Corporation obtaining approval, the name of its representative, and the address of its main office

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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Maize tolerant to glufosinate herbicide (*bar, Zea mays* subsp. *mays* (L.) Iltis) (DLL25, OECD UI: DKB-8979Ø-5)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

I. Information collected prior to assessing Adverse Effect on Biological Diversity

1. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

i) Composition and origins of component elements

The composition of the donor nucleic acid and the origins of component elements that was used for the development of maize tolerant to glufosinate herbicide (*bar, Zea mays* subsp. *mays* (L.) Iltis) (DLL25, OECD UI: DKB-8979Ø-5) (hereinafter referred to as "this recombinant maize") are shown in Figure 1 and Table 1.

The nucleotide sequences of component elements of the donor nucleic acid are shown in Annex 1.

ii) Functions of component elements

Functions of component elements of donor nucleic acid that was used for the development of this recombinant maize are shown in Table 1.

(a) Phosphinothricin acetyltransferase (PAT), which is encoded by *bar* gene, the target gene, inactivates phosphinothricin (PPT), an active ingredient of glufosinate herbicide, which confers the glufosinate herbicide tolerance to plant body.

The *bar* gene was isolated from *Streptomyces hygroscopicus* (ATCC21705), a microorganism in soil. The PAT protein, which is encoded by the *bar* gene, is an enzyme to catalyze the acetylation of dimethyl phosphinothricin, an intermediate stage of biosynthesis of antibiotics bialaphos (phosphinothricin alanylalanine) produced by *Streptomyces hygroscopicus*. In the process of inactivation of PPT, PAT protein catalyzes the reaction of transferring the acetyl group of acetyl CoA to the amino acid group of PPT, an active ingredient of glufosinate herbicide, which acetylates the PPT and resultantly inhibits the activity of glufosinate herbicide (Reference 10).

It is known that PAT protein possesses high substrate specificity (Reference 10). The substrate affinity to phosphinothricin is known 30 times higher than the affinity to dimethyl phosphinothricin, which resembles the phosphinothricin in structure, and 300 times higher than the affinity to glutaminc acid, which also resembles in structure (Reference 10). Consequently, it is considered unlikely that PAT protein will acetylate any natural products in the cells of maize.

(b) In order to investigate whether the PAT protein shares functionally important amino acid sequence with known allergens, the PAT protein was compared with allergens in the database (GenPept, PIR, SwissProt). As a result, the PAT protein did not share structurally related homologous sequences with any of the

known allergens examined.

(2) Information concerning vector

i) Name and origin

The origin of plasmid vector pDPG165 used for the development of this recombinant maize is plasmid pUC 19 from *Escherichia coli* (Reference 11).

ii) Properties

The total number of base pairs of this plasmid vector is 4,609 bp. It possesses *bla* gene as a selectable marker gene for construction vector in *E. coli*. The infectivity of this plasmid vector is not known. The nucleotide sequences of component elements are shown in Annex 1.

(3) Method of preparing living modified organisms

i) Structure of the entire nucleic acid transferred in the recipient organism

The component elements of this plasmid vector transferred in the recipient organism are shown in Table 1. In addition, the location and section broken by restriction enzyme of the component elements of the nucleic acid in the vector are shown in Figure 1.

