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Summary of the risk assessment of the genetically modified soybeans

(*Glycine max* L. Merrill) GTS 40-3-2

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 1 st of December 2003

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

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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 sequences

The plants intended for release are progeny of the transgenic line GTS 40-3-2, which has been deregulated in the USA since 1994. In 2002, genetically modified soybeans were cultivated on approx. 36.5 million hectares worldwide; more than 50% of the annual global cultivation is accounted for by transgenic soybeans. With a few exceptions, almost exclusively glyphosate-tolerant soybeans were cultivated.

Since 1996, this genetically modified soybean has been approved for placing on the market within the EU according to Directive 90/220 EEC. This approval also covers application as animal feed and foodstuffs. Harvest products of these soybeans are imported to the EU and processed to animal feed and foodstuffs. Based on previous experience from cultivation and the use of harvest products, no harmful effects on human health or the environment have been observed.

(a) The *epsps* gene derived from *Agrobacterium* strain CP4

The *epsps* gene codes for a 5-enylpyruvylshikimate-3-phosphate synthase (EPSPS). In plants and microorganisms, this enzyme catalyses the reaction of shikimate-3-phosphate with phosphoenolpyruvate to yield 5-enolpyruvylshikimate-3-phosphate, an intermediate stage in the biosynthesis of some aromatic compounds such as the amino acids tyrosine and tryptophan. The EPSPS naturally occurring in soybean plants is inhibited by the herbicide's active ingredient glyphosate (commercial product e.g. Roundup®). The plants die off after application of the corresponding herbicide. By contrast, the equivalent enzyme from *Agrobacterium* strain CP4 is not inhibited, so that the biosynthesis of aromatic metabolites is maintained at sufficient levels even after treatment of the plants with glyphosate-based herbicides. During the construction of the plasmid pV-GMGT04, the *epsps* gene from *Agrobacterium* strain CP4 was fused with the DNA sequence for the chloroplast transit peptide (CTP) of the EPSPS from *Petunia hybrida*. As a result, the transport of EPSPS into the chloroplasts is ensured in the plant cells. The transit peptide is usually cleaved on import. The 35S promoter with a duplicated enhancer region from cauliflower mosaic virus (CaMV) and the termination signal of the nopaline synthase gene (*nos* gene) from *Agrobacterium tumefaciens* serve as regulatory sequences.

In the genetically modified soybeans, the newly formed CP4 EPSPS catalyses the same reaction as the equivalent enzymes that occur naturally in soybeans and other cultivated crops. Since no adverse health effects have been attributed to the *Petunia hybrida*-derived EPSPS transit peptide, 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 fusion protein (in this case CTP and CP4 EPSPS).

When treating the genetically modified plants with glyphosate-based herbicides, the degradation product amino methyl phosphonic acid (AMPA) may form in the genetically modified soybean plants, which is then either non-selectively bound to natural plant constituents or degraded further to native plant metabolic products, thus becoming a part of the plant's metabolism.

In documents dealing with further deliberate release trials with genetically modified plants into which the *epsps* gene from *Agrobacterium* strain CP4 has been transferred, reference is made to toxicological studies indicating that AMPA has no adverse toxicological effect on aquatic and terrestrial animal species, it is not oncogenic, teratogenic or mutagenic and only exhibits low acute toxicity (oral LD_{50} in rats is 8,300 mg/kg). Glyphosate and AMPA are excreted by animals and humans in non-metabolised form.

Consumption of the plants and the harvest products is not intended. In the opinion of the Central Committee on Biological Safety (ZKBS), the EPSPS introduced by means of transformation within the scope of the proposed deliberate release is not expected to pose any risk to human or animal health or to the environment.

(b) Additional fragments of the transformation plasmid pV-GMGT04

The plasmid pV-GMGT04 used for transformation is derived from pUC119, which contains, in addition to the origin of replication (*ori*) and the *lac* sequences, a kanamycin resistance gene (*npt*II). Furthermore, the vector pV-GMGT04 contains the β-glucuronidase gene (*gus* gene) from *E. coli* and two separately present copies of the coding region of the gene for the 5-enylpyrovylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* strain CP4, of which a copy is fused to the chloroplast transit peptide (CTP) from *Petunia hybrida* at its N terminus.

Due to the transformation method, only a part of the plasmid was integrated into the plant genome. Of the above-mentioned elements of the plasmid pV-GMGT04, only the *epsps* gene, including the control elements for expression and the CTP from *P. hybrida,* was integrated into the genome of the soybean line GTS 40-3-2. This was proved by cross-breeding experiments as well as by restriction mapping, DNA hybridisation, PCR analyses and DNA sequence analyses. The description of the results of these studies summarised in the application documents is plausible.

The insert transferred into the transgenic soybean line GTS 40-3-2 was examined in more detail after having been approved for placing on the market. The results of the examinations can be summarised as follows:

- Of the 35S promoter of the CaMV, a part of the duplicated enhancer comprising 354 base pairs (bp) was not transferred into the soybean genome. However, the promoter exhibits sufficient activity to impart agronomically useful tolerance against the herbicide Roundup to the genetically modified soybean plants.

