



Notification 6786-01-0158

**Summary of the risk assessment on
genetically modified peas (*Pisum sativum*; Eiffel)
within the framework of a proposed deliberate release
carried out by the German Competent Authority
Berlin, 15 April 2005**

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 plants effected by the transferred nucleic acid sequence

a) The *Vfaap1* gene

The *Vfaap1* gene from *Vicia faba* encodes an amino acid permease. Amino acid permeases transport amino acids from the plant vascular tissue into the symplasts of plant cells. They

are differentially expressed according to plant development and tissue, and exhibit a range of specificity patterns for amino acids. In the donor organism, *Vicia faba*, the *Vfaa1* gene is expressed predominantly in the storage parenchyma cells of the cotyledons, particularly in early development. The strongest expression corresponds with the formation of storage protein transcripts. VfAAP1 mediates the transport of a range of amino acids (mainly cysteine, arginine, glutamine, serine, leucine, methionine, histidine, glycine and threonine) with particular emphasis on cysteine.

In the genetically modified pea, expression of the *Vfaap1* gene is controlled by the promoter of the legumin B4 gene (*leB4* gene) from *Vicia faba*. This promoter regulates the seed-specific expression of the genes under its control. Expression in pollen has also been observed. In immature seeds of the transgenic pea line proposed for release, expression of the gene was verified by Northern blot. Other parts of the plant have not yet been examined for expression.

In greenhouse studies on the genetically modified pea line 14/10 no differences in habitus (growth and flowering time) were found in comparison to the control line 14/3. The number and weight of the seeds and their protein content as well as the C:N ratio were also defined. With the exception of an increase in protein content, the genetically modified plants displayed normal phenotype.

Both the target gene *Vfaap1* and the *leB4* gene promoter used are derived from *Vicia faba* (field bean or broad bean). In Germany *Vicia faba* is cultivated as a pulse crop on about 21,000 hectares of land (2004). Its primary uses are as protein-rich fodder (field bean) and as a vegetable (broad bean). As long as the usual methods of preparation are observed, the broad bean has no known toxic effect on humans. Neither the peas nor any other parts of the genetically modified plants cultivated in the framework of this field trial are intended for human or animal consumption.

In the context of the proposed field trial the formation of the amino acid permease of the field bean in pea seeds and the resulting increase in the seed protein content are not expected to pose any threat to the health of humans or animals or to the environment.

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

The pPZP200/VfAAP1 plasmid used in the co-transformation of the pea only contains the construct of the target gene within its T-DNA borders.

The pCAMBIA3300D plasmid, which was also used in the co-transformation, contains a marker gene for selecting the genetically modified plant tissue (*bar* gene) and also nucleotides of the *lacZ* gene from *E. coli*. As a result of the integration of the T-DNAs of both transformation plasmids at different locations in the plant genome and as a result of the breeding steps taken following regeneration of the fertile plants, the *bar* gene is no longer contained in the plants intended for release. Any unexpected additional components of the T-DNA of the pCAMBIA3300 plasmid in the genetically modified plants are of bacterial origin and would not be active in plants.

(c) Sequences located outside the T-DNA

As a rule, only DNA located within the border regions is integrated into the plant genome during Agrobacteria-mediated transformations. However, a transfer of DNA fragments outside the border regions has been reported. The results of a Southern blot analysis submitted with this application show that regions outside the T-DNA of the pPZP200/VfAAP1 plasmid were not transferred to the genetically modified pea. However, if the genetically modified plants were to contain any unexpected additional components of the pPZP200/VfAAP1 plasmid (streptomycin/spectinomycin resistance genes, regions of plasmid replication, stability and

mobilisation from the pBR322 and pVS1 plasmids), these would not be active in plants. The same applies to components of the pCAMBIA3300 plasmid.

(d) Position effects and context changes; allergenicity

Genes which have been 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 nucleotide sequences neighbouring the integration site ("position effect"). Under field conditions the level of expression may be influenced by environmental factors, for instance, by temperature. In this particular case this could mean that the characteristics of the genetically modified peas are not modified to the same degree in the field as under climate-chamber or greenhouse conditions. This is not expected to pose a risk to the environment or to human or animal health.

The insertion of foreign genes may influence the expression or regulation of the plant's own genes at or near the site of insertion. Such processes may alter plant metabolic pathways. However, during the course of the work carried out to date on these genetically modified plants, no observations were made that would suggest such an event.