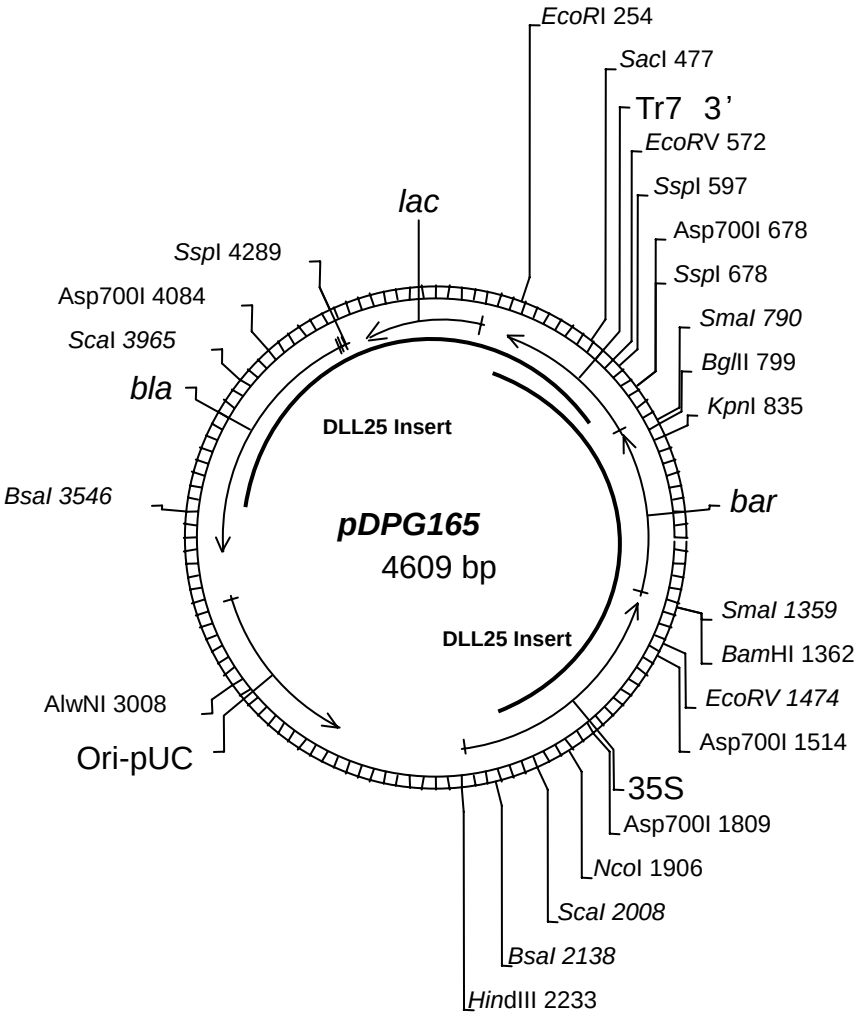

Figure 1 Plasmid vector pDPG165 [1]

[1] All the rights pertinent to the information in the diagram above and the responsibility for the content rest upon Monsanto Japan Limited.

Table 1 Component elements of pDPG165 to be used for introduction and their origin and function [2]

Component elements	Origin and Function
bar gene expression cassette	
35S	A promoter from cauliflower mosaic virus (CaMV) (Reference 12). Makes target genes expressed in all the tissues constantly.
bar	The gene derived from *Streptomyces hygroscopicus*, encodes phosphinothricin acetyltransferase (PAT) (Reference 10). The glufosinate tolerance is conferred to the plant body due to the expression of this gene.
Tr7 3'	3' untranslated region from T-DNA transcript product 7 of *Agrobacterium tumefaciens* (Reference 13). It terminates transcription and induces polyadenylation.
Other component elements	
lac	Consists of partial coding sequence for *lac* repressor derived from *E.coli*, *lac* promoter, and partial coding sequence for β-galactosidase (*lacZ*) (Reference 10). Used as a selective marker in cloning experiments in *E.coli*. The *lac* promoter is not expressed in this recombinant maize since it never function in any plant body.
bla	A gene derived from *E.coli* plasmid pBR322, encoding β-lactamase (Reference 13; Reference 10). It confers the resistance to ampicillin and other penicillin to bacteria.
ori-pUC	The replication origin derived from *E. coli* plasmid pUC19. Permits autonomous replication of vectors in *E. coli* (Reference 10).

[2] All the rights pertinent to the information in the table above and the responsibility for the content rest upon Monsanto Japan Limited.

ii) Method of transferring nucleic acid transferred in the recipient organism

Plasmid vector pDPG165, in the circular DNA state, was introduced by particle gun bombardment into the regeneration line derived from embryo culture callus that is classified into dent type.

iii) Processes of rearing of living modified organisms

(a) The callus to which pDPG165 was introduced was grown on a tissue culture media containing glufosinate, and then the recombinant plant was selected. From the selected callus, the regenerated plant was obtained.

