Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0198

Summary of the risk assessment of genetically modified maize

(*Zea mays*) GA21

carried out by the German Competent Authority

within the framework of a proposed deliberate release

Berlin, 15 May 2009

Explanatory note to this document:

The following text is a summary of the risk assessment of genetically modified organisms intended for use in an experimental field trial (deliberate release) in Germany. The text forms part of the official authorisation issued in response to an application for the deliberate release of genetically modified organisms in Germany in accordance with Directive 2001/18/EC and the German Genetic Engineering Act (Gentechnikgesetz, GenTG). The authorisation was issued by the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL [Federal Office of Consumer Protection and Food Safety], as the German Competent Authority under the law on genetic engineering, and comprises the chapters:

Only the original German authorisation document is legally binding. The following extract is a courtesy translation of chapter III.1.2., prepared for the Biosafety Clearing House.

Dienstsitz Braunschweig
Bundesallee 50, Geb. 247
38116 Braunschweig
Tel: +49 (0)531 21497-0
Fax: +49 (0)531 21497-299

Abt. Pflanzenschutzmittel
Messeweg 11/12
38104 Braunschweig
Tel: +49 (0)531 299-5
Fax: +49 (0)531 299-3002

Dienstsitz Berlin
Mauerstraße 39-42
10117 Berlin
Tel: +49 (0)30 18444-000
Fax: +49 (0)30 18444-89999

Referatsgr. Untersuchungen
Diedersdorfer Weg 1
12277 Berlin
Tel: +49 (0)30 18412-0
Fax: +49 (0)30 18412-2955

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

(a) The *mepsps* gene

The modified *epsps* gene from maize codes for a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Both the endogenous EPSPS protein and the mEPSPS protein expressed in the maize plants as a result of the genetic modification 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 the secondary plant metabolism. The mEPSPS protein, which is expressed additionally in the genetically modified (GM) maize, catalyses the same reaction as corresponding enzymes that occur naturally in maize and other crop plants. In contrast to the endogenous EPSPS protein, the modified EPSPS protein is not inhibited by glyphosate, so that the GM maize can tolerate applications of glyphosate herbicides.

In the GM maize, expression of the gene for a glyphosate-tolerant EPSPS from maize takes place constitutively under the control of the rice actin promoter (McElroy *et al.*, 1990) and the *nos* terminator sequence from *Agrobacterium tumefaciens*. The first non-coding exon and intron of the actin gene from rice is included in the transcription unit with the aim of increasing the level of gene expression, which can probably be attributed to RNA processing events (Luehrsen & Walbot, 1991). The upstream position of the optimised transit peptide causes the post-translational import of the mEPSPS protein into the chloroplasts. The transit peptide is generally cleaved on import (processing).

The CP4 EPSPS protein additionally expressed in the GM maize catalyses the same reaction as corresponding enzymes that occur naturally in maize and other crop plants. Since no adverse health effects have been attributed to the chloroplast transpeptide sequences of the ribulose-1,5-biophosphate carboxylase from maize and sunflower, or to any other currently known signal peptides, whether processed or unprocessed, it can be assumed that the same applies to polypeptides from optimised transpeptides and enzymes (in this case mEPSPS). There is no reason to expect that the newly formed EPSPS protein might have a harmful effect.

The mode of action of the EPSPS proteins expressed in GA21 maize as a result of the genetic modification is not expected to pose any risk to human or animal health or to the environment. The use of GA21 maize in food and feed has been approved in the EU since 28 March 2008.

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

(b) DNA fragments located outside the target sequences

Transformation of GA21 maize was achieved by particle bombardment with a purified NotI fragment from the plasmid pDPG434. The transfer of additional plasmid fragments is therefore unlikely. The absence of elements of the plasmid backbone was confirmed by Southern blot analyses.

As a result, no further evaluation of these elements is necessary.

(c) 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 GM 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 studies carried out so far with the 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-GM plants there is always the possibility that these events might influence plant metabolic pathways. With respect to these traits, the GM plants proposed for release here do not differ fundamentally from non-GM 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 previous field trials with plants that express the *epsps* gene under the control of non-tissue-specific promoters, no evidence of increased plant allergenicity was recorded.

The GM maize referred to in the application for deliberate release is not intended for use in the production of food or feed within the scope of the field trial. In general, maize pollen does not play a significant role in triggering pollen allergies.

