Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

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Summary of the risk assessment of the genetically modified maize

(Zea mays L.) T25

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 14 May 1998

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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III.2 Appraisal of and reply to objections
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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 synthetic *pat* gene

In the genetically modified maize plants, the synthetic *pat* gene codes for a phosphinothricin acetyltransferase (PAT).

L-phosphinothricin is a glutamic acid analogue and inhibits glutamine synthetase in plants. The inhibition of glutamine synthetase leads to apoptosis resulting from accumulated ammonium. This is why phosphinothricin (glufosinate ammonium) is used as the active ingredient in the non-selective herbicide Basta®. Phosphinothricin contains the enantiomers D- and L-phosphinothricin in a 1:1 ratio. D-phosphinothricin does not act as a glutamine synthetase inhibitor.

Unlike in non-genetically modified plants treated with phosphinothricin, the use of phosphinothricin in genetically modified plants causes L-phosphinothricin to be acetylated by the phosphinothricin acetyltransferase (PAT), thereby creating N-acetyl-L-phosphinothricin, which has no herbicidal effect. This makes the genetically modified plants tolerant to the herbicide phosphinothricin. The substrate specificity of phosphinothricin acetyltransferase is high. Even the phosphinothricin analogue glutamate is hardly acetylated. D-phosphinothricin is not metabolised by phosphinothricin acetyltransferase.

The genetically modified maize plants will be treated with phosphinothricin. Due to its good water solubility, N-acetyl-L-phosphinothricin thereby formed in the plants is distributed in the plants during further plant growth, while its concentration is reduced with increasing biomass. There are no indications of N-acetyl-phosphinothricin being further metabolised in the genetically modified plants.

Any N-acetyl-phosphinothricin still present in those parts of the genetically modified plants that remain on the field enters the soil during decomposition, where it is converted back into L-phosphinothricin by microorganisms. D/L-phosphinothricin is degraded in the soil, also by microorganisms.

According to the available data, N-acetyl-L-phosphinothricin has a significantly lower toxicity than phosphinothricin. Phosphinothricin is approved by the Federal Biological Research Centre (Biologische Bundesanstalt) under the German Plant Protection Act (Pflanzenschutzgesetz). As part of the authorisation process, the herbicide was assessed for toxicity and ecotoxicity.

No adverse effects are expected to result from the consumption of parts of the genetically modified plants containing phosphinothricin acetyltransferase by animals or humans. In the event of oral intake, it can be assumed that this enzyme would be fully degraded in the digestive tract, as is generally the case with proteins.

After the end of the trial, the genetically modified plants will be disposed of and are not intended to be used for human consumption or animal feed. Maize grain harvested for testing or sowing will be transferred to a corresponding facility and is also not intended to be used for human consumption or animal feed. Even unintentional consumption by animals or humans is not expected to have any adverse health effects.

(b) The coding sequence of the α-fragment of the β-galactosidase

In the vector pUC/Ac (parent vector: pUC18) used for the transformation of the maize plants,

the synthetic *pat* gene was inserted into the coding sequence of the α-fragment of the β-galactosidase. The native enzyme β-galactosidase splits β-D-galactosides into galactose and the related alcohol compound. The physiologically most important substrate is lactose, which is hydrolysed into galactose and glucose. The first 146 amino-terminal amino acids of the β-galactosidase are referred to as the α fragment. The α fragment by itself is not enzymatically active; however, complementation in suitable hosts is possible. The sequence coding for the α fragment of the β-galactosidase was interrupted by the insertion of the *pat* gene, preventing it from coding for an α fragment capable of complementation in *E. coli* bacteria that contain the vector pUC/Ac. While cloning the *pat* gene in *E. coli*, this allowed the selection of those bacteria in which the gene had been integrated into the vector pUC18.

The interrupted sequence of the α fragment of the β-galactosidase is under the control of a bacterial promoter. This sequence does not code for a functional gene product. The presence of this sequence is not expected to cause any changes in the genetically modified maize plants.