Mobile genetic elements (transposable elements), which when transposed within the genome can exert effects on existing plant genes at the target site, occur naturally in plants. The inactivation of genes or alterations in gene regulation also take place in a range of other naturally occurring processes such as point mutations, deletions or translocations and are traditionally used in plant breeding. Therefore, even in non-genetically modified plants such events can always influence plant metabolic pathways. With regard to these properties the genetically modified plants 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 possible allergenic action of a protein on the basis of the amino acid sequence. As already described under (a), the target gene and the promoter sequence originate from a plant species that is used in foodstuffs and animal feed. Given this fact, the use of these sequences is not expected to result in any adverse effects on human health. Pea pollen is only dispersed to a small degree by wind and generally does not play a noteworthy role in triggering pollen allergies.

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

P. sativum is a summer annual plant with a one-year life cycle. Sexual reproduction takes place by seed. Seeds in the stock can be dispersed if the pods open before harvesting. To prevent dispersal through seeds falling from the pod or being spread by animals, the applicant intends to cover the individual pods with small bags and to harvest them manually. The long-term establishment of crops such as the pea outside the cultivation area is unlikely because germinating peas compete poorly against the vegetation (weed growth) present at the site of germination. Plants of the summer pea varieties found in Germany are sensitive to frost and die off at temperatures of between -5 bis -9°C. In frost-free regions seeds that have germinated in autumn have the potential to overwinter. Under certain climate conditions (mild winter) pea seeds may persist in the soil. The emergence of seedlings from such seeds would, however, be rare and these would be only weakly competitive; furthermore, they would be identified during the planned post-trial monitoring of the release site. Vegetative reproduction does not take place. There is no evidence for the spread of cultivated peas in the environment. Thus establishment of these plants outside cultivated areas is not anticipated.

Owing to the planned covering of the pods before maturation and the manual harvesting of the plants described in the experimental design of the present application, an unintentional spread of the genetically modified pea plants is highly unlikely. In the event that plant material remains on the release site, no more plants would be able to regenerate from the residue. Consequently, the genetically modified plants are not expected to persist or establish in the environment.

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

P. sativum is almost exclusively self-pollinating. The percentage of cross-pollination, which is primarily carried out by insects (mainly wild bees), can be up to 1-3%. Therefore the possibility of pollen transfer to other varieties of garden pea (*P. sativum* ssp. *hortense*) or feed pea (*P. sativum* ssp. *arvense*) cannot be ruled out, although it is largely limited by the prevalence of self-pollination. The applicant plans a minimum separation distance of 20 m to the next nearest area cultivated with peas; peas from the gene bank stock of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) are grown about 500 m from the release site. These distances are considered sufficient to prevent pollen transfer from the genetically modified pea plants to other peas. In Central Europe, no wild species of the genus *Pisum* that could hybridise *P. sativum* occur. There is no evidence of hybridisation with other plant species.

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

The inserted sequences are stably integrated into the chromosomes of the recipient organisms. There is no evidence that the transfer of genetic information from plants or its expression in micro-organisms takes place under natural conditions. However, 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 which are so distantly related in terms of taxonomy is actually possible, it could 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.

(a) The *Vfaap1* gene construct

The sequences from the *Vfaap1* gene and the *leB4* promoter present in the construct originate from *Vicia faba* and are therefore already commonly found in the environment. A horizontal gene transfer in micro-organisms is therefore much more likely to occur from non-genetically modified organisms. In the event of a horizontal gene transfer in microorganisms the *Vfaap1* construct is also not expected to be functional in these microorganisms.

(b) Sequences located outside the T-DNA

Based on the study results contained in the notification documents, it is unlikely that nucleic acid fragments located outside the T-DNA border regions of the plasmid pPZP200/VfAAP1 have been transferred into the genome of the genetically modified peas. However if, contrary to expectations, these nucleic acid fragments were transferred, the likelihood that they would spread by transfer between bacteria would far outweigh the probability of spreading by hori-

zontal gene transfer from the genetically modified plants to micro-organisms. Therefore, the potential presence of these nucleic acid fragments in the plant chromosome would not be expected to contribute to an increase in the overall frequency of horizontal gene transfer. The same applies to the pCAMBIA3300 plasmid.