Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0178

Summary of the risk assessment of the genetically modified

wheat (*Triticum aestivum*)

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 23 November 2006

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

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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.

Dienstsitz Braunschweig
Bundesallee 50, Geb. 247
38116 Braunschweig
Tel: +49 (0)531 21497-0
Fax: +49 (0)531 21497-299

Abt. Pflanzenschutzmittel
Messeweg 11/12
38104 Braunschweig
Tel: +49 (0)531 299-5
Fax: +49 (0)531 299-3002

Dienstsitz Berlin
Mauerstraße 39-42
10117 Berlin
Tel: +49 (0)30 18444-000
Fax: +49 (0)30 18444-89999

Referatsgr. Untersuchungen
Diedersdorfer Weg 1
12277 Berlin
Tel: +49 (0)30 18412-0
Fax: +49 (0)30 18412-2955

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

(a) The *HvSUT1* gene

The *HvSUT1* gene encodes a transport molecule of the cell membrane which mediates the energy-dependent transport of saccharose (sucrose) across cell membranes against a concentration gradient. Sucrose transporters are thought to be among the basic components of all higher plants, since saccharose is the universal transport form of all photoassimilates. In barley tissue, messenger RNA that encodes the HvSUT protein was found at the central exchange point between the maternal (parent plant) and filial (grain) parts of the plant. Induction of the *HvSUT1* gene is associated with an increase in sucrose content in the developing grain, with the production of enzymes that play a role in starch formation, and with starch accumulation in the endosperm during corn formation. The HvSUT1 protein, therefore, appears to be an important controlling element for the import of sucrose into the endosperm. The function and mode of action of this gene has been closely examined in expression studies on baking yeast. Apart from the transport of sucrose, the gene is not known to have any additional functions.

In the genetically modified plants this gene is expressed under the control of the *hordein B1* promoter and terminator of the barley-derived *hordeinB1* gene. This promoter regulates the accumulation of hordein – a storage protein found in maturing barley grains. Expression of the inserted foreign gene in immature seeds of the genetically modified plants was demonstrated by RT-PCR mediated mRNA detection.

Southern blot analyses on progeny of the transformants showed that one copy of the gene was transferred into the genome of the HOSUT wheat line.

Sucrose transporters from wheat have already been described in developing grains. The expression of *HvSUT1* in wheat, therefore, does not give rise to fundamentally novel traits in the genetically modified plants. The protein is naturally produced in barley grain considered safe for human and animal consumption. Whether the increased influx of sucrose in the developing grain leads to metabolic effects other than the detected increase in protein content is not known. However, it can be assumed that expression of the transporter will not lead to the formation of any novel substance group. To date no allergenic or toxic properties have been reported for these transport proteins.

It is assumed that the activity of the newly formed transport protein in the genetically modified wheat is the cause of the increased protein storage in the seed. In the greenhouse, *HvSUT1*-expressing wheat plants (HOSUT line) show no alteration in habitus when compared with non-genetically modified control lines. The genetically modified wheat proposed for release here is not intended for consumption.

The scale of the proposed release is very small. Under these conditions, no adverse effects on human health or the environment are expected.

(b) The *VfAAP1* gene

The *VfAAP1* gene from *Vicia faba* codes for an amino acid permease. In most higher plants, organically bound nitrogen is transported in the form of amino acids. Amino acid permeases are responsible for the transport of amino acids from the plant vascular tissue into the plant cell symplasts. They are expressed at different levels, depending on the specific stage of development and type of tissue, and exhibit varying affinities to the different amino acids. Amino acid permeases have already been described in a wide range of plants and can probably be found in all higher plants. In the donor organism *Vicia faba* the *VfAAP1* gene is expressed primarily in the cotyledon storage parenchyma cells during the early stages of development. The strongest expression corresponds with the formation of storage protein transcripts. VfAAP1 mediates the transport of a range of amino acids (mainly cysteine, arginine, glutamine, serine, leucine, methionine, histidine, glycine and threonine) with a particular emphasis on cysteine. The VfAAP1 protein has been biochemically well characterised in heterologous expression studies on baker's yeast.