(c) The β-lactamase gene

The β-lactamase gene was inserted into the maize plants as a component of the vector pUC18. PCR analyses with specific primer combinations performed by the company AgrEvo demonstrate that the 3´ terminal end of the β-lactamase gene was integrated into the maize genome. PCR analyses with primers complementary to the 5´ terminal region of the β-lactamase gene did not yield any amplified DNA fragments, either in 5´-3´ or in 3´-5´ direction. This result indicates that this region of the plasmid pUC18 was split for integration. Therefore, the incomplete β-lactamase gene contained in the genetically modified maize plants is not expected to code for a functional enzyme.

The company Hoechst performed tests with the progeny of the T_{25} transformation event to investigate the β-lactamase activity. Such activity was not observed in proteins extracted from leaves of the genetically modified plants.

(d) Regulatory sequences

Integrated into the genome, the genetically modified maize plants contain the 35S promoter and terminator of the cauliflower mosaic virus (CaMV), the promoter of the β-lactamase gene and the promoter of the lactose operon from the plasmid pUC18.

In the genetically modified plants, the 35S promoter and terminator sequences regulate the expression of the coding sequence of the phosphinothricin acetyltransferase located between them. Further information on the effects associated with the formation of this enzyme in the plants can be found in III.1.2.1 (a).

(e) The origin of replication of the plasmid pBR322

The results of the PCR analyses presented show that the region of the plasmid pUC18 in which the origin of replication is located was integrated into the maize genome. For the purpose of evaluation, the origin of replication was therefore assumed to have been integrated into the plant genome.

The origin of replication of pBR322 allowed propagation of the vector pUC/Ac in *E. coli* bacteria from which the vector was isolated after propagation and then used for maize transformation. The origin of replication is derived from the plasmid pMB1, which belongs to the group of ColE1-like plasmids. The ColE1 replicon has a narrow host range limited to E. coli and some related bacterial species. The origin of replication of pBR322 is non-functional in the cells of the maize plants.

(f) 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 expression level may be influenced by environmental factors, for instance, by temperature. In this particular case, this could mean that the genetically modified plants do not tolerate phosphinothricin to the same degree in the field as under climate-controlled or greenhouse conditions. The application of phosphinothricin could result in damage to the genetically modified plants. This does not represent a risk to the environment or to human and animal health. In terms of the other traits transferred, the change in the expression level also does not pose a risk to the environment or to human and animal health.

The insertion of foreign genes may influence the expression or regulation of endogenous plant genes at or near the site of insertion. Such processes can affect plant metabolic pathways. During propagation of genetically modified plants in the greenhouse and in other deliberate release trials with the genetically modified maize 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 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 regard, the genetically modified plants to be deliberately released 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 potential allergenicity of a protein on the basis of its amino acid sequence. In previous experiments with the genetically modified maize plants under climate controlled and/or greenhouse conditions as well as in deliberate release trials in Germany and abroad with plants that express the phosphinothricin acetyltransferase, no evidence was found to suggest an increased allergenic potential of the pollen of these plants. The company Hoechst performed tests to ascertain the PAT content and PAT activity in the pollen of the genetically modified maize plants. Neither the protein nor the corresponding enzyme activity was detected.

III.1.2.2. Evaluation of the ability of the genetically modified maize 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 inserted into the maize plants and seeds merely confers tolerance to the herbicide phosphinothricin. It can be assumed that the persistence characteristics have not been altered.

In the deliberate release trial submitted for approval, the maize will be allowed to reach grain maturity. The emergence of volunteer maize has not been observed in Central Europe, even in grain maize that is harvested when fully mature. If – contrary to agricultural experience – genetically modified maize plants were to emerge on the release site after the end of the release period, they would be identified and destroyed in the course of the proposed post-trial monitoring period. These measures ensure the spatial and temporal limitation of the release project. The applicant proposed a cultivation gap and a post-trial monitoring period of initially one year. The proposed duration of the post-trial monitoring period for identifying any re-emerging maize plants prescribed in the supplementary conditions is regarded as sufficient.

For disposal, the genetically modified maize plants as well as the non-genetically modified maize plants that have reached grain maturity will be shredded and worked into the soil for decomposition. 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.