- In addition to the complete *epsps* gene from *Agrobacterium* strain CP4, which is contained in the insert described in the application, the soybean line 40-3-2 and its progeny contain two additional fragments of the *epsps* gene, i.e.

  - a 72-bp-long fragment, which is integrated into the soybean genome separately from the originally described insert, but still segregates together with it in subsequent progeny,

  - a 250-bp-long fragment, which is attached to the 3’ end of the terminator signal of the *nos* gene from *Agrobacterium tumefaciens* and a 16-bp-long sequence of the transformation vector.

mRNA transcripts of the two fragments were not detected in Northern blot analyses. Therefore, it can be assumed that the two fragments are not expressed in the genetically modified soybeans.

A 534-bp-long DNA fragment, for which no homologies with other DNA sequences have so far been found, is attached to the 3’ end of the 250-bp-long fragment. This fragment may be soybean DNA which was rearranged in the course of the transformation process.

A comparison of the amino acid sequences, which were derived from three reading frames of the verified nucleic acid sequences, with relevant polypeptide databases revealed no indications of significant amino acid sequence homologies with any known allergens or toxins.

DNA and RNA studies of genetically modified soybeans cultivated in 1992 for the application-relevant field trials demonstrated that the above-mentioned additional fragments were present in both the plants cultivated in 1992 and the plants on which the current “Roundup Ready” types are based. According to that, the structure of the insert derived from latest information was already present in the soybean plants whose characteristics had been described in the application for marketing approval (C/UK/94/M3/1).

(c) Regulatory sequences

Integrated into the genome, the genetically modified plants contain regulatory sequences that are functional in plants. 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.

(d) 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 glyphosate-containing herbicides 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 the two CP4 EPSPS 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 propagation of genetically modified plants in the greenhouse and during the cultivation of genetically modified soybean 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 potential allergenicity of a protein on the basis of its amino acid sequence. During the cultivation of this genetically modified soybean in other countries and the use of harvest products in animal feed and foodstuffs, 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

Soybean is an annual herbaceous cultivated plant of subtropical origin. Due to its temperature requirements and lack of tolerance to cold, its cultivation is limited to warm climates. Within the European Union, soybean is only cultivated in appreciable amounts in southern countries, particularly in Italy and France. Being a domesticated plant, soybean is dependent upon human cultivation; it is not capable of establishing in the natural flora. Soybean plants are not hardy; the seeds exhibit no secondary dormancy. In sufficiently moist environments, seeds which have fallen out during cultivation or harvesting germinate immediately after harvesting. Therefore, the emerging plants are expected to die off during the winter or be destroyed in the course of usual soil cultivation measures.

The type of genetic modification, the results of the applicant's investigations as well as several years of experience from the large-scale cultivation of this plant in other countries give no reason to assume that the genetically modified soybeans differ from non-genetically modified soybeans in terms of their ability to persist, establish in the environment or potential transfer of genetic material to other soybean plants.

The genetically modified soybean or the transferred gene is not expected to establish or spread in the European flora, nor are the genetically modified beans expected to persist in the environment.

3.2. Pollen dispersal

Soybeans are self-pollinating; cross-pollination in the field is only possible to a small extent (< 1%). Directive 69/208/EEC of the European Council on the marketing of oil and fibre plant seed prescribes no minimum distance of soybean seed crops to adjacent soybean fields. The few species that can be crossed with soybean (*Glycine gracilis, G. soja*) are not native to Europe so that hybridisation is not expected.

3.4 Spreading of soybean seed

It is almost impossible for seeds of the genetically modified plants to be spread by birds without losing germination capacity. If seed should nevertheless be dispersed by birds or other animals as a result of human carelessness, this would constitute exceptional cases. Based on the above elaborations on pollen dispersal, persistence and establishment, the genetically modified soybeans are not expected to permanently establish in the environment. Such exceptional cases pose no risk within the meaning of Section 1 of the German Genetic Engineering Act (GenTG).

III.1.2.4. Assessment of the possibility of horizontal gene transfer of the inserted foreign genes from the genetically modified plants to microorganisms

The inserted sequences are firmly integrated into the chromosomes of the recipient organisms. No evidence exists to suggest that the transfer of genetic information from plants or its expression in microorganisms 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 that are as distantly related in terms of taxonomy as plants and microorganisms 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.

(a) The *epsps* gene

The *epsps* gene is an integral part of plants and microorganisms. The *epsps* gene used in the proposed release trial is derived from an *Agrobacterium*. Agrobacteria are ubiquitously present in the environment. Nucleic acid sequences coding for chloroplast transit peptides, particularly for the *epsps* gene, have been described in a number of different plant species. Even in the event of a transfer of the *epsps* gene or the *ctp* gene fragment from the genetically modified plants to microorganisms, the overall frequency of the genes is unlikely to increase significantly in the environment.

(c) Regulatory sequences

Even if the other regulatory sequences used in the construct were to be transferred, this is not expected to increase the overall frequency of the corresponding DNA fragments. These regulatory sequences originate from CaMV and *Agrobacterium tumefaciens*. CaMV is a plant-infecting, double-stranded DNA virus commonly found in plants. *Agrobacterium tumefaciens* is a ubiquitous soil bacterium.