Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0179

Summary of the risk assessment of the genetically modified

maize (*Zea mays*)

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 21. May 2007

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.

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

a) The *epsps* gene

In these genetically modified maize plants the expression of the genes for a glyphosate-tolerant EPSPS derived from *Agrobacterium* sp. strain CP4 takes place constitutively under the control of the CaMV *35S* promoter and the Act1 promoter from rice (Oryza sativa). The presence of the introns (see I. 1.1.) in both transcription units is aimed at enhancing gene expression. The upstream position of the EPSPS chloroplast transit peptide derived from *Arabidopsis thaliana* (CTP2) causes the post-translational import of the CP4 EPSPS into the chloroplasts. The transit peptide is generally cleaved on import (processing).

Both the endogenous EPSPS and the CP4 EPSPS introduced into the maize plants by means of transformation catalyse the reaction of shikimate-3-phosphate with phosphoenolpyruvate in the chloroplast to yield 5-enolpyruvylshikimate-3-phosphate, an intermediate stage in the biosynthesis of aromatic amino acids and other aromatic substances of secondary plant metabolism. In contrast to the endogenous EPSPS, the CP4 EPSPS is not inhibited by glyphosate.

The additional expression of CP4 EPSPS in the GM maize catalyses the same reaction as corresponding, naturally occurring enzymes in maize and other crop plants. Since no adverse health effects have been attributed to the *Arabidopsis thaliana*-derived transit peptide EPSPS CTP2, or to any other currently known signal peptides, whether processed or unprocessed, it can be assumed that the same applies to transit peptide-enzyme compounds (in this case CP4 EPSPS). There is no reason to expect that the newly formed EPSPS would have a toxic effect.

The mode of action of EPSPS introduced by means of transformation is not expected to pose risks to human or animal health or to the environment.

The *pat* gene

Expression of the transferred gene for PAT takes place under the control of the constitutive CaMV *35S* promoter and the CaMV *35S* terminator. The gene for PAT codes for an enyzme that confers resistance to the agent L-phosphinothricin. The herbicidal component of glufosinate ammonium is L-phosphinothricin (L-PPT). In plants L-PPT binds to the active site of glutamine synthetase. As a result, the breakdown of surplus ammonium in the plant is blocked, causing the plant to die off. PAT converts the herbicidal substance L-PPT to N-acetyl-L-phosphinothricin (N-acetyl-L-PPT), which has no herbicidal effect. *Pat* expression in the 1507 maize plants allows the continued breakdown of surplus ammonium by glutamine

synthetase. As a result, the 1507 maize plants possess in-built tolerance to the herbicide glufosinate ammonium. Field trials with 1507 maize plants have demonstrated that this tolerance persists when glufosinate ammonium is applied at concentrations of 1600 g a.i./ha – an amount four times that applied in practice.
There is no evidence to suggest that the PAT expressed in the 1507 maize plants performs any other physiological activities. Therefore, apart from the production of PAT in the 1507 maize plants and – in the case of glufosinate ammonium application – the above-described metabolisation of L-PPT, we can assume that no other effects on the plant metabolism will occur. This assumption is based mainly on the results of compositional analyses conducted within the context of applications for placing on the market. Moreover, the evaluation of agronomic parameters and phenotypic characterisation of the 1507 maize plants failed to produce evidence that PAT expression would produce effects on plant development or plant metabolism. The mode of action of the *pat* gene introduced by means of transformation is not expected to pose a threat to human or animal health or to the environment. In the maize line 59122, PAT is expressed by a gene construct which is almost identical to that contained in maize 1507; the assessment therefore corresponds to that for maize 1507.

The *cry1F* gene

Expression of the transferred gene for Cry1F takes place constitutively under the control of the ubiZM1(2) promoter and the ORF25PolyA terminator.
The c*ry1F* gene codes for a *Bt* toxin. There is no evidence of enzymatic activity of the *Bt* toxin expressed in the 1507 maize plants. Therefore we can assume that, apart from the formation of *Bt* toxin in the 1507 maize plants, no other effects on the plant metabolism will occur. This assumption is mainly based on the results of compositional analyses conducted within the scope of applications for placing on the market. Moreover, the evaluation of agronomic parameters and phenotypic characterisation of the 1507 maize plants failed to produce evidence that expression of the *Bt* toxin would produce effects on plant development or plant metabolism.