In the GM wheat, expression of the *VfAAP*1 gene in the SUTAP line takes place under the control of the SUT1 promoter from barley and its own termination signal. In the XAP line, expression is controlled by the *1Ax1* promoter from wheat and the octopine synthetase terminator from *Agrobacterium tumefaciens.* Both promoters trigger the endosperm-specific expression of the *VfAAP1* gene*.* The expression of the gene in immature seeds of the GM wheat lines proposed for release was demonstrated by quantitative RT-PCR analysis. Other parts of the plant have not yet been examined for expression.

Southern blot analyses of the progeny of the transformants showed that one copy of the gene was transferred into the genome of the wheat lines SUTAP and XAP.

In greenhouse studies, the plants of the GM wheat lines SUTAP and XAP were not found to differ from non-GM plants in habitus, seed morphology or germination capacity. In the greenhouse, the lines SUTAP and XAP come into flower 2-3 weeks earlier than plants of the HOSUT line and non-GM control lines. They accumulate an increased amount of protein in the seeds.

The target gene VfAAP1 derives from *Vicia faba* (field bean or broad bean). In Germany, *Vicia faba* is cultivated as a pulse crop on about 21,000 hectares of land (2004). Its primary uses are as protein-rich fodder (field bean) and as a vegetable (broad bean). As long as the usual methods of preparation are observed, the broad bean has no known toxic effect on humans. Neither the wheat grains nor any other parts of the GM plants cultivated in the framework of this field trial are intended for human or animal consumption.

Since amino acid permeases occur ubiquitously in higher plants, the insertion of the foreign gene into the receptor plants will not give rise to any essentially novel traits. The expression of *VfAAP1* in the plant is not expected to result in the production of a new class of substances.

In the context of the proposed field trial, the formation of the amino acid permease of the field bean in wheat seeds and the resulting increase in the seed protein content are not expected to pose any hazards to the health of humans or animals or to the environment.

(c) The *bar* gene

The bar gene from *Streptomyces hygroscopicus* encodes the enzyme phosphinothricin-N-acetyltransferase (PAT) and is driven by the maize *Ubi-1* promoter and the 35S termination sequence of the cauliflower mosaic virus. This marker gene confers tolerance to phosphinothricin (glufosinate), the active ingredient in the herbicide Basta®, and was transferred for selection purposes in the production of the GM plants. Several copies of the *bar* gene were inserted into the HOSUT line. The number of copies present in the SUTAP and XAP lines was not determined. The *bar* gene is not expressed in the HOSUT line, presumably due to cosuppression.

L-phosphinothricin is a glutamine acid analogon that inhibits glutamine synthetase in plants. Inhibition of glutamine synthetase causes accumulation of ammonium ions, thus leading to cell death. For this reason phosphinothricin is used as the active ingredient in the non-selective herbicide Basta®. Basta® contains the enantiomers D- and L-phosphinothricin at a ratio of 1 : 1. D-phosphinothricin does not act as a glutamine synthetase inhibitor.

In contrast to the effect of Basta® treatment on non-GM plants, if these GM plants were treated with the same herbicide, L-phosphinothricin would be acetylated by phosphinothricin acetyltransferase, thereby producing N-acetyl-L-phosphinothricin, which has no herbicidal effect. As a result the GM plants are Basta®-tolerant. Phosphinothricin acetyltransferase is a highly substrate specific enzyme. Even the phosphinothricin analogon glutamate is barely converted. D-phosphinothricin is not metabolised by phosphinothricin acetyltransferase.

N-acetyl-phosphinothricin present in the genetically modified plant material remaining in the field following completion of the trials enters the soil during decomposition and here it is converted back into L-phosphinothricin by soil microorganisms. D/L-phosphinothricin is likewise broken down in the soil by microorganisms.