III.1.2.3. Assessment of the possibility of pollen-mediated transfer of the inserted genes 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 generally dispersed by wind. An 8-metre wide border strip of non-genetically modified maize plants proposed by the applicant is regarded as suitable to limit the dispersal of maize pollen from the release site. However, the development of individual maize grains on non-genetically modified maize plants through pollination by genetically modified plants beyond a distance of 8 m cannot be ruled out.

For the reasons explained in III.1.2.1, it is assumed that even the potential consumption of maize seeds and any resulting plants that may develop through pollination by the genetically modified plants is not expected to have any adverse health effects.

As explained in III.1.2.2, maize seeds and any resulting plants that may develop through pollination by the genetically modified plants would not be hardy and thus not capable of persisting in the environment.

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

The inserted sequences are firmly integrated into the chromosomes of the recipient organisms. No evidence exists to suggest that the transfer of genetic information from plants or its expression in microorganisms 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.

Insofar as we assume that an exchange of genetic material between organisms that are as distantly related in terms of taxonomy as seed plants and bacteria is actually possible, it can 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 any heterologous genetic material, including all forms of plant DNA.

The synthetic *pat* gene can also be expressed in bacteria by the CaMV promoter and confers resistance to the herbicide phosphinothricin. Studies on the breakdown of phosphinothricin in soil have demonstrated that enzymes that acetylate and thereby inactivate phosphinothricin are commonly found in soil bacteria. The application of phosphinothricin as an herbicide is not expected to change the composition of soil microflora, since phosphinothricin is rapidly inactivated in soil and is an antibiotic that has very limited effectiveness against most bacteria. Even if herbicide application were to lead to the selection of resistant bacteria, the origin and distribution of the resistance would be accounted for by the bacteria themselves and would not be traced back to the transfer of genes from the genetically modified plants to microorganisms. The potential horizontal transfer of genes would not contribute to any increase in the overall frequency of this resistance mechanism in bacteria.

In the genetically modified maize plants, the gene for the α fragment of the β-galactosidase is interrupted by the insertion of the *pat* gene, preventing the formation of a functional gene product, as already explained in III.1.2.1. This would also be the case in bacteria receiving the sequence for the α subunit by horizontal gene transfer from the genetically modified plants.

The genetically modified maize plants contain an incomplete copy of the β-lactamase gene from pUC18, which is positioned downstream of a bacterial promoter. The PCR analyses performed by the company AgrEvo submitted along with the application demonstrate that the 3´ terminal end of the β-lactamase gene was integrated into the maize genome. PCR analyses with primers complementary to the 5´ terminal region of the β-lactamase gene did not yield amplified DNA fragments, either in 5´-3´ or in 3´-5´ direction. This result indicates that this region of the plasmid pUC18 was split for integration. According to this, the 5´ terminal region and the 3´ terminal region would be spatially separated and non-functional in the maize genome. Given these circumstances, even horizontal gene transfer is not expected to result in the expression of a functional gene product.

TEM-1-β-lactamase genes, which confer resistance to a number of β-lactam antibiotics, are found in a number of Enterobacteriaceae and some other gram-negative bacterial species. The exchange of antibiotic resistance genes between bacteria is possible by effective transfer mechanisms. Even in the event of horizontal gene transfer from the genetically modified plants to microorganisms, the overall frequency of this resistance mechanism in the environment would not be noticeably increased.

The origin of replication of pUC18 (pMB1 replicon) is assumed to be integrated into the chromosomes of the genetically modified maize plants. This nucleic acid sequence is non-functional in the genetically modified plants, but is expected to have a function as origin of

replication in certain bacteria. The pMB1 replicon belongs to the ColE1-type plasmids, whose host range is limited to a number of gram-negative bacteria. Basically, this replicon is capable of replicating in *E. coli* and closely related species of bacteria such as *Serratia* or *Salmonella*. In most gram-negative soil bacteria, replication does not take place. ColE1-type plasmids occur frequently in enterobacteria. Gene transfer from enterobacteria to other bacteria is considered far more likely than a horizontal gene transfer from the genetically modified plants to bacteria. Therefore, the potential presence of the origin of replication of pUC18 in the plant chromosome is not expected to contribute to an increase in the overall frequency of horizontal gene transfer.