Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0192

Summary of the risk assessment of the

genetically modified sugar beet (*Beta vulgaris*) H7-1

carried out by the German competent authority

Berlin 31 March 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
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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.

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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 gene for glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)

In the genetically modified sugar beet plants the expression of the gene for glyphosate-tolerant EPSPS derived from *Agrobacterium sp*. strain CP4 takes place under the control of the 35S promoter of the figwort mosaic virus and the E9-3' terminator sequence from *Pisum sativum*. The nucleic acid sequence of the *epsps* gene was optimised for expression in plants.

Both the endogenous EPSPS and the EPSPS introduced into the sugar beet 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. In contrast to the endogenous EPSPS, the EPSPS inserted into the genetically modified sugar beet plants is not inhibited by glyphosate. The upstream position of the transit peptide CTP2 of the EPSPS derived from *Arabidopsis thaliana* causes the post-translational import of the chimeric protein into the chloroplasts.

No risks to human or animal health or to the environment are expected to result from the mode of action of the EPSPS inserted by means of transformation in the proposed deliberate release. In the genetically modified sugar beet plants the newly formed EPSPS catalyses the same reaction as that catalysed by corresponding, naturally occurring plant enzymes.

In accordance with the German Plant Protection Act the herbicide Roundup®-Ready is approved for use in a range of agronomic applications, including post-emergence weed control in glyphosate-tolerant sugar beets. As part of the licensing process the herbicide and its metabolites were assessed for toxicity and ecological impact.

Here too, no adverse effects are expected to result from the consumption of parts of the genetically modified sugar beet plants containing the glyphosate-tolerant EPSPS protein. 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. This is documented in numerous studies on the digestibility of CP4 EPSPS as well as rodent feeding studies, which are available from previous applications for placing on the market. Feeding studies in which in glyphosate-tolerant sugar beets were fed to pigs and cattle are also available. Since no health-damaging potential has 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 the transit peptide-enzyme compound.

(b) Functional regulation sequences in plants

Integrated into the genome, the genetically modified plants contain regulation sequences that are functional in plants; these are the 35S promoter from the figwort mosaic virus and the 3´ termination signal derived from gene 9 of *Pisum sativum*. As promoter and terminator, they regulate the expression of the coding sequences mentioned above, which are located between the promoter and the terminator. Additional functions have not been identified; additional effects in the genetically modified plants are not anticipated.

(c) DNA fragments from outside the T-DNA of the transformation vectors

The vectors used to generate the genetically modified sugar beet plants by *Agrobacterium*-mediated transformation contain the following outside the T-DNA border regions: The bacterial gene *aad* for streptomycin/spectinomycin resistance (enzyme: aminoglycoside-3-adenyltransferase), the sequences “ori-322” for replication in E. coli, as well as an additional ori-V for the replication of the binary vector (in this particular case in *Agrobacterium tumefaciens*). Based on the information contained in the application it can be assumed that these DNA fragments were not transferred into the genome of the H7-1 lines. Even in the event of transfer, no effects on the plant metabolism are to be expected.

(f) 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 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 genetically modified plants do not tolerate glyphosate to the same degree in the field as under climate-controlled or greenhouse conditions. It is possible that the application of Roundup®-Ready could result in damage to the genetically modified plants. 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 the plant’s own genes at or near the site of insertion. Such processes can affect plant metabolic pathways. However, during the cultivation these genetically modified plants within a number of previous deliberate release trials, 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. In the proposed field trials the genetically modified sugar beet plants do not come into flower and, as a result, do not produce pollen. In previous experiments with these genetically modified plants, and also in earlier deliberate release trials with other genetically modified plants that express the corresponding gene under the control of non-tissue-specific promoters, no evidence was found to suggest an increased allergenic potential of the plants.

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

As a result of the proposed measures, the genetically modified sugar beets are not expected to spread to areas outside the release site, nor are they expected to persist or establish in the environment.

Towards the end of the vegetation period the released sugar beet plants will be harvested while still in a vegetative state, either by hand or mechanically. A portion of the beet harvest will be transferred to laboratories for analysis (content evaluation, determination of yield). If the yield intended for analysis is found to contain plant material still capable of propagation, it is deemed adequate in terms of safety if it is inactivated during the course of the analysis. Inactivation is, in any case, an inherent part of the analysis process.

Surplus harvest material (beets) and other excess vegetative plant material from the genetically modified beets are to be destroyed, for example, by shredding. The resulting material is to be worked into the soil. Alternatively, plant material will be transferred to steaming or composting facilities for disposal. In view of these precautions, the regeneration of genetically modified plants from material remaining on the release site is not expected.