According to the data on hand, the level of toxicity exhibited by N-acetyl-L-phosphinothricin is significantly lower than that of phosphinothricin (= the active agent in the herbicide Basta®).

Basta® has been authorised for use under the German Plant Protection Act by the Federal Biological Research Centre for Agriculture and Forestry (BBA) and the Federal Office of Consumer Protection and Food Safety (BVL). An assessment of the toxicological and ecological impact of the herbicide was conducted within the context of the approval process.

Furthermore, if parts of the genetically modified plants are consumed by humans or animals the phosphinothricin-acetyltransferase contained in these plants is not expected to cause any harmful effects. If ingested orally it can be assumed that the enzyme, as for proteins in general, would be degraded in the digestive tract. The phosphinothricin-acetyltransferase does not possess any of the typical properties (thermal stability, stability in the digestive tract) of allergenic proteins from food, nor does it have any sequence homology with known allergens. According to the application, the use of Basta® as a herbicide within the scope of the experimental release is not planned.

(d) Additional DNA fragments contained in the introduced transformation plasmids

To accomplish transformation the intact transformation plasmids were inserted using microprojectile bombardment technology. Therefore, the possibility that additional DNA fragments were transferred to the GM wheat plants cannot be ruled out.

The pJFBar plasmid

The pJFBar plasmid derives from the binary vector pPZP111 and contains the following genetic elements outside the border regions:

- a bacterial chloramphenicolacetyl transferase gene (Cm^R gene, *cat* gene), which confers resistance to the antibiotic chloramphenicol;
- the *bom* sequence from pBR322 for mobilising the plasmid from *E. coli* in *Agrobacterium tumefaciens*;
- the origins of replication ColE1 and pVS1, which enable replication in *E. coli* and *Agrobacterium*.

The application does not contain details on the transfer of the chloramphenicolacetyl transferase gene. However, if this gene were transferred, it can be assumed that it would not be expressed in plants since it is driven by a prokaryotic promoter. Consequently, no effects on the plant metabolism are expected. There are no indications that the replication regions of CoIE1 and pVS1 or the *bom* sequence have any function in higher plants.

The plasmid HOSUT/pPZP200

The plasmid HOSUT/pPZP200 employed in the transformation of the wheat plants derives from the binary vector pPZP200. It contains the following genetic elements outside the border regions:

- a bacterial aminoglycoside-3''-adenyltransferase gene (Sp/Sm^R gene, *aadA* gene), which confers resistance to the antibiotics streptomycin and spectinomycin;
- the *bom* sequence from pBR322 for mobilisation of the plasmid from *E. coli* in *Agrobacterium tumefaciens*;
- the origins of replication ColE1 and pVS1 for replication in *E. coli* and *Agrobacterium,* respectively.

Southern blot analysis was unable to demonstrate the presence of the *aadA* gene in the HOSUT line transformed with this plasmid. The remaining elements are to be evaluated in the same manner as the plasmid pJFBar.

The plasmids SUTAP/pUC18 and XAP/pUC19

- The *amp^r* gene that encodes a β–lactamase
- Nucleotides of the *lacZ* gene from *E. coli*
- The origin of replication ColE1 *(ori)*

Southern blot analysis demonstrated the presence of the *amp^r* gene that codes for a β–lactamase in the genome of the XAP27 and SUTAP-78 lines. Based on the data available it cannot be ruled out that the *amp^r* gene was also integrated into the lines SUTAP-60 and -69. All of the above-mentioned fragments play a role in expression or gene regulation in bacteria and do not have any function in plants. If any of these DNA fragments were to be transferred to the genome of the GM wheat, they would not be expressed. Thus no adverse effects on human health or the environment are expected.

(e) 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 characteristics of the genetically modified plants are not modified to the same degree in the field as under climate chamber or greenhouse conditions. 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 and can potentially lead to alterations in plant metabolic pathways. Such processes may influence plant metabolism pathways. During previous greenhouse trials with the genetically modified wheat plants, the SUTAP and XAP lines flowered three weeks earlier than the HOSUT line and the non-GM control lines. Neither the germination capacity of the seeds, their morphology nor the general appearance of the plants had altered. Flowering three weeks early is a desirable trait for plant breeding and one that would be of value for cultivation and use; this trait would not pose any risks to human or animal health or to the environment.

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 and were first identified in maize. 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 respect the genetically modified plants do not differ fundamentally from non-genetically modified plants.

Given the current state of knowledge, it is impossible to make reliable predictions about the possible allergenic action of a protein on the basis of its amino acid sequence. The transport proteins produced in the genetically modified wheat belong to the group of proteins that make up the basic transporter components of all metabolically active organisms. To date no allergenic effects or toxic properties have been reported for this protein group. Since they play a key role in the metabolism of such a wide range of organisms, no increased allergenic potential is expected for these transporter proteins. The products of this trial are not intended for use in foodstuffs or animal feed.

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

Wheat has a long history of cultivation as a crop plant; hexaploid wheat is not known to exist as wild-type wheat. It only appears as a wild plant on roadsides and other ruderal sites in the vicinity of existing cultivated areas. As a weakly competitive plant, wheat is not known to establish in natural, intact plant communities.

Experience gained from greenhouse and open field trials did not produce any evidence to suggest that the genetically modified wheat differs from non-genetically modified wheat in its ability to establish in the environment as a result of the genetic modification.

On completion of the generative phase wheat plants die off. From the produced seed new plants can emerge. During harvesting the seeds (grains) are separated from the wheat ears by threshing. After entering secondary dormancy they can survive under favourable conditions for up to two years in the soil without losing the ability to germinate. Under favourable conditions these seeds may germinate in subsequent stocks of cultivated plants. There are no indications that this genetic modification leads to altered persistence in comparison with non-genetically modified wheat.

The applicant plans to harvest the ears of the GM wheat and the non-GM control plants by hand early enough to prevent spontaneous seed spillage. Harvested plant material not required for analysis will be destroyed by autoclaving. After the harvest, the stalks will be burned off as stipulated and the remaining plant residue will be shredded and worked into the soil with a cultivator.

After completion of the proposed trial the release site is to be either left fallow or cultivated with plant species that allow the identification of potentially emerging wheat. In the post-trial monitoring period, any emerging wheat plants are to be removed before flowering during the planned cultivation gap. The post-trial monitoring area is to include a three-metre wide strip surrounding the border rows. Both the cultivation interval and the post-trial monitoring period are to be extended if volunteer wheat plants are observed in the final year of the release.

In studies carried out to date with the genetically modified wheat and in observations of the morphological traits of the plants under greenhouse conditions, the applicant reports that no differences between the genetically modified and the non-genetically modified plants were found. In the greenhouse the SUTAP and XAP lines come into flower about three weeks earlier than the HOSUT line or the non-GM control lines. The GM wheat was not found to exhibit any increased vitality or fertility which would promote the persistence or invasiveness of the genetically modified plants in the environment. Accordingly, the risk that the GM wheat would persist or establish in the environment is extremely slight.

The development of these GM wheat plants with increased seed protein content is ultimately expected to lead to the production of higher quality seeds for animal feed.

There is no evidence to suggest that the introduced trait would alter the general inability of the cultivated plants to compete against wild plant species. For these reasons neither the establishment nor the uncontrolled persistence of the genetically modified plants is to be expected.

III.1.2.3. Assessment of the likelihood of pollen-mediated transfer of the genes inserted into the GM plants to other plants

Wheat (*Triticum aestivum*) is the most important crop cultivated in the temperate zones. It is an annual, mostly awnless, cereal grass with both summer and winter varieties. The upright stem comprises a distichous arrangement of alternating spikelets each containing 3-6 androgenous florets, of which only about 3 produce seeds. The flowering period of each individual floret is extremely short, lasting approximately one hour. Due to the staggered onset of flowering in the individual florets of the spikelet, in the entire head and in the different heads on the main stem and on the auxiliary stems of the same plant, it can take over a week for all the florets of an entire wheat plant to flower. As a rule self-pollination occurs before the flower opens, although to a certain extent allogamy is possible, depending on genotype and climatic conditions during flowering. The rate of cross-pollination is said to be around 1-3%, but under warm and dry weather conditions the rate for some genotypes may be higher. On enquiry, the Federal Plant Variety Office (*Bundessortenamt*) quoted a cross-pollination rate of 1-3% for varieties cultivated in Germany.

