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 resistant to Lepidoptera and tolerant to glufosinate herbicide (*cry1F*, *pat*, *Zea mays* subsp. *mays* (L.) Iltis) (*B.t.* Cry1F maize line 1507, OECD UI: DAS-Ø15Ø7-1)
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

I. Information concerning preparation of living modified organisms

In the maize resistant to Lepidoptera and tolerant to glufosinate herbicide (*cry1F*, *pat*, *Zea mays* subsp. *mays* (L.) Iltis) (*B.t.* Cry1F maize line 1507, OECD UI: DAS-Ø15Ø7-1) (hereinafter referred to as "Cry1F line 1507"), *cry1F* gene to confer the resistance to Lepidoptera and *pat* gene to confer the tolerance to glufosinate herbicide are inserted.

1. Information concerning donor nucleic acid

(1) Composition and origins of component elements

Composition of the donor nucleic acid and the origins of component elements are shown in Table 1.

Table 1 Composition of donor nucleic acid and origins of component elements

Component elements	Size (kbp)	Origin and function
cry1F gene expression cassette		
UBIZM1(2) Promoter	1.98	Ubiquitin constitutive promoter[1)] derived from *Zea mays* (including intron and 5' untranslated region).
cry1F	1.82	A gene that encodes Cry1F protein derived from *Bacillus thuringiensis* var. *aizawai*. Optimized to activate the expression in plant body.
ORF25PolyA Terminator	0.72	A terminator to terminate transcription from *Agrobacterium tumefaciens* pTi5955.
pat gene expression cassette		
CAMV35S Promoter	0.53	35S constitutive promoter[1)] derived from cauliflower mosaic virus (CaMV).
Pat	0.55	A gene that encodes phosphinothricin acetyltransferase (PAT protein), derived from *Streptomyces viridochromogenes*. Optimized to activate the expression in plant body.
CAMV35S Terminator	0.21	35S terminator to terminate transcription from cauliflower mosaic virus (CaMV).

1) Constitutive promoter: A promoter that drives the expression of target genes in all sites in plant body.

(2) Functions of component elements

1) Functions of individual component elements of donