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 var. altissima*) GT77

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 15 February 2002

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]
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III.1. Requirements for approval according to section 16 GenTG [German Gene Technology Act]
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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. and was prepared for the Biosafety Clearing-House.

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

(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 constitutively 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 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 the equivalent, naturally occurring plant enzymes.

In accordance with the German Plant Protection Act, the herbicide Roundup® is approved by the Federal Biological Research Centre for use in a range of agronomic applications, including preharvest application in grain. As part of the licensing process, the herbicide and its metabolites were assessed for toxicity and ecotoxicological impact. Based on the toxicological and ecotoxicological data, the residues or metabolites of the herbicide glyphosate contained in the genetically modified sugar beet plants are not expected to pose a risk to human and animal health or the environment.

Likewise, 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. Since no adverse health effects have been attributed to the transit peptide of EPSPS derived from *Arabidopsis thaliana*, 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 complex.

(b) The *uidA* gene (*gus* gene)

The *gus* gene from *E. coli,* which is contained in the genetically modified sugar beets line GT77, is controlled by the 35S promoter of the CaMV (here: "enhanced") and the E9-3' terminator sequence from *Pisum sativum.*

The *gus* gene was introduced into the sugar beet genome as a reporter gene for the histochemical proof of successful transformation. The enzyme β-glucuronidase cleaves glucuronides and is found in the tissue of vertebrates and invertebrates as well as in bacteria. Plants also exhibit minor endogenous β-glucuronidase activity, which can, however, be suppressed

using appropriate methods. After adding a corresponding substrate, the enzyme activity can be verified in transgenic tissue. The expression of the *gus* gene is not expected to confer a selective advantage to plants.

The consumption of plant parts by animals is not expected to have any harmful effects, since the GUS enzyme is assumed to be degraded in the digestive tract.

(c) The gene of glyphosate oxidoreductase (*gox* gene)

In the genetically modified sugar beet line GT77, only one fragment of the GOX expression cassette, consisting of the promoter, the sequence for the transit peptide and approx. 70% of the coding region of the *gox* gene from *Ochrobactrum anthropi (= Alcaligenes sp.)*, was integrated into the plant genome. The *nos* terminator and the *npt*II gene were not transferred. The *gox* gene fragment was not expressed in the genetically modified sugar beet line GT77. The gene product, the glyphosate oxidoreductase, was not verified.

Even if GOX protein were to be formed in the genetically modified sugar beets, this is not expected to involve any harmful effects. There is no reason to expect that the transit peptide of the small sub-unit of Ribulose-1,5-bisphosphate-carboxylase/oxygenase from *Arabidopsis thaliana* or the GOX protein would have a toxic effect. When plant parts are consumed by humans or animals, the enzyme is expected to be fully degraded in the digestive tract, as is generally the case with proteins.

When treating the plants with the herbicide Roundup®, the GOX enzyme in the genetically modified plants would cause the herbicide's active ingredient glyphosate to be degraded to native plant metabolic products via amino methyl phosphonic acid (AMPA) and glyoxylate. Glyoxylate is a metabolite that naturally occurs in plants; by contrast, AMPA is a metabolic product that is formed by the degradation of glyphosate. The metabolite AMPA also forms when the herbicide is applied to plants that are not tolerant to the herbicide. This metabolite also forms during the degradation of the herbicide by soil-based microorganisms. Roundup® is an herbicide that is approved by the Federal Biological Research Centre according to the Plant Protection Act. The toxicological assessment of Roundup and its metabolites was already discussed under (a).

(d) Functional regulatory sequences in plants

Integrated into the genome, the genetically modified plants contain regulatory 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 indentified; additional effects in the genetically modified plants are not anticipated.

(e) DNA fragments of the transformation vectors located outside the T-DNA

Outside the T-DNA border regions, the vector used to generate the genetically modified sugar beet plants by *Agrobacterium*-mediated transformation contains 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 provided in the application, it can be assumed that these DNA fragments were not transferred into the genome of the GT77 line. Even in the event of transfer, no effects on the plant metabolism are to be expected.

(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 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. The application of Roundup® could result in damage to 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 native plant genes at or near the site of insertion. Such processes can affect plant metabolic pathways. However, during the cultivation of 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 potential allergenicity of a protein on the basis of its amino acid sequence. In the proposed field trials, 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, 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 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 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, e.g. by subsequent topping of the beets in the beet laboratory. 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 by shredding or by appropriate chemical measures (herbicides). The resulting material is to be subsequently worked into superficial soil layers. 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 subsequent 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 the supplementary provision II.7 [of the notification 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 plants only have a selective advantage over other plants in areas where glyphosate is used as an 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 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 field trials 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 beet plants to microorganisms

The inserted sequences are integrated into the chromosomes of 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 and 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 all forms of heterologous genetic material, including all forms of plant DNA.

The genetically modified plants contain the EPSPS gene derived from *Agrobacterium sp.* strain CP4, whereby the coding region of this gene is fused to a plant “leader peptide” sequence at its N terminus. Such “leader peptide” sequences would be non-functional in bacteria. EPSPS genes are ubiquitously present in soil microorganisms. Studies on the breakdown of glyphosate in soil have demonstrated that 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 resistance to streptomycin und spectinomycin as well as the bacterial origins of replication ori-322 and oriV. Based on the results of the studies submitted, the presence of these sequences in the genetically modified plants is not assumed. Since they frequently occur in bacteria and the exchange of nucleic acids between microorganisms is possible by effective transfer mechanisms, it can be assumed that even if the sequences were present in the genetically modified plants, horizontal gene transfer between genetically modified plants and bacteria would not significantly increase the overall frequency of these sequences in the environment.

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

In order to generate the original transformants from which the genetically modified sugar beet plants intended for release originate, sterile cotyledons were inoculated with agrobacteria containing the genes to be transferred between the border regions of the corresponding binary vector plasmids. In contrast to the common wild-types of *A. tumefaciens*, the *Agrobacterium* strain used is disarmed, i.e. it no longer has the capacity to induce tumours. Following transformation, antibiotic treatment was carried out to eliminate the agrobacteria. Furthermore, the plants intended for release were propagated by seed. As a result of this generative propagation, any agrobacteria that survived the antibiotic treatment were removed from the genetically modified sugar beet lines.