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 hardy. Maize does not have the ability to persist under Central European climate conditions. The genetic material introduced into these maize plants/seeds imparts tolerance to the herbicidal agent glyphosate. It can be assumed that the persistence traits of these plants have not been altered.

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 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 required by provision II.10 [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 trials all surplus harvest material from the release site as well as all emerging border row plants and any maize plants growing within the buffer zone required by provision II.8, which should ensure a distance of at least 200 m to all other maize stocks, will be destroyed. This can be done by shredding the harvest material, crushing the grains, and working the resulting material into the soil. Alternatively, the shredded material can be transferred to a biogas plant for further inactivation. Even if some of the maize grain were to escape being broken down in the shredding process, it can still be assumed that under field conditions no persistent plants would develop from this grain. If the shredded material goes through the fermentation process in a biogas plant the same result would be expected.

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

Due to the lack of a crossing partner in the flora of Central Europe, the transfer of the genes inserted into the GM maize plants to other plant species can be ruled out. As a result, the following passage focuses solely on the potential transfer of pollen from the GM maize plants to other maize plants.

Maize pollen is normally dispersed by wind, whereby the maximum pollen drift distance is in no way identical to the maximum outcrossing distance. This is due to pollen sensitivity to weather conditions such as heat, humidity and UV radiation, all of which quickly cause sex cells in the pollen grain to die off. This explains why the recorded pollen drift distance is normally much greater than the recorded outcrossing distance. Another important factor for outcrossing is the size of the pollen donor and recipient population areas. For example, the smaller the pollen donor area, the shorter the outcrossing distances. The flowering period of the recipient population also plays a role. The longer the female flowers are receptive, the higher the rate of outcrossing, given simultaneous flowering. For this reason, outcrossing data from the earlier literature is often redundant, since modern high-yield varieties have a narrower cross-pollination time frame than older maize varieties. At distances of 200 m only a negligibly low level of outcrossing is anticipated. 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 from other varieties.

Provision II.8 [of the decision on this application] requires the observance of a minimum isolation distance of 200 m around the perimeter of the release site to all other maize stocks. In addition, the applicant plans to sow 4 border rows of a non-GM maize variety of comparable maturity around the release plot. This measure, coupled with the isolation distance of 200 m required by provision II.8, will ensure that the risk of pollen transfer to other maize populations is adequately addressed.

Given the grounds mentioned in III.1.2.1., it follows that maize seeds or plants emerging from these seeds, which may be generated as a result of fertilisation with pollen from the GM plants, are not expected to pose a health hazard, even if consumed. In the EU, GA21 maize is authorised as a genetically modified foodstuff and as animal feed.

As explained in III.1.2.2., maize seeds and/or plants emerging from these seeds, which may be generated as a result of fertilisation with pollen from the GM plants, are not winter-hardy and are therefore not capable of establishing in the environment.

III.1.2.4. Assessment of the possibility of transfer of the inserted foreign genes from the GM plants to soil 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 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.

Insofar as 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 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 can always result in the uptake of all forms of heterologous genetic material, including plant DNA.

(a) The *mepsps* gene

In soil bacteria the inactivation of glyphosate and the expression of glyphosate-tolerant EPSP synthases is a naturally occurring process. Bacteria with a corresponding resistance are widespread in the environment. Even in the event of a transfer of this gene from the GM plants to microorganisms, no discernible increase in the overall frequency of the related phenotype of a glyphosate-tolerant form of EPSPS in the environment would result.

In the construction of the inserted expression cassettes copies of the *epsps* gene were fused with the DNA sequence for a chloroplast transit peptide from maize and sunflower. DNA sequences that code for chloroplast transit peptides have been described for a range of different plant species and are therefore widespread in the environment. Moreover, transit peptide sequences of this type would be non-functional in bacteria.

(b) Regulation sequences

Even in the event of a transfer of the regulation sequences used in the constructs, there would be no reason to expect a significant increase in the overall frequency of the corresponding DNA sequences. These regulation sequences originate from *Agrobacterium tumefacien, Oryza sativa,* and are commonly found in plants and soil organisms.

As a result, such a transfer is unlikely to have ecological consequences.

(c) Additional DNA fragments located outside the target sequences

Transformation of GA21 maize was achieved by particle bombardment with a purified NotI fragment from the plasmid pDPG434. The transfer of additional plasmid fragments is therefore unlikely. The absence of elements of the plasmid backbone was confirmed by Southern blot analyses.

As a result, no further evaluation of these elements is necessary.