The mode of action of the CryF1 protein introduced by means of transformation is not expected to pose a hazard to human or animal health. In view of the selective mechanisms of action of *Bt* toxins due, amongst other things, to receptor-specific binding in the intestinal tract of susceptible insects, no adverse effects on the environment are expected to result from the release of these maize plants.

The *cry34Ab1* and *cry35Ab1* genes

The genes for the proteins Cry34Ab1 and Cry35Ab1 introduced into maize 59122 are expressed constitutively under the control of the ubiZM1(2) and the TA peroxidasePRO promoters, respectively. In both cases expression is terminated by the PIN II terminator.

The genes c*ry34Ab1 and cry 35Ab1* encode proteins of 14 and 44 kDA, respectively, which when coexpressed are toxic to susceptible insects. Feeding studies indicate that the larvae of the chrysomelidae family of beetles (e.g. *Diabrotica sp.*) are killed off by a combination of the proteins Cry34Ab1 and Cry35Ab1.

There is no evidence of enzymatic activity of the *Bt* toxins expressed in the 59122 maize plants. Therefore we can assume that, apart from the formation of *Bt* toxin in the 59122 maize plants, no other effects on the plant metabolism will occur.

The mode of action of the Cry34Ab1 and Cry35Ab1 proteins introduced by means of transformation is not expected to pose a hazard to human or animal health. In view of the selective mechanisms of action of *Bt* toxins due, amongst other things, to receptor-specific binding in the intestinal tract of susceptible insects, no adverse effects on the environment are expected to result from the release of these maize plants.

The proteins CP4 EPSPS, PAT, Cry1F, Cry34Ab1 and Cry35Ab1 are coexpressed in the hybrid 59122x1507xNK603; CP4 EPSPS is expressed in the chloroplasts, PAT, Cry1F, Cry34Ab1 and Cry35Ab1 in the cytoplasm. An interactive effect of the proteins *in planta* can be ruled out, since the Bt protein is not metabolically active and the enzymatic activity of PAT and CP4 EPSPS is clearly limited. Moreover, given that all of these proteins are broken down by gastric fluid in mammals, coexpression of the proteins CP4 EPSPS, PAT, Cry1F, Cry34Ab1 and Cry35Ab1 in the hybrid is not expected to pose a threat to human or animal health or to the environment.

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

The plasmid pPHP17662 employed in the transformation of maize 59122 contains only the target construct within the T-DNA borders.

(c) Sequences located outside the T-DNA

As a rule, only DNA located within the border regions is integrated into the plant genome in *Agrobacterium*-mediated transformations. However, a transfer of DNA fragments outside the border regions has been reported. The plasmid pPHP17662 used to transform the 59122 maize also contains genes that confer resistance to the antibiotics tetracycline and spectinomycin outside the border regions.

The results of Southern blot analyses attached to the application show that none of the regions outside the T-DNA of the transformation plasmid were transferred to the GM maize line 59122.

(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 characteristics of the genetically modified plants are not modified to the same degree in the field as under climate chamber or greenhouse conditions. This does not represent 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 or near the site of insertion. Such processes may affect plant metabolic pathways. However, in previous studies carried out with these GM plants no observations were made that would suggest such an event.

Mobile genetic elements (transposable elements) that can exert effects on existing plant genes at the target site when transposed within the genome are naturally occurring 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 influence plant metabolic pathways. In this respect the genetically modified plants do not differ fundamentally from non-genetically modified plants.

Given the current state of knowledge, it is impossible to make reliable predictions about the possible allergenic action of a protein on the basis of its amino acid sequence. However, in numerous earlier field trials with plants that express either the *epsps* gene or the *pat* gene under the control of non-tissue-specific promoters, no evidence of increased plant allergenicity was recorded. Likewise, there is no evidence of increased allergenicity of the Bt protein expressed in plants.
The GM maize proposed for release is not intended for use in the production of food or feed within the scope of the planned trial.