Although wheat pollen is dispersed by wind, the likelihood of its spreading is limited due to the weight of the pollen grains. The probability of cross-pollination is similarly limited, since only a relatively small amount of pollen is produced. Moreover, wheat pollen is only fertile for a very short period. Under optimal conditions the fertile period lasts for about three hours; under field conditions, less than 30 minutes. Studies on the dispersal of wheat pollen showed a seed set of about 10% on pollen sterile wheat plants that were planted about 30 m from the pollen source. In contrast, field trials showed that the seed set on pollen sterile wheat plants declined to 10% at distances of only 1-3 m. The furthest outcrossing distance documented to date was 300 m, recorded during an experiment with a biological detection system. According to the information available to the BVL, no other outcrossings have been documented. In the case mentioned above, the outcrossing rate was 0.005%.

As a measure to protect against unwanted incrossing in wheat propagation areas, seed legislation requires that these areas be separated from neighbouring cereal stocks by a buffer zone (the width of which is not given). Other minimum separation distances must not be observed. The seed for hybrid wheat varieties permitted in Germany is produced abroad.

The necessary pollen sterility is not genetically induced, but is achieved by the application of gametocides that are not permitted in Germany. Therefore, the cultivation of hybrid wheat does not imply increased outcrossing potential.

The most commonly grown wheat in our region (*Triticum aestivum* or bread wheat) is hexaploid. Additional types grown with a regional focus include durum wheat (*Triticum durum*, tetraploid, for pasta products) and, occasionally, spelt (*Triticum spelta*, hexaploid, e.g. for pearl barley or semolina). Other species of wheat such as poulard (*Triticum turgidum*, tetraploid), emmer (*Triticum dicoccum*, tetraploid) and einkorn (*Triticum monococcum*, diploid), however, are only found very occasionally in agricultural areas. Pollen-sterile wheat is not used for cultivation purposes.

As an important crop, wheat has long been the subject of crossing experiments with crossing partners both within the genus *Triticum* and from other species. Hexaploid wheat types and species can be intercrossed to produce fertile progeny. In contrast, the fertility of the F1 from crossings between hexaploid and tetraploid species is often extremely limited, and progeny resulting from crossings between hexaploid and diploid species are usually sterile. One exception is the cross between *T. aestivum* and *T. turgidum* (tetraploid), which produces fertile F1.

Of the possible crossing partners for intergeneric hybrids of *T. aestivum* mentioned in the consensus document of the OECD, species of *Agropyron, Elymus, Hordeum*, *Leymus*, *Setaria* and *Sorghum* as well as *Secale cereale* (rye) and triticale, and also *Aegilops* are found in Germany. Crossings of the mentioned wheat species and types with other species are often only achieved by applying special techniques (pollination by hand, male-sterile lines, embryo rescue methods) and produce mostly sterile offspring. The risk of spontaneous hybridisation occurring under field conditions is considered to be extremely low. Apart from the genetic incompatibility of the crossing partners, other requirements, such as the synchronous flowering of both partners, must be met before successful hybridisation can be achieved under natural conditions. Agrotriticum, an intergeneric hybrid from *Triticum aestivum* and *Agropyron* sp., which is said to be back-crossable with both parent species, is not cultivated in Germany. The appearance of spontaneous hybrids from rye and wheat (triticale) has only been reported in older publications. This probably has to do with the open flowering mode of the cultivars used at that time. In the cases described, rye was the pollen donor and wheat the pollen acceptor. The spontaneous appearance of triticale in neighbouring rye fields is therefore not expected. Naturally occurring hybrids between wheat cultivars and barley or oat cultivars have not been reported.