(b) A plasmid was introduced in this recombinant maize by particle gun bombardment, so confirmation of remaining Agrobacterium was not carried out.

(c) The evaluation of pedigree selection was started in 1990, and field experiments were carried out from 1991 to 1993. Finally, an excellent line was selected. In the field experiments in 1994, the morphological and growing characteristics of this line were investigated (see Figure 2 for the line to be tested). Based on these results, necessary approval was obtained and general commercial cultivation was implemented until 1999, however it has been discontinued since 1999.

The following shows the approvals received from organizations in Japan.

December, 1997:	Based on the “Guideline for the use of recombinant in agriculture, forestry and fisheries”, the compatibility to the guideline regarding recombinant being imported to Japan (used for processing and feed) was certified by the Ministry of Agriculture, Forestry and Fisheries. (The compatibility of the guideline to the posterity, DLL25-DK566 was certified.)
June, 1999:	Based on the “Guideline for the use of recombinant in agriculture, forestry and fisheries”, the compatibility to the guideline regarding recombinant being imported to Japan (used for processing and feed) was certified by the Ministry of Agriculture, Forestry and Fisheries.
March, 2000:	The safety of use of the cultivar for feed was approved in accordance with “Guideline for the safety evaluation of feed derived from recombinant-DNA plants, 6-(2)” by the Ministry of Agriculture, Forestry and Fisheries.
March, 2001:	The safety of use of the cultivar for food, in accordance with “Safety Evaluation Criteria for Food and Additives derived from Recombinant-DNA Techniques” was certified by the Ministry of Health, Labour and Welfare.

March, 2003:	The safety of the use of the cultivar as feed, following "Procedure to Check the Safety of Feed and Additives Produced by Recombinant-DNA Techniques" was certified by the Ministry of Agriculture, Forestry and Fisheries.

Figure 2 The process of rearing the maize DLL25 tolerant to glufosinate herbicide

[Not made available or disclosed to unauthorized person]

(4) State of existence of nucleic acid transferred in cells and stability of expression of traits caused by the nucleic acid

i) Location of copies of the introduced nucleic acids

On the chromosome

ii) Number of copies of the introduced nucleic acids and inter-generational inheritance stability of copies of the introduced nucleic acids

As a result of Southern blotting analysis to examine the genes transferred to this recombinant maize, it was confirmed that one copy of the DNA fragment is inserted into the genome of this recombinant maize at one site (Annex 2, Annex 3). The inserted gene consists of *bar* gene cassette ([35S]-[*bar*]-[Tr7]), *bla* gene fragment, *lac*, and Tr7 (polyadenylic sequence) (Figure 3, Annex 2). The *bla* gene was considered not to function because the 3' region was found broken. In addition, as a result of Western blotting analysis, there was no β-lactamase, a product of *bla* gene, detected in this recombinant maize (Annex 3). The *lac* consists of partial coding sequence for *lac* repressor derived from *E.coli*, *lac* promoter, and partial coding sequence (*lacZ*) for β-galactosidase, though the *lac* promoter does not function in plant body, therefore *lacZ* is not expressed in this recombinant maize. In addition, it was proved in Southern blotting analysis on the multiple generations (marked with * in Figure 2) that the transferred genes are stably inherited in posterity (Annex 3).

iii) Nearby or separate location of multiple copies, if present, on the chromosome

This item is not applicable due to the one copy (Annex 2).

iv) Inter-individual or inter-generational expression stability under natural conditions

It was proved as a result of Western blotting analysis on the multiple generations (the generations underlined in Figure 2) that the transferred genes of this recombinant maize are stably inherited in posterity (Annex 3). In addition, it was also confirmed during the process of selection that the tolerance to glufosinate herbicide was expressed stably in the multiple generations.

v) Existence of transmission routes and its scale, if it is possible that nucleic acids introduced via viral infection or other routes might be transmitted to wild animals and plants

It has been confirmed that this recombinant maize does not contain any DNA sequence allowing transmission; therefore there is no possibility that the transferred DNA fragment might be transmitted to any wild animals and wild plants.

