Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0190

Summary of the risk assessment of the genetically modified maize (*Zea mays*) 98140 within the framework of a proposed deliberate release carried out by the German Competent Authority

Berlin, 21 April 2008

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.

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

a) The *gat4621* gene

The transferred expression cassette contains the *gat4621* gene, which was derived from the soil bacterium *Bacillus licheniformis* and synthesised using a gene shuffling method. The gene codes for a glyphosate-N-acetyltransferase protein which confers tolerance to glyphosate-containing herbicides.

The expression of the modified glyphosate-N-acetyltransferase protein from *Bacillus licheniformis* takes place under the control of a constitutive promoter resulting in tolerance to glyphosate.

The enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) catalyses the reaction of shikimate-3-phosphate with phosphoenolpyruvate to form 5-enolpyruvylshikimat-3-phosphate, an intermediary stage in the biosynthesis of aromatic amino acids and other aromatic substances of secondary plant metabolism. This metabolic pathway is inhibited by the glyphosate agent, causing the plant to die off.

Glyphosate-N-acetyltransferase expression in the genetically modified 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 genetically modified 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.

No similarities between the protein GAT4621 and other acetyltransferases from bacteria or fungi could be identified.

A single-dose mouse feeding study was carried out with the expressed protein. A once-off dose of 1640mg GAT protein per kg body weight was administered to the 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 using transformation methods.

b) 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.

The 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. 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 genetically modified 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 using the expressed protein. A once-off 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 using transformation methods.

(c) Position effects and context changes; allergenicity

Genes which have been 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 integration site environment (“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 maize plants are not modified to the same degree in the open field as under climate-controlled 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 the plant's own genes at or near the site of insertion. Such processes may alter plant metabolic pathways. However, during the course of the work carried out to date on these genetically modified 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. 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 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 possible allergenic action of a protein on the basis of its amino acid sequence.

The genetically modified maize is not intended for human or animal consumption within the framework of the proposed field trials.

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

Maize plants and maize seeds are not hardy. Maize can not persist under Central European climate conditions. The genetic material inserted into the maize plants or seeds confers resistance to the herbicide agent glyphosate and to acetolactate synthase-inhibiting herbicides such as, for example, sulfonylurea. 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 the case of grain maize, which is harvested when fully mature. Should genetically modified maize plants accumulate in the experimental area after the end of the release period, they would subsequently be recorded and destroyed in the course of the required cultivation interval and post-trial monitoring, as set down in provisions II.9 [of the decision on this application]. These measures help to ensure the spatial and temporal limitation of the proposed deliberate release.

On conclusion of the proposed series of experiments both the genetically modified and the non-genetically modified maize plants will be broken down by shredding and worked into the soil, where they will be left to decompose. Even if some of the maize grain is not fully destroyed by shredding, it can be assumed that under open-field conditions no persistent plants could develop from the plant residue.

The persistence or establishment of maize grain from silage or from the biogas fermentation process can be ruled out.

The non-genetically modified maize plants of the border rows will be disposed of in the same manner as the genetically modified trial plants.

III.1.2.3. Assessment of the possibility of the pollen transfer of genes inserted into the genetically modified plants to other plants

Since maize has no crossing partner in the Central European flora, a transfer of the inserted genes from the genetically modified maize plants to other plant species is not possible. The following passage, therefore, only addresses the possibility of pollen transfer from the genetically modified maize plants to other maize plants.

Maize pollen is normally spread by wind. In the production of hybrid maize seeds, seed legislation prescribes – in the absence of other isolation measures - a minimum isolation distance of 200 m to other maize fields to adequately minimize cross-breeding with pollen of other varieties. In addition to maintaining an isolation distance of 200 m to commercial maize crops, the applicant plans to sow four border rows of non-genetically modified maize around the release site. The possibility of pollen transfer to other maize populations will be adequately addressed by these measures, thereby providing for the protection of resources in accordance with § 1 (No. 1) of the German Genetic Engineering Act (GenTG).

III.1.2.4. Assessment of the possibility of transfer of the inserted foreign genes from the genetically modified plants to soil micro-organisms by horizontal gene transfer

The gene expression cassettes GAT4621 and ZM-HRA

The inserted sequences are stably integrated into the genome of the recipient organisms. No evidence exists to suggest that the transfer of genetic information from plants or its expression in micro-organisms 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, 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 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 gene expression cassettes in the genetically modified plants contain regulatory elements which are optimised for plant metabolism. Therefore, in the event of a transfer, these expression cassettes would not have the capacity to function in bacteria.

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 occur naturally 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.

Therefore, even in the unlikely event of a transfer of the *gat4621* or *zm-hra* genes to microorganisms, the resulting genetic constellation would not be assessed any differently from the uptake of corresponding natural genes or gene fragments. The uptake of the genes as a result of horizontal transfer has not been found to confer a selective advantage.