggest that this enzyme is toxic to plants, animals, microorganisms or humans.

(c) Border sequences from Ti-plasmids, T-DNA sequences and regulatory sequences

The genetically modified oilseed rape plants were generated by means of transformation with the binary vector pCGN3828. Depending on the gene products of the *vir* region of a helper plasmid present in the *Agrobacterium* strain used for the transformation, which was not transferred to the plants, these sequences from *A. tumefaciens* caused the integration of the genes located between the border regions into the chromosomes of the oilseed rape plants. These border regions of the Ti plasmids have no function in the genetically modified oilseed rape plants and are not expected to cause any changes in the plants.

The T-DNA of the vector pCGN3828 also contained the *lacZ'* fragment, in the multiple cloning site of which the chimeric ACP-TE gene was inserted, and an approx. 300 bp long se-

quence from the TnT transposon. These nucleic acid sequences are not expected to pose a potential hazard.

Furthermore, the genetically modified plants also contain the following regulatory sequences:

- the 35S promotor of CaMV,
- the promotor region of the napin gene from *B. napus*,
- the terminator region of the napin gene from *B. napus*,
- the *tml* 3' terminator sequence from the *A. tumefaciens* plasmid pTiA6.

The promotor and termination sequences regulate the expression of the genes located between them: the ACP-TE gene and the *nptII* gene. Statements regarding the effects of the expression of these sequences in the plants are found under points III.1.2.1.a) and b).

(d) Sequences located outside the T-DNA

As a general rule, only DNA sequences located within the border regions are integrated into the plant genome in *Agrobacterium*-mediated transformations. However, the transfer of DNA sequences from outside the border regions has been reported in the scientific literature.

According to the results of the studies submitted by the applicant, vector sequences which correspond to a maximum of 600 base pairs of the replication origin of the pRI plasmid from *Agrobacterium rhizogenes* are present in two of the T-DNA copies integrated into the plant genome. There is no evidence to indicate that this origin of replication has a function in higher plants. Other sequences of the binary vector were not transferred.

(e) Position effects and context changes; allergenicity

The level of expression of genes that have been integrated into the plant genome by genetic engineering methods is dependent on the insertion site on the chromosome and/or on the environment around the insertion site (position effect). Under field conditions the level of expression may be additionally influenced by environmental factors, for instance, by temperature. In the present case this could mean that the alteration in the lauric acid content of the seeds of the genetically modified plants might not occur to the same extent as observed in other field trials conducted outside Germany. This is not expected to pose a risk to the environment or to human or animal health.

The insertion of foreign genes may influence the expression or regulation of native plant genes at and/or near the site of insertion. Such processes may alter plant metabolic pathways. However, during propagation of the genetically modified plants in the greenhouse and in other field trials with these genetically modified plants, no observations were made that would indicate such an event.

Mobile genetic elements (transposable elements), which when transposed within the genome can exert effects on existing plant genes at the target site, occur naturally in plants. The inactivation of genes or alterations in gene regulation also take place in a range of other naturally occurring processes, e.g. point mutations, deletions or translocations, and are traditionally used in plant breeding. Therefore, even in non-genetically modified plants such events can always influence plant metabolic pathways. With regard to these properties, the genetically modified plants proposed for release here do not differ fundamentally from non-genetically modified plants.

With the current state of knowledge it is not possible to predict the potential allergenic effect of a protein on the basis of its amino acid sequence. However, from previous experiments with genetically modified oilseed rape plants that express the acyl carrier protein thiosterase conducted in climate chambers or greenhouses, as well as from deliberate release experiments conducted outside Germany, there is no evidence of increased allergenicity through pollen of these plants.

In this context, it should be mentioned that oilseed rape plants resulting from breeding of the F1 hybrid 23-198 have already been authorised for unrestricted commercial production and use in Canada and the USA, after being assessed by the competent authorities in those countries.

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

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

Summer oilseed rape is an annual, winter oilseed rape a perennial plant. Following the generative phase the plant dies off; new plants can only emerge from the seeds produced. If they become buried deep in the soil and enter secondary dormancy, rape seeds can persist in the ground for over 20 years. More recent studies show that alterations in the fatty acid composition of the storage lipids of the seeds can affect seed persistence in oilseed rape. However, whether the lauric acid produced in these genetically modified rapeseeds does in fact influence their capacity to persist is not yet known.

In any case, the persistence of seeds from the genetically modified oilseed rape and from any potentially occurring oilseed rape hybrids can be minimised by taking appropriate measures after every harvest to ensure that any seeds released are brought to germination during the same vegetation period. The plants that emerge from these seeds can easily be destroyed. Corresponding measures are planned by the applicant and/or are the subject of provision II.6..

After the above measures have been implemented, while the soil is being prepared for future conventional agricultural use, as planned, any seeds from oilseed rape or oilseed rape hybrids that remain in the soil are brought close to the soil surface where they can germinate. The resulting plants will be identified and destroyed in the course of the planned control measures which are to take place during and after the end of the trial. A post-trial monitoring period of three years is considered sufficient to control for re-growth of oilseed rape plants. If genetically modified oilseed rape plants or oilseed rape hybrids continue to emerge during the third post-trial monitoring year, the monitoring period is to be extended by a further year. To ensure that re-emerging plants can be detected, no oilseed rape plants should be cultivated on the release site during the monitoring phase following completion of the proposed trial. This cultivation pause and the monitoring measures carried out during the trial ensure that any potentially re-emerging oilseed rape plants and oilseed rape hybrids can be identified.