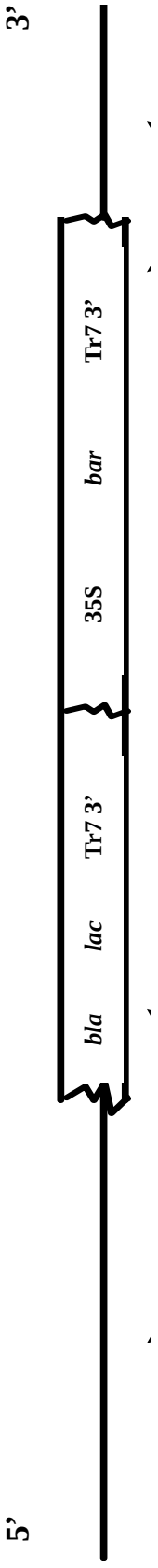

Figure 3 Genetic map of inserted genes in DLL25 [3]

[3] All the rights pertinent to the information in the diagram above and the responsibility for the content rest upon Monsanto Limited Japan.

(5) Methods of detection and identification of living modified organisms and their sensitivity and reliability

For the detection and identification of this recombinant maize, a qualitative PCR method has been developed where the DNA sequences of the inserted genes and the nearby regions of the plant genome are used as primers. This method makes it possible to specifically detect this recombinant maize (Annex 4).

(6) Difference from the recipient organism or the taxonomic species to which the recipient organism belongs

i) This recombinant maize is given tolerance to glufosinate herbicide with the consistent expression of PAT protein, which is encoded by the bar gene, in various regions of the plant body. In practice, the non-recombinant control maize died due to the influence of glufosinate herbicide, while this recombinant maize grew normally (Annex 3).

ii) [4] Isolated fields tests were carried out in the National Institute for Agro-Environmental Sciences (NIAES) in 1998 using the inbred line of this recombinant maize (Figure 2) and the inbred line of non-recombinant control maize, which is genetically equivalent to this recombinant maize (Annex 3). Differences between this recombinant maize and the non-recombinant control maize, the recipient organism, have been examined primarily based on the results of the tests in 1998. However, also based on the results of isolated field tests carried out in 1997 in the NIAES (Annex 2) and the results of field experiments conducted in 1994 in 12 sites in the US, comprehensive examination has been conducted (Annex 3).

(a) Morphological and growth characteristics

For this recombinant maize and the non-recombinant control maize, evaluation was conducted regarding germination rate, uniformity of germination, time of tasseling, time of silking, maturation time, plant type, tiller number, total number of ears, number of useful ears, culm length, height of ear and plant weight at harvesting time. Statistically significant difference was not observed between this recombinant maize and the non-recombinant control maize lines in any of the characteristics except height of ear (Annex 3). Regarding height of ear, statistically significant difference was found between this recombinant maize and the non-recombinant control maize, and the average value of height of ear was 80.4 cm for this recombinant maize and 95.4 cm for the non-recombinant control maize (Annex 3). On the other hand, statistically significant difference was not observed between the first cross line of this recombinant maize and the first cross line of the non-recombinant control maize, which is genetically equivalent to this recombinant maize (Annex 2).

[4] All the rights pertinent to the information in the paragraphs (a) through (g) following this section and the responsibility for the content rest upon Monsanto Japan Limited.

(b) Cold-tolerance and heat-tolerance at the early stage of growth

Cold-tolerance test at the early stage of growth was not conducted in the isolated field tests. However, in the field experiments conducted in 1994 in 12 sites in the US, and during the period of commercial cultivation from 1997 to 1999, it was not reported that this recombinant maize, which grew up to seedlings after having dropped in the fields during harvesting time, could overwinter and survive into the beginning of following spring.

(c) Wintering ability and summer survival of the matured plant

Maize is a summer type annual plant, and after ripening it usually dies out in winter, and it does not regrow and propagate vegetatively, or produce seeds. It was observed that dying started after ripening at the end of the tests carried out in the isolated field for this recombinant maize in practice. Based on the above, an overwintering test for the matured plant of this recombinant maize was not carried out.