Coupled with the provisions of the decision on this application, the measures proposed in the application documentation ensure that a minimum separation distance of 120 m to other areas on which soft or hard wheat is sown will be observed.

All participating institutions agree that the described measures adequately reduce the risk of outcrossing to neighbouring crop cultivations.

If the modified DNA were to outcross to a species of the aforementioned plant genera intended for consumption (e.g. rye, barley) despite the proposed measures and under consideration of the biological properties of wheat, the evaluation carried out under III.1.2.1. (a)-(d) of the characteristics transferred indicates that no harmful effects on human health or the environment would be expected to result. Any isolated hybridisation events that might occur between the genetically modified plants and wild plants in spite of the described measures would most likely not lead to the spread of the transferred genes to wild plant populations, since this would require the subsequent backcrossing of the hybrid with the wild plant species.

III.1.2.4. Assessment of the likelihood of horizontal gene transfer of the inserted foreign genes from the GM plants to microorganisms

The inserted sequences are stably integrated into the chromosomes of the recipient organisms. There is no evidence 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 possible in principle, 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 as plants and microorganisms 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 there is always the possibility that such an exchange could result in the uptake of all forms of heterologous genetic material, including all forms of plant DNA.

(a) The *HvSUT1* gene

The *HvSUT1* gene that was transferred to the GM plants is derived from barley – a widely cultivated crop plant. Therefore this gene can also be spread as a result of horizontal gene transfer from non-GM organisms.

(b) The *VfAAP1* gene

Amino acid transporters are also ubiquitous in plants. The gene transferred to the GM wheat derives from field bean (*Vicia faba*), which is grown extensively in Germany. There is a possibility that this gene may also be spread by horizontal gene transfer from non-GM organisms.

(c) The *bar* gene

Inactivation of phosphinothricin by acetylation is a naturally occurring process in soil microorganisms. Bacteria with a corresponding resistance are widespread in the environment. Therefore there is a possibility that the *bar* gene may also be spread by horizontal gene transfer from non-GM microorganisms. In the event of a transfer of the *bar* gene from the GM plants to microorganisms, no detectable increase in the overall frequency of this resistance in the environment would result.

(d) Additional DNA fragments located on the transformation plasmids

The GM wheat plants may contain the following genetic elements, which are located on the plasmids used for transformation:

- a bacterial chloramphenicolacetyl transferase gene (Cm^R gene, *cat* gene) that confers resistance to the antibiotic chloramphenicol;
- the *ß-lactamase* gene for resistance to the antibiotic ampicillin;
- the gene for the α-fragment of the β-galactosidase from *E. coli*;
- the *bom* sequence and the *nic* sequence from pBR322 for mobilisation of the plasmid from *E. coli* in *Agrobacterium tumefaciens*;
- the origins of replication ColE1 and pVS1 for replication in *E. coli* and *Agrobacterium,* respectively;
- parts of the plasmids pUC18 or pUC19 (ori of replication).

The *Cm^R* gene derives from the transposon Tn9 and codes for an acetyltransferase that catalyses the acetyl-CoA-dependent acetylation of the antibiotic chloramphenicol and in doing so counteracts its antibacterial effect (Proctor and Rownd, 1982). Today, chloramphenicol is only applied clinically in isolated cases due to the risk of inducing aplastic anaemia, and it is not authorised for use in the treatment of animals for the production of food in the EU. Chloramphenicol-resistant microorganisms are widespread in the environment.

In its statement of 6.7.1999 on the biological safety of antibiotic resistance genes in the genome of genetically modified plants, the ZKBS (Central Committee on Biosafety) allocated the *Cm*^{R} gene to the group I antibiotic resistance genes that are (a) already widespread in soil and enterobacteria and (b) whose relevant antibiotics are have no, or only little significance in human and veterinary medicine, so that it can be assumed that the potential presence of these antibiotic resistance genes in the genome of genetically modified plants would have no impact on the distribution of these antibiotic-resistance genes in the environment.