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

Maize plants and maize seeds are not hardy. Maize does not have the ability to persist in Central European climate conditions. The genetic material introduced into these maize plants/seeds confers resistance to infestation by certain coleopteran and lepidopteran insects and imparts tolerance to the herbicidal agents glyphosate and glufosinate ammonium. It can be assumed that the persistence traits of these plants have not been altered.

Genetically modified maize may reach grain maturity during the vegetation period. The establishment of volunteer maize has not been observed in the flora of Central Europe, even in grain maize that is harvested when fully mature. If genetically modified maize plants were to emerge in the experimental area after the end of the release period, they would be subsequently detected and destroyed in the course of the required cultivation gap and post-trial monitoring, as set down in provision II.9 [of the decision on this application]. These measures help to ensure the spatial and temporal limitation of the proposed release trial.

On conclusion of the proposed trial series, both the GM and the non-GM maize plants will be shredded and incorporated into the soil to rot. Even if some of the maize grain escapes being broken down in the shredding process, it can still be assumed that under field conditions no persistent plants would develop from this grain.

The non-GM maize plants from the border rows are to be disposed of in the same manner as the GM trial plants.

III.1.2.3. Assessment of the possiblilty of pollen-mediated transfer of the inserted gene from the genetically modified maize plants to other plants

The possibility that the genes introduced into the GM maize plants might be transferred to other plant species can be excluded, since maize has no crossing partner in the flora of Central Europe. Therefore, the focus here is placed solely on the risk of pollen transfer from the genetically modified maize plants to other maize plants.

Maize pollen is normally dispersed by wind. In the production of hybrid maize seeds, seed legislation stipulates – in the absence of other isolation measures - a minimum separation distance of 200 m to other maize fields to adequately minimize incrossing by pollen of other varieties.

The applicant plans to observe an isolation distance of 200 m to commercially grown maize stocks, as well as the planting of a trap crop consisting of 4 rows of non-GM maize. These measures will ensure that the risk of pollen transfer to other maize populations is adequately addressed.

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

(a) The expression cassettes of the genes *epsps*, *pat*, *cry1F*, *cry35Ab1* and *cry34Ab1*

The inserted sequences are stably integrated into the genome of the recipient organisms. There is no evidence that the transfer of genetic information from plants and its expression in microorganisms can take 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.

If we assume that an exchange of genetic material between organisms which are so distantly related in terms of taxonomy as plants and bacteria is actually possible, it should then 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 GM plants contain copies of the CP4 *epsps* gene, the *pat* gene, the *cry1F* gene and the *cry34Ab1* and *cry35Ab1* genes, whereby the coding region of the *epsps* gene is N-terminally fused to plant transit peptide sequences. These transit peptide sequences would be non-functional in bacteria.

The expression of glyphosate-tolerant EPSP synthases is a naturally occurring process in soil microorganisms. Bacteria with a corresponding resistance are commonly found in the environment.

The inactivation of phosphinothricin by acetylation is a naturally occurring process in soil microorganisms. Bacteria with a corresponding resistance are widespread in the environment. Therefore this resistance may also be spread by horizontal gene transfer from non-GM microorganisms. Even in the case of a transfer of the *pat* gene from the GM plants to microorganisms, the overall distribution of this resistance in the environment would not increase significantly.

The *cry1F*, *cry34Ab1* and *cry35Ab1* genes originate from *Bacillus thuringiensis*, a ubiquitous soil bacterium. Even in the case of a transfer of these genes from the GM plants to microorganisms, no detectable increase in the overall frequency of these genes in the environment would result. A gene transfer of this type is not likely to have ecological consequences.

(b) Additional fragments located within the transferred DNA

Apart from the expression cassettes mentioned in (a), the DNA fragments used to transform the maize lines 1507, NK603 and 59122 only contain a number of short nucleotide fragments with the recognition sequences for restriction endonucleases, which are important for molecular biology studies. These short fragments are not known to have any further functions.

(c) Sequences located outside the T-DNA (in the case of line 59122)

Based on the results of the investigations described and submitted along with the application, it can be assumed that nucleic acid fragments located outside the T-DNA border regions of the plasmid PHP17662 were not transferred to the genome of the GM maize plants.