(d) Fertility and size of the pollen

The tolerance to glufosinate herbicide is the only characteristics conferred to this recombinant maize and there exists no insect that can be affected by dispersion of pollens nor wild relative that can be crossed in Japan, therefore this item is not applicable.

(e) Production, shedding habit, dormancy and germination rate of the seed

Regarding the production of this recombinant maize, ear length, ear diameter, row number per ear, grain number per row, and 100-kernel weight after sib-mating were examined. As a result, no statistically significant difference was observed between this recombinant maize and the non-recombinant control maize in any of the characteristics examined (Annex 3).

Regarding shedding habit of the seed, shedding habit was not observed in the natural condition, since the ears of this recombinant maize and the non-recombinant control maize were covered with skins at the time of harvesting.

No examination was carried out on the germination rate of harvested seeds in the isolated field tests, though it was reported in the field experiments carried out in 1994 in 12 sites in the US and in the commercial cultivation implemented from 1997 to 1999 that no difference was observed in the number of individuals, which seemed to germinate and grow from the seeds dropped after harvesting, between this recombinant maize and the non-recombinant control maize. As a result, it was considered that this recombinant maize is comparable to the non-recombinant control maize in the dormancy and germination rate of the seeds.

(f) Crossability

Crossability test was not performed for this recombinant maize, since no wild relatives that can be crossed grow in Japan.

(g) Productivity of harmful substances

Succeeding crop tests and soil microflora tests were performed for this recombinant maize and the non-recombinant control maize. Statistically significant difference was not observed in any of the items (Annex 3). In addition, as part of the isolated field tests carried out in 1997, succeeding crop test, soil microflora test and weed vegetation survey were conducted in the cultivation zones for this recombinant maize and the non-recombinant control maize. As a result, no difference was observed between this recombinant maize and the non-recombinant control maize (Annex 2). In the field in the US, following the harvesting of this recombinant maize in 1999, plant body of this recombinant maize was plowed into the field and soybean and wheat were cultivated in the same field in the next year, though no inhibited growth was reported. In addition, as a result of cultivation of other crops in the field following this recombinant maize during the period of commercial cultivation, no inhibited growth was reported.

2. Information concerning the Use of living modified organisms

(1) Content of the Use

Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them

This recombinant maize will not be cultivated by intent both at home and abroad in the future.

(2) Information obtained from Use abroad

For this recombinant maize, tests were performed between 1990 and 1994 in the fields in the US to observe the characteristics including morphological and growth characteristics, characteristics regarding yield, characteristics regarding the ability to become self-seeding, and sensitivity to pests. As a result, no definite difference was observed in all the items examined between this recombinant maize and the non-recombinant control maize (Annex 3).

Commercial cultivation of this recombinant maize was implemented for three years from 1997 to 1999. Since 2000, commercial cultivation has been discontinued in every country. The area for commercial cultivation was very small.

Table 2 Cultivation area in acre for this recombinant maize in overseas [Confidential information]

Country								

Commercial cultivation of this recombinant maize was continued until 1999 and, in 1996 and 1997, seeds for cultivation were produced. Dekalb Genetics Corporation maintained a monopoly on development through to cultivars rearing and seeds production of this recombinant maize, and the seeds of this recombinant maize retained in stock by Dekalb Genetics Corporation at the end of commercial cultivation were all disposed of by incineration. Therefore, at present, Monsanto Company retains no seed of this recombinant maize. Since 2000 when the commercial cultivation was discontinued, there have been no occasions in which any seeds of this recombinant maize line have been mixed up with the maize lines that Monsanto Company has been rearing. In addition, in the production of seeds of this recombinant maize, this recombinant maize has been always selected as female parent and not used as pollen parent; therefore it is considered unlikely that this recombinant maize might be mixed with any other varieties due to dispersion of pollens in the site of seeds production. In the future, commercial cultivation, sales and distribution of this recombinant maize will not be performed.

