Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

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Summary of the risk assessment of the genetically modified sugar beet

(*Beta vulgaris* L. ssp. *vulgaris*) TAD13, TAD 18, TAD28, TAD33 and TAD44

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 07 May 1999

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

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 sugar beets effected by the transferred nucleic acid sequences

(a) The synthetic *pat* gene

In the genetically modified sugar beets, 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) is used as the active ingredient in the non-selective herbicide Basta® (Liberty®). Basta® 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 Basta®, the use of Basta® 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 Basta®. The substrate specificity of phosphinothricin acetyltransferase is high. Even the phosphinothricin analogue glutamate is hardly acetylated. D-phosphinothricin is not metabolised by phosphinothricin acetyltransferase.

Due to its good water solubility, N-acetyl-L-phosphinothricin formed in the genetically modified plants after treatment with Basta® 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 (= active ingredient in the herbicide Basta®). Basta® 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 and its metabolites were assessed for toxicity and ecotoxicity. Based on the toxicological and ecotoxicological data on phosphinothricin and N-acetyl-L-phosphinothricin, the residues or metabolites of the herbicide Basta® contained in the genetically modified sugar beet plants are not expected to pose a risk to human and animal health or the environment.

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.

(b) The *dmamp1* gene

The *dmamp1* gene from *D. merckii* codes for a cysteine-rich polypeptide of 50 amino acids with a calculated weight of approx. 5 kD. Based on it structural and functional properties, it is counted among the group of plant defensins. Defensins are found in a number of plant species, including sugar beets. Plant defensins have in common that they inhibit fungal growth.

In *D. merckii*, DmAMP1 is formed in the seeds; it protects the developing seedling against fungal infestation.

In the genetically modified sugar beets, DmAMP1 is expressed under the control of the 35S promoter in conjunction with the double enhancer region of this promoter. As is the case with defensins naturally found in sugar beets, the DmAMP1 defensin in the genetically modified sugar beets is also formed in the leaves. Studies under greenhouse conditions have demonstrated that the DmAMP1 formed in the leaves of the genetically modified sugar beets is sufficient to reduce fungal infestation.

DmAMP1 is active against a great number of fungal species as well as some bacterial species. Previous studies with plant defensins did not reveal any harmful effects on human and plant cell cultures. α-amylases in the digestive tract of insects are not inhibited either. The evaluation of changes in the genetically modified plants effected by the transferred nucleic acid sequences has shown that the consumption of plant parts of the genetically modified sugar beets is not expected to have any adverse health effects in animals or humans. Based on the available information, harmful effects on the objects of legal protection pursuant to Section 1 No. 1 GenTG are not expected in the course of the deliberate release trial.

(b) The *his3* gene; the *ded1* gene fragment

In the genetically modified plants, the *his3* gene derived from *Saccharomyces cerevisiae* is under the control of the *his3* promoter and the *his3* termination region from *S. cerevisiae*.

The *his3* gene codes for the enzyme imidazoleglycerol-phosphate dehydratase (IGPD), which catalyses the conversion of imidazoleglycerol-phosphate into imidazoleacetol-phosphate during histidine biosynthesis. The *his3* gene was inserted into the transformation vector to allow the selection of transformed bacteria; it is non-functional in the genetically modified plants. IGPD enzymes are ubiquitously present and occur naturally in plants. In addition, they exhibit strong homologies in various organisms (bacteria, fungi, plants). The *his3* gene thus does not confer any new traits to the plants. The *his3* gene has no plant-specific promoter sequences and, based on the information provided by the applicant, no transcripts are found in plants with the intact *his3* gene. The *his3* gene is thus not expected to be expressed in the genetically modified plants. Even in the event of expression, a change in plant metabolism is not expected to occur.

The 6-bp fragment of the *ded1* gene from *S. cerevisiae* containing the coding region is also not expected to have an influence on the metabolism of the genetically modified plants.

(c) *lacZ* and *lacI* sequences from *E. coli*; the origin of replication of the plasmid pMB1 (ColE1 *ori*) from *E. coli*

The presence of the *lacI* and *lacZ* sequences as well as the origin of replication of the plasmid pMB1 is not expected to have an influence on the metabolism of the genetically modified sugar beets.

