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Summary of the risk assessment of the genetically modified

maize (*Zea mays* L.) 1507, 59122, 98140, 1507x59122, 98140x59122, 98140x1507 and 98140x1507x59122

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 28 April 2010

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

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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 plants effected by the transferred nucleic acid sequences

a) The *pat* gene

The transferred gene for PAT is expressed under the control of the constitutive CaMV 35S promoter and the CaMV 35S terminator.

The *pat* gene codes for the enzyme phosphinothricin-N-acetyltransferase, which confers resistance to the agent L-phosphinothricin. In herbicides, L-phosphinothricin (= glufosinate) is mainly deployed as the ammonium salt glufonisate ammonium. 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. The expression of *pat* in the 1507 maize plants allows the continued breakdown of surplus ammonium by glutamine synthetase. As a result, the 1507 maize plants possess tolerance to the herbicide glufosinate ammonium. Field trials with 1507 maize plants have demonstrated that this tolerance persists even when glufosinate ammonium is applied at concentrations of 1600 g a.i./ha – an amount four times that typically applied in practice.

There is no evidence 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, it can be can assumed that no other effects on the plant metabolism will occur. This assumption is mainly based on the results of compositional analyses. 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.

In 59122 maize, PAT expression is controlled by an almost identical gene construct to that used in 1507 maize and therefore the assessment corresponds to that for 1507 maize.

In the opinion of the BVL and in agreement with the ZKBS, the mode of action of the *pat* gene introduced by transformation is not expected to pose a threat to human animal health or to the environment.

b) The *cry*1F gene

The transferred gene for Cry1F is expressed constitutively under the control of the ubiZM1(2) promoter and the ORF25PolyA terminator.

The *cry*1F 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 in the context of applications for placing on the market. In addition, 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.

In the opinion of the BVL and in agreement with the ZKBS, 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. Owing to 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.

c) The *cry*34Ab1 and *cry*35Ab1 genes

Expression of the genes for Cry34Ab1 and Cry35Ab1 contained in the 59122 maize plants takes place constitutively under the control of the ubiZM1(2) and the TA-PeroxidasePRO promoter, respectively, and the PIN II terminator.

The *cry*34Ab1 and *cry*35Ab1 genes code for a 14-kDa protein and a 44-kDa protein, respectively, which together have a toxic effect on sensitive insects. Feeding studies indicate that in particular the larvae of beetles of the family *Chrysomelidae* (e.g. *Diabrotica spp*.) 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. It can therefore be assumed that apart from the formation of the *Bt* toxin in the 59122 maize plants there will be no further impact on plant metabolism.

In the opinion of the BVL and in agreement with the ZKBS, the mode of action of the Cry34Ab1 and Cry35Ab1 proteins introduced by transformation is unlikely to pose a hazard to human or animal health. Owing to 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.

d) The *gat*4621 gene

The transferred expression cassette contains a gene derived from a glyphosate-N-acetyltransferase gene (*gat*) from the soil bacterium *Bacillus licheniformis*. The *gat*4621 gene was synthesised from different natural variants of the gene using a gene shuffling method. The gene codes for a glyphosate-N-acetyltransferase protein providing tolerance to herbicides containing glyphosate.

The expression of the modified glyphosate-N-acetlytransferase protein from *Bacillus licheniformis* is under the control of a constitutive promotor resulting in tolerance to glyphosate.

The enzyme 5-enolpyruvylshikimate-3-phosphate-synthase (EPSPS) catalyses 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. This metabolic pathway is inhibited by the active substance glyphosate, causing the plant to die off.

Glyphosate-N-acetyltransferase expression in the genetically modified (GM) maize plants leads to detoxification of the glyphosate agent. This is brought about by the transfer of an acetyl group from acetyl CoA to the amine group of glyphosate. The resulting N-acetylglyphosate does not have the ability to inhibit the activity of the EPSPS enzyme, and the GM plants are able to grow despite glyphosate treatment.

To evaluate the safety of the expressed protein the applicant compared the amino acid sequences of the protein with sequences from several different databases with regard to potential allergenicity and toxicity. In bioinformatic analyses of the protein, no significant similarity to known or suspected allergens was identified.

A single-dose mouse feeding study was carried out with the expressed protein. A single dose of 1640mg GAT protein per kg body weight was administered to mice. The animals were observed for 14 days, after which they were sacrificed and examined. These studies did not reveal any evidence of acute toxicity for the expressed protein.

In the opinion of the BVL and in agreement with the Central Commission for Biological Safety (ZKBS) no threat to human or animal health or to the environment is expected to result from the release of the enzyme inserted by means of transformation.

e) The *zm-hra* gene

The transferred expression cassette contains a modified *zm-hra* gene derived from maize. This gene encodes a modified acetolactate synthase, which confers tolerance to several acetolactate synthase-inhibiting herbicides such as, for example, sulfonylurea.

Expression of the modified maize acetolactate synthase protein takes place under the control of a constitutive promoter, conferring tolerance to a range of acetolactate synthase-inhibiting herbicides.

The acetolactate synthase enzyme (ALS) plays a key role in the biochemical pathways of the branched-chain amino acids leucine, isoleucine and valine. The application of ALS-inhibiting herbicides blocks this synthesis pathway. The lack of the aforementioned amino acids interferes with protein synthesis, causing the plant to die off.

In contrast to the parental line, the activity of the modified variant of the endogenous maize ALS inserted into the proposed maize line is not inhibited by the relevant herbicides. In the

GM maize plants the biosynthetic pathway for branched-chain amino acids remains uninterrupted and the plants can develop unhindered.