II. Review by persons with specialized knowledge and experience concerning Adverse Effect on Biological Diversity

A review was made by persons with specialized knowledge and experience concerning Adverse Effect on Biological Diversity (called Experts) for possible Adverse Effect on Biological Diversity caused by the use in accordance with the Type 1 Use Regulation for Living Modified Organism applied based on the "Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms". Results of the review are listed below.

1. Item-by-item assessment of Adverse Effect on Biological Diversity

(1) Competitiveness

Maize (*Zea mays* subsp. *mays* (L.) Iltis), to which the recipient organism belongs, has been used, including for cultivation, etc., in Japan, though there is no report that it has become self-seeding in Japan.

This recombinant maize is given a trait to be tolerant to glufosinate herbicide due to the PAT protein which is expressed by the transferred *bar* gene. However, it is not generally considered that glufosinate exerts pressure for selection under a natural environment; therefore it is unlikely that these characteristics cause this recombinant maize to be dominant in competitiveness and affect wild animals and wild plants. In addition, as a result of the confined field trial in Japan, it was confirmed that there is no significant difference between this recombinant maize and a non-recombinant maize line with regard to various traits relating to competitiveness except height at node of bearing 1st ear. The height was, on average, 80.4 cm for this recombinant maize and 95.4 cm for the non-recombinant maize but it is considered unlikely that this significant difference can cause this recombinant maize to be predominant over the non-recombinant maize in competitiveness.

Based on the above understanding, the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to competitiveness is valid.

(2) Productivity of harmful substances

Regarding the maize, to which the recipient organism belongs, there is no report that it produces harmful substances to affect wild animals and wild plants.

This recombinant maize possesses a trait to produce PAT protein that inactivates glufosinate herbicide and this protein is not reported as harmful to wild animals and wild plants. In addition, PAT protein possesses high substrate specificity and thus it is considered not to affect the metabolic system of the recipient organism.

Productivity of harmful substances of this recombinant maize (including secretion from roots to affect the other plants, and secretion from roots to affect microorganisms in soil) has been investigated in the confined field trial in Japan,, but there is no significant difference between this recombinant maize and non-recombinant maize. In addition, based on the reasons listed below, it is considered extremely low that productivity of harmful substances of the above-ground part might affect the other wild animals and

wild plants.

i) Commercial cultivation of this recombinant maize has been discontinued since 2000 in everywhere in the world, seeds in stock were all disposed of by incineration, and intentional cultivation will not be conducted in the future even in foreign countries. Consequently, the use in Japan is limited to possible mixing of trace quantity with other maize seeds imported for cultivation, provision as food and/or feed and other purposes.

ii) There is no report that PAT protein is harmful to wild animals and wild plants.

iii) This recombinant maize possesses the tolerance to herbicide and no difference has been observed between this recombinant maize and the non-recombinant maize in the growth characteristics which can cause predominance in competitiveness.

iv) In the field tests in the US, no inhibited growth was observed in soybean and other crops cultivated in the following year after this recombinant maize was harvested and the plant body was plowed in the field in the previous year.

Based on the above understanding, no wild species that can be affected by this recombinant maise is specified, the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to productivity of harmful substances is valid.

(3) Crossability

In Japan, the growth of wild relatives (teosinte) that can be crossed with maize in natural environment has not been reported. Based on the above understanding, no wild species that can be affected by this recombinant maize is specified, it is judged that the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to crossability is valid.

2. Conclusion

Based on the above understanding, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this recombinant cotton in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion above is valid.

Annex List

Annex 1 Sequence of the Genetic Elements in pDPG165

[Not made available or disclosed to unauthorized person]

Annex 2 Program for application of recombinant plant: Maize DLL25 (B16)-DK566 tolerant to glufosinate herbicide (Application in an open system)

[Not made available or disclosed to unauthorized person]

Annex 3 Program for application of recombinant plant: Maize (DLL25 line) tolerant to glufosinate herbicide (Application in an open system)

[Not made available or disclosed to unauthorized person]

Annex 4 Sampling Protocol and General PCR Method for Determining Levels of Unintended Event Presence

[Not made available or disclosed to unauthorized person]