(d) Regulatory sequences functional in plants

Integrated into the genome, the genetically modified sugar beets contain regulatory sequences that are functional in plants from the cauliflower mosaic virus (CaMV), the tobacco mosaic virus (TMV), from *Agrobacterium tumefaciens*, *Escherichia coli* and *Saccharomyces cerevisiae*. These sequences regulate the expression of the above-mentioned genes. Additional functions have not been identified; additional effects in the genetically modified plants are not anticipated.

(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 glyphosate to the same degree in the field as under climate-controlled or greenhouse conditions. The application of Basta® (Liberty®) could result in damage to the genetically modified plants. Fungal infestation as a result of reduced resistance to fungi may entail loss in the yield of the genetically modified plants. This does not represent 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. Based on the information provided by the applicant, during greenhouse experiments with the 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 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 the proposed field trial, the genetically modified sugar beet plants do not reach the flowering stage and, as a result, do not produce pollen. In previous experiments with these genetically modified plants as well as in earlier deliberate release trials with other genetically modified plants that express the *pat* gene, no evidence was found to suggest an increased allergenic potential of the plants. The applicant refers to studies comparing the amino acid sequence of the DmAMP1 peptide with those of other known allergens. Based on the information provided by the applicant, these studies revealed no evidence to suggest an allergenic or toxicologically relevant potential of DmAMP1.

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

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 mechanically and topped while still in a vegetative state. After taking any necessary samples (e.g. for residue or content analysis in corresponding laboratories), the remaining beets and other plant parts will be inactivated in the field using appropriate methods (e.g. by shredding and working into superficial soil layers or by using rotary tillers). In view of these precautions, the regeneration of genetically modified plants from material remaining on the release site is not expected.

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 this plant material is inactivated during the course of the analysis. Inactivation is, in any case, an inherent part of the analysis process.

The genetically modified beets will be treated and cultivated according to good experimental practice in agriculture. 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 germinable 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 subsequent years. Nevertheless, should a few germinable seeds persist in the soil, which could lead to the emergence 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 period prescribed in the supplementary conditions. Even if individual genetically modified sugar beet seeds were to be dispersed, the uncontrolled spread of the genetically modified plants is not anticipated. These plants only have a selective advantage over other plants in areas where glufosinate (phosphinothricin) is used as an herbicide. The plants could be destroyed by mechanical methods (e.g. hoeing) or by using non-glufosinate herbicides.

III.1.2.3. Assessment of the possibility of pollen-mediated transfer of the inserted genes from the genetically modified sugar beet 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 recognised during the field trial and are destroyed before flowering. 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 horizontal gene transfer of the inserted foreign genes from the genetically modified sugar beets to microorganisms

The inserted sequences are integrated into the chromosomes of the recipient organisms. 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 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.

In soil microorganisms, the inactivation of phosphinothricin by acetylation is a naturally occurring process. Bacteria with a corresponding resistance are commonly found in the environment. This resistance can therefore also be spread by horizontal gene transfer from non-genetically modified microorganisms. Even if the *pat* gene were to be transferred from the genetically modified plants to microorganisms, the overall frequency of this resistance in the environment would not be significantly increased.

Plant defensin genes are found in a great number of plant species and are widespread. This trait could thus also be transferred by horizontal gene transfer from non-genetically modified organisms.

The *his3* gene from *S. cerevisiae* codes for an enzyme (imidazoleglycerol-phosphate dehydratase, IGPD) that is required for histidine biosynthesis and is ubiquitously present in microorganisms. In addition, the various IGPD enzymes are very homologous. The transfer of the *his3* gene from the genetically modified plants to microorganisms would not confer any new traits to these microorganisms.

The coding regions of the *lacZ* gene and the *lacI* gene from *E. coli* are incomplete, preventing the formation of a functional gene product. This would also be the case in bacteria receiving these genes by horizontal gene transfer.

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 pMB1 in the plant chromosome is not expected to contribute to an increase in the overall frequency of horizontal gene transfer.

Even if regulatory sequences used in the construct were to be transferred, there is no reason to fear that the overall frequency of the respective DNA sequences will increase. These regulatory sequences are derived from CaMV, TMV, *A. tumefaciens, E. coli* and *S. cerevisiae.* CaMV and TMV are plant-infecting viruses commonly found in plants. The phytopathogenic bacterium *A. tumefaciens* as well as *E. coli* and *S. cerevisiae* are also widespread in the environment.