Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0169

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, 19. May 2006

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]
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III.2 Appraisal of and reply to objections
IV. Costs
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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.

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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

The *epsps* gene codes for an enylpyruvylshikimate-3-phosphate synthase (EPSPS). 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 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. As a result, the genetically modified maize can tolerate applications of glyphosate-based herbicides.

In the genetically engineered maize, expression of the *epsps* gene derived from *Agrobacterium* sp. strain CP4 takes place under the control of the Act1 promoter (*Oryza sativa*). The *act1* intron from rice is included in the transcription unit with the aim of enhancing gene expression. A second copy of the *epsps* gene is expressed under the control of the e35S promoter from the cauliflower mosaic virus. Inclusion of the *hsp70* intron from *Zea mays* in the transcription unit is aimed at increasing the level of 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.

The additional expression of CP4 EPSPS in genetically modified maize catalyses the same reaction as the corresponding enzymes that occur naturally in maize and other cultivated crops. 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 inserted by means of transformation is not expected to pose any risk to human or animal health or to the environment.

(b) The *cry*3Bb1 gene

The *cry3*Bb1 gene codes for a coleopteran-specific protein toxin (*Bt* toxin). The protein expressed in the genetically modified organism does not show any evidence of enzymatic activity. It can therefore be assumed that apart from the formation of *Bt* toxin in the genetically modified plants, this gene is unlikely to have any further impact on the plant metabolism.

The additional gene present in genetically modified maize plants, which codes for the Cry3Bb1 protein from *Bacillus thuringiensis* ssp *kumamotoensis*, is constitutively expressed under the control of the CaMV 35S promoter. The intron of the rice actin 1 gene enhances transcription efficiency. The insecticidal action of *cry* proteins develops after the insect ingests the crystal protein: Following solubilisation in the alkaline environment of the intestinal tract of the larval insect, protolytic cleavage of the so-called δ-endotoxin takes place. This endotoxin then permeates the peritrophic membrane and binds to specific receptors in the epithelium of the midgut, altering the electrolyte permeability of the intestine and leading to a disturbance in the pH value of the digestive tract. The insect ceases to feed and dies. Receptors for δ-endotoxin do not exist in the digestive tract of mammals.

In feeding studies attached to applications for placing MON863 maize on the market, no evidence of adverse effects from the presence of Bt proteins in feed administered to rats, chickens and mice was found. Similarly, these documents did not reveal any evidence that Cry3Bb1 possesses allergenic potential. MON863 was developed using the same *cry3Bb1* gene construct used to generate the MON88017 maize referred to in the present application. The material harvested during the proposed deliberate release is not intended for use in the production of foodstuffs or animal feed.

In a two-year field study conducted in the USA the effects of Cry3Bb1 protein-producing *Bt* maize on soil microbial biomass and activity and on the structure of the microbial community in the soil was determined. No differences between the genetically modified maize and the control lines were established. Similarly, in studies on the impact of the CRY3Bb1 protein on a predatory coleopteran species, the protein was not found to have any negative impact on this beneficial species.

The mode of action of the Cry3Bb1 protein introduced by means of transformation is not expected to result in risks 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 sensitive insects, no adverse effects on the environment are expected.

(c) The *cryIA(b)* gene

The *cryIA(b)* gene codes for a lepidopteran-specific protein toxin (*Bt* toxin). The protein expressed in the genetically modified organisms shows no evidence of enzymatic activity. It can therefore be assumed that apart from the formation of the *Bt* toxin in the genetically modified plant, this gene is not likely to have any further impact on the plant metabolism.

The additional gene contained in the genetically modified maize plants, which codes for the CryIA(b) protein from *Bacillus thuringiensis* ssp *kurstaki*, is expressed under the control of the CaMV 35S promoter. The insecticidal action of *cry* proteins develops after the insect ingests the crystal protein: Following solubilisation in the alkaline environment of the intestinal tract of the larval insect, protolytic cleavage of the so-called δ-endotoxin takes place. This endotoxin then permeates the peritrophic membrane and binds to specific receptors in the epithelium of the midgut, altering the electrolyte permeability of the intestine and leading to a shift in the pH value of the digestive tract. The insect ceases to feed and dies. Receptors for δ-endotoxin do not exist in the digestive tract of mammals.

In feeding studies attached to applications for placing MON810 maize on the market, no evidence of adverse effects from the presence of Bt proteins in feed administered to rats, chickens and mice was documented. Similarly, in an assessment of the dossier Cry1A(b) was not found to have any allergenic potential.

The material harvested within the scope of the proposed deliberate release is not intended for use as food or feed.

Field trials conducted by the German Federal Agricultural Research Centre (*Bundesforschungsanstalt für Landwirtschaft*) determined the effects of the CryIA(b) protein on the microbial biomass present in the soil, microbial activity and the structure of the microorganism community. Differences between the genetically modified maize and the control lines were not detected.

Given the selective mode of action of *Bt* toxins, i.e. by binding to specific receptors in the intestinal tract of susceptible insects, no risks to human or animal health or to the environment are anticipated.

(d) Additional sequence fragments located on the DNA insert

According to the information supplied by the applicant, the plasmid used to transform the parental lines contains, in addition to the constructs mentioned in (a) and (b), only short nucleic acid fragments that serve as so-called polylinker recognition sequences for DNA- cleaving restriction endonucleases in molecular biology studies. No further functions are known for these nucleic acid fragments.

(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 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. In previous studies carried out with the genetically modified plants within the scope of the authorisation and cultivation of the parental line MON810 as well as in deliberate release trials with the parental line MON88017 in Germany and France (2004, 2005), and in trials with the hybrid in France (2005) 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 and were first identified in maize. 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 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 the numerous releases of plants that express the *epsps* 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 with regard to the CryIA(b) and Cry3Bb1 proteins expressed in the plants.

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

Maize plants and maize seeds are not hardy. Maize does not have the ability to persist in Central European climate conditions. The genetic material inserted into the maize plant confers resistance to certain coleopteran and lepidopteran insects and imparts glyphosate herbicide tolerance to the plant. It can be assumed that the persistence characteristics 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 accumulate in the experimental area after the end of the release period, they would be subsequently recorded and destroyed in the course of the required cultivation gap and post-trial monitoring, as set down in provision II.8 [of the decision on this application]. These measures help to ensure the spatial and temporal limitation of the release project.

On conclusion of the proposed trial series, the GM maize plants as well as the non-GM maize plants will be broken down by shredding. The resulting plant material will either be: a) worked into the ground and left to rot, b) composted on the release site, or c) transported to a biogas plant for disposal. 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.

Provision II.9 [of the decision on this application] states that the non-GM border row maize plants are to be disposed of in the same manner as the GM maize plants.

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

Since maize has no crossing partner in the flora of Central Europe, the possibility of a transfer of the genes introduced into the genetically modified maize plants to other plant species can be ruled out. Therefore, the focus here is 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.

Provision II.7 details the requirements applying to the proposed isolation measures on the release site. The implementation of these measures will ensure that the risk of pollen transfer to other maize populations is adequately addressed.

The applicant plans to sow a 6 m wide border row of non-genetically modified maize around the perimeter of the release plot. In combination with the specified isolation distance of 200m, these measures will ensure that the risk of pollen transfer to other maize cultivations 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*, *cry3Bb1* and *cryIa(b)*

The inserted sequences were integrated into the chromosome of the recipient organism during transformation and were passed on to the hybrids by crossing single-trait lines. Studies on the transformation capacity of soil bacteria under natural conditions also suggest that the transfer of plant genetic material to soil microorganisms is theoretically possible, although such a transfer would constitute a very rare event.

Insofar as we assume that a genetic exchange between organisms that are so distantly related in terms of taxonomy as spermatophytes and microorganisms 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.

The genetically modified plants contain one copy each of the CP4 *epsps* gene, the *cry3Bb1* gene and the *cryIa(b)* gene, whereby the coding region of the *epsps* gene is N-terminally fused to the plant transpeptide sequences. These transpeptide sequences would be non-functional in microorganisms.

The expression of glyphosate-tolerant EPSP synthases is a naturally occurring process in soil microorganisms. Bacteria with corresponding resistance are widespread in the environment. The *cry3Bb1* and *cryIa(b)* genes are derived from *Bacillus thuringiensis*, another ubiquitous soil bacteria. Even in the event of a transfer of these genes from the genetically modified plants to microorganisms, no significant increase in the overall distribution of these genes in the environment would result. A transfer of these genes is unlikely to have ecological consequences.

(b) Additional fragments located within the transferred DNA

Apart from the expression cassettes mentioned in (a), the plasmid used in the transformation of MON88017 only contains 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.