The genetically modified beet seeds are to be sown using drilling machines. After sowing, the drilling machines are to be cleaned on the release site to ensure removal of any residual genetically modified seed. Following emergence of the seedlings, surplus plants are to be removed by hoeing or by weeding. Since the plants will not reach the flowering stage, no new seeds will be produced during the course of the experiments. Under certain circumstances, particularly when incorporated into deeper soil layers, sugar beet seeds can remain viable for several years. However, based on general farming experience, planted seed which does not germinate is considered inactive and will therefore be incapable of germinating in subse-

quent years. Nevertheless, should a few viable seeds persist in the soil – which could lead to the appearance of genetically modified sugar beet plants following completion of the experimental release - these plants would be detected in the course of the proposed post-trial monitoring described in the application and stipulated in provisions II.10 [of the decision on this application]. Even if individual genetically modified sugar beet seeds were to be dispersed, the uncontrolled spread of the genetically modified plants is not anticipated. These measures help to ensure the spatial and temporal limitation of the release project. These plants only have a competitive advantage over other plants in areas where glyphosate is used as a herbicide. The plants could be destroyed by mechanical methods (e.g. hoeing) or by using non-glyphosate herbicides.

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

Sugar beet is a biennial plant which normally only flowers in the second year, following a cold spell. The applicant plans to harvest the sugar beet plants at the end of the first year of growth while they are still in a vegetative state. Potential beet bolters on the release site are easily recognized during field trials and they must be destroyed before flowering, as stipulated in provision II.9. Therefore, a discharge of genetically modified sugar beet pollen is not anticipated within the framework of the proposed deliberate release.

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

The inserted sequences are chromosomally integrated into the recipient organisms. From the results of studies on the transformation ability of soil bacteria under natural conditions it can be concluded 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 that are as distantly related in terms of taxonomy as seed plants and bacteria actually takes place, 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 all forms of heterologous genetic material, including all forms of plant DNA.

The genetically modified plants contain the *epsps* gene derived from the *Agrobacterium sp.* strain CP4, whereby the coding region of this gene is fused with the plant leader peptide sequence at its N-terminus. Such leader peptide squences would be non-functional in bacteria.

epsps genes are ubiquitously present in soil microorganisms. Studies on the breakdown of glyphosate in soil have demonstrated the metabolic activities of microbes which cause the decomposition and inactivation of glyphosate are widespread. Even if herbicide application were to lead to the selection of a group of glyphosate-degrading bacteria, the origin and distribution of the metabolic activity 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 noteworthy increase in the overall frequency of glyphosate-degrading metabolic activities in bacteria.

Located outside the T-DNA borders, the binary vector used to produce the genetically modified sugar beet plants contains the *aad* gene, which confers tolerance to streptomycin and spectinomycin, as well as the bacterial replication origins 322 and oriV. Based on the results of the studies submitted, the presence of these sequences in the genetically modified plants can be ruled out.

III.1.2.5. Agrobacteria used to generate the genetically modified plants

Agrobacterium-mediated binary transformation systems were used to generate the genetically modified plants. It was demonstrated that the lines intended for release do not contain any backbone sequences from the vector used for transformation. It can therefore be assumed that the plants are free of the Agrobacteria used in the transformation.

In contrast to the common wild-type *A. tumefaciens*, the *Agrobacterium* strains used are “disarmed”, i.e. they no longer have the capacity to induce tumours. In the unlikely, but theoretically conceivable, event that the inserted foreign genes are transferred to a cell of another plant via these Agrobacteria, the plant would have to spontaneously regenerate into a whole, fertile plant for the foreign genes to enter the germ cells. This is the only way that these genes could be passed on to the plant offspring. Such an event is not expected to occur under natural conditions.

Assuming that the presence of small amounts of recombinant Agrobacteria in the genetically modified plants cannot be ruled out, the potential transfer by conjugation of the binary plasmids contained in the Agrobacteria to wild-type Agrobacteria (*A. tumefaciens* or *A. rhizogenes*) present in the environment would also have to be considered, since these could, in turn, pass on the foreign genes to individual cells of other plants. In the case of infection and subsequent transformation via wild-type *A. tumefaciens* or *A. rhizogenes* a crown gall or hairy root tumour would develop from the transformed plant cell. Under natural conditions such a tumour would not be expected to give rise to a plant.

Following transformation antibiotic treatment was performed to eliminate the Agrobacteria. Furthermore, the plants intended for release were propagated by seed and have gone

through several generation cycles since production. As a result of this generative propagation any Agrobacteria that survived the antibiotic treatment were removed from the genetically modified sugar beet lines. No transfer of Agrobacteria from seed has been observed to date.