The *ß-lactamase* gene is widespread in microorganisms. About 35% of all clinical *E. coli* isolates from humans are resistant to ampicillin, 90% of which can be attributed to a ß-lactamase-mediated mechanism of action. Similarly, 74% of all cattle- and pig-derived *E. coli* isolates display resistance to ampicillin. In its statement of 6.7.1999 on the biological safety of antibiotic-resistance genes in the genome of GM plants, the ZKBS allocated the *amp*^{r} gene to the Group II antibiotic-resistance genes which are (a) widespread in microorganisms and (b) their relevant antibiotics are currently only applied in specific areas of human and veterinary medicine, so that it can be assumed that the presence of these antibiotic-resistance genes in the genome of GM plants would have only minimal, if any, impact on the distribution of these antibiotic-resistance genes in the environment.

In its report of 2 April 2004 on the use of antibiotic resistance genes as marker genes in genetically modified plants, the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA) classed the *Cm*^{R} and *amp*^{r} genes among the group of genes permitted only for use in experimental release trials; these genes should not be present in genetically modified plants intended for the market. It should be borne in mind that the proposed deliberate release experiment is to be conducted on a limited area and for a specified period only.

The α-peptide of the *lacZ* gene for a β–galactosidase: The multiple cloning site pUC18/19 is located within the coding sequence for the α-fragment of the β–galactosidase from *E. coli*. The native β–galactosidase enzyme splits β–D-galactosidase into galactose and the corresponding alcohol complex. On its own, the α-fragment is not enzymatically active. Besides, the α-fragment in the GM wheat would be interrupted by the insertion of the cloned gene into the multiple cloning site, so that no functional gene product can be formed. The same would also apply for bacteria that receive the gene by horizontal gene transfer.

The replication region of the plasmid pVS1 from *Pseudomonas aeruginosa* contains the genetic information for replication and stability and enables replication of this plasmid in *Agrobacterium tumefaciens.* For this nucleic acid fragment, the likelihood of spreading by transfer between bacteria is far greater than the likelihood of spreading by horizontal gene transfer from the GM plants to microorganisms.

The pUC replicon is one of the ColE1-type plasmids with a host range that is restricted to a few gram-negative bacteria. Essentially, the replicon can be replicated in *E. coli* and in other closely related species of bacteria such as, for example, *Serratia* or *Salmonella*. In most gram-negative soil bacteria no replication occurs. ColE1 plasmids are quite common in enterobacteria. The likelihood of enterobacterium-mediated gene transfer to other bacteria is considered far greater than the likelihood of horizontal gene transfer from the genetically modified plants to bacteria. Therefore, the possible presence of the pUC origin of replication in the plant chromosome is not expected to contribute to an increase in the overall frequency of horizontal gene transfer.

(e) Regulation sequences

In the event that regulation sequences used in the construct are transferred, there is no reason to fear that the overall distribution of the respective DNA fragments will increase. These regulation sequences used are derived from *Zea mays*, *Triticum aestivum*, *Hordeum vulgare*, *Agrobacterium tumefaciens* and the cauliflower mosaic virus. CaMV is a plant-infesting, double-stranded DNA virus commonly found in plants. Maize and rice are common crops in agricultural regions around the world. *A. tumefaciens* is also ubiquitous in the environment. In wild-type agrobacteria the sequences of interest are located on Ti plasmids that may be exchanged between different Rhizobiaceae. Maize, barley and wheat are all commonly grown crops in agricultural regions around the world.

(f) Additional DNA sequences

As a result of the use of microprojectile bombardment technology for transformation additional fragments of the transformation plasmids may have been integrated into the genome of the GM wheat plants. These fragments are non-encoding and have no regulatory function; a horizontal gene transfer of these fragments to microorganisms would therefore not be of any significance.