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

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

Because they have no apparent selective advantage over other plants, the genetically modified oilseed rape plants are not expected to develop altered plant sociological traits nor to be capable of populating other biotopes as a result of the inserted genes. Therefore, even in the event of the emergence of individual genetically modified oilseed rape seedlings and the possible transfer of pollen to non-genetically modified plants, no long-term, sustainable spread of the genetically modified oilseed rape is expected. The temporal and spatial limitation of the release is thus sufficiently guaranteed.

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

Oilseed rape stocks are about two thirds self-pollinating and one third cross-pollinating. This oilseed rape pollen is dispersed by insects, especially bees, and by wind. To minimise undesirable foreign pollination in agricultural seed production, seed legislation calls for isolation distances of 100 m for certified seed and 200 m for basic seed. The applicant plans to implement these minimum isolation distances for the cultivation of the genetically modified oilseed rape plants for propagation. When cultivating plants for seed production, it is not possible to have an additional border row. The release sites which the applicant proposes to use as breeding yards during the deliberate release trial shall be subject to the isolation measures required by provision II.6. However, it must be assumed that to a limited extent oilseed rape pollen can be carried beyond the proposed isolation distance.

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

The possibility of oilseed rape from self-harvested seed being grown in the vicinity of the release site as a catch crop for green manure or for the production of green fodder cannot be ruled out. In this type of one-time reproduction, the plants do not normally reach the flowering stage. Therefore, genetically modified seed cannot be produced and spread by this means.

Pollination of individual flowers of non-genetically modified oilseed rape and a one-time reproduction of this oilseed rape would result in the temporary appearance in the vicinity of the release site of isolated oilseed rape plants with increased lauric acid content. Since the transferred characteristics confer no apparent selective advantage, this is not expected to pose a risk to the environment or to agriculture.

During extraction of the rapeseed oil (e.g. also as a foodstuff) from seeds that may have emerged as result of the pollination of individual oilseed rape flowers with pollen from the genetically modified oilseed rape plants, the produced enzymes would be separated from the oil along with the rest of the proteins. The produced fatty acid, lauric acid, is also a component of oils and fats of other origins which are used for human consumption. The proteins would remain in the pressing residue, the so-called "press cake", which is used as animal

fodder. Therefore, the potential pollination of individual flowers with pollen from the genetically modified oilseed rape plants is not expected to have adverse effects on human health.

Swede (*B. napus* var. *napobrassica*) belongs to the same species as oilseed rape. One can assume that oilseed rape and swede are cross-compatible.

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

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

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

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

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

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

The following holds for all theoretically possible hybrids between the genetically modified plants and non-genetically modified crop plants or wild plants: the ACP-TE gene would not

confer a selective advantage to the plants. Therefore, there are no grounds for concern about the unintended spread of such plants.

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

The inserted sequences are integrated into chromosomes of the recipient organisms. From studies on the transformation capacity of soil bacteria under natural conditions it can be concluded that a transfer of plant genetic material to soil microorganisms is theoretically possible, although it must be assumed that such a gene transfer would constitute an extremely rare event.

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

The ACP-TE gene originates from the plant *U. californica* and shows homology with naturally occurring thioesterases in oilseed rape which have a different substrate specificity. Thioesterases have been described in various different plants. Bacteria could also – in case of a hypothetical gene transfer – take up similar thioesterase genes from other plants. There is no evidence to indicate that the uptake of the ACP-TE gene would confer any selective advantage on bacteria – even in the event of a horizontal gene transfer – such that a noticeable increase in the overall frequency of this gene in the environment would result.

As already described under point III.1.2.1.(b), antibiotics that are inactivated by the neomycin phosphotransferase are of little relevance to human medicine, but they are widely used in veterinary medicine. As a precautionary measure, it was examined whether the therapeutic use of the relevant antibiotics would be affected by a potential horizontal gene transfer of the *nptII* gene.

Even in the event of a transfer of the other regulatory sequences used in the constructs, an increase in the overall frequency of the corresponding DNA sequences is not to be expected. The napin promoter and the napin terminator occur naturally in oilseed rape. The other regulatory sequences were derived from *A. tumefaciens* and CaMV. *A. tumefaciens* is widespread in soils and the sequences mentioned above are found in wild-type *Agrobacteria* on Ti-plasmids, which can be exchanged between different species of *Rhizobiaceae* by means of conjugation. The theoretical possibility of a transfer of the CaMV-derived sequences from these plants would not represent a new situation in comparison to the naturally occurring situation, since CaMV is already found in plants as a double-stranded plant-infecting DNA virus.

The genetically modified plants contain a nucleic acid fragment which originates from a region of the binary plasmid pCGN3238 outside the T-DNA (see point III.1.2.1.d). This is a partial sequence of the replication origin of the pRI plasmid from the gram-negative bacterium *A. rhizogenes*. Since the conjugative exchange of nucleic acids between microorganisms is common, the likelihood of this sequence spreading by transfer between bacteria is far greater than the likelihood of spreading by horizontal gene transfer from the genetically modified plants to microorganisms.

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

To generate the genetically modified oilseed rape plants, pieces of hypocotyl were inoculated with *Agrobacteria* containing the genes intended for transfer between the border regions of the binary vector plasmid. In contrast to the common wild-type *A. tumefaciens*, the *Agrobacterium* strain used is “disarmed”, i.e. it no longer has the capacity to induce tumours. After transformation had occurred, antibiotic treatment was carried out to eliminate the *Agrobacteria*.

The seeds of the genetically modified oilseed rape lines intended for deliberate release were obtained by generative propagation of the original transformants. As a result of the reproduction via seeds, any *Agrobacteria* that might remain after the antibiotic treatment have been removed from the genetically modified oilseed rape lines.