To evaluate the safety of the expressed protein the applicant compared the amino acid sequences of the protein with sequences from several different databases with regard to potential allergenicity and toxicity. In bioinformatic analyses of the protein no significant similarity to known or suspected allergens was identified.

A comparison of the ZM-HRA protein sequence revealed similarities to the ALS enzymes of a number of crop plants and wild plants, as well as a weaker homology to ALS sequences from bacteria and fungi. None of the protein similarities examined involved toxins or anti-nutritive substances, nor did they reveal any evidence of potential health risks.

A single-dose mouse feeding study was carried out with the expressed protein. A single dose of 1236mg ZM-HRA protein per kg body weight was administered to the mice. The animals were observed for 14 days, after which they were sacrificed and examined. The studies did not reveal any evidence of acute toxicity for the expressed protein.

In the opinion of the BVL and in agreement with the Central Commission for Biological Safety (ZKBS), no threat to human or animal health or to the environment is expected to result from the release of the enzyme inserted by means of transformation.

f) Combined expression

The proteins PAT, Cry1F, Cry34Ab1, Cry35Ab1, GAT4621 and ZM-HRA are co-expressed in the hybrid 98140x1507x59122 and partially in the hybrids 1507x59122, 98140x59122 and 98140x1507. 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, GAT4621 and ZM-HRA is clearly limited. In mammals these proteins are broken down by gastric fluid. The co-expression of PAT, Cry1F, Cry34Ab1, Cry35Ab1, GAT4621 and ZM-HRA in the hybrid plants is not expected to pose a threat to human or animal health or to the environment.

g) Position effects and context changes; allergenicity

The level at which genes which have been integrated into the plant genome by genetic engineering methods are expressed depends on the chromosomal site of integration and on the sequences neighbouring the integration site (“position effect”). Under field conditions the level of expression may also be influenced by environmental factors, for instance, by temperature. In the present case this could mean that the characteristics of the GM maize plants are not altered 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 native plant genes at or near the site of insertion. Such processes may alter plant metabolic pathways. However, in the course of the work carried out to date on these GM plants, no evidence of such an event has been observed.

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 commonly used in plant breeding. Such events can therefore influence plant metabolic pathways at any time, even in non-GM plants. To that extent the GM plants do not differ fundamentally from non-GM plants in relation to these characteristics.

The EFSA has assessed the safety of the maize lines 1507, 59122, 98140, 1507x59122, 98140x59122, 98140x1507 and 98140x1507x59122 for use as food and found no evidence of increased allergenicity in comparison to conventional maize.

The GM maize is not intended for use as food or feed in the proposed field trials.

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

Maize plants and maize seeds are not winter-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 glyphosate tolerance to the herbicidal agents glyphosate, glufosinate ammonium and acetolactate-synthase inhibiting herbicides. It can be assumed that the persistence traits of these plants have not been altered.

The GM 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 GM maize plants were to emerge in the trial area after the end of the release period, they would be subsequently detected and destroyed in the course of the cultivation gap and post-trial monitoring period required by Provision II.9. These measures 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 are to be shredded and incorporated into the soil to rot, as planned. Alternatively, the harvest material can be shredded and transported to a biogas plant. 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 a pollen-mediated transfer of the inserted genes from the GM maize plants to other plants

Since maize has no crossing partner in the flora of Central Europe, a transfer of the genes introduced into the GM maize plants to other plant species can be ruled out. Therefore the following passage deals solely with the risk of pollen transfer from the GM 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 minimum isolation distance to all other non-GM maize crops defined in Provision II. 12 ensures the spatial limitation of the field trial.

III.1.2.4. Evaluation of the possibility of horizontal gene transfer of the inserted foreign genes from the GM plants to micro-organisms

(a) The *pat, cry*1F*, cry*35Ab1*, cry*34Ab1*, gat*4621 and *zm-hra* genes

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 micro-organisms 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.

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 must 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 *pat* gene, the *cry*1F gene, the *cry*34Ab1 and *cry*35Ab1 genes, the *gat*4621 gene and the *zm-hra* gene.

The soil bacterium *Bacillus licheniformis* is widespread in the environment. Thus, it can be assumed that the original forms of the transferred glyphosate-N-acetyltransferase (GAT4621) occur in nature and display a similar mode of action. The *zm-hra* gene is a modified endogenous maize gene with a strong similarity to acetolactate synthase genes derived from maize and other plants. The metabolic enzyme ALS is commonly found in the environment and it occurs in many forms.

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

The *cry*1F*, cry*34Ab1 and *cry*35Ab1 genes originate from *Bacillus thuringiensis*, a ubiquitous soil bacterium. Even in the case of a transfer of this gene from the GM plants to micro-organisms, no detectable increase in the overall frequency of these genes in the environment would result. A gene transfer of this type is unlikely to have ecological consequences.

(b) Regulatory sequences

There is no reason to fear that a transfer of the regulatory sequences used in the constructs would lead to an increase in the overall frequency of the respective DNA fragments. These regulatory sequences originate from *Agrobacterium tumefaciens,* cauliflower mosaic virus, maize, potato and wheat and are commonly found in plants and soil organisms.

Such a gene transfer is therefore unlikely to have ecological consequences.

(c) Additional fragments located within the transferred DNA

In addition to the expression cassettes listed under (a), the DNA fragments used in the transformation of the maize lines 1507, 59122 and 98140 contain only several short nucleotide segments with the recognition sequences for restriction endonucleases, which are important for molecular biology work. These short segments are not known to have any other functions.

(d) Sequences located outside the T-DNA (in the case of 59122 and 98140)

Based on the results of the studies presented in the application dossier, it can be assumed that the nucleic acid fragments of the plasmids PHP17662 and PHP24279 located outside the T-DNA border regions were not transferred into the genome of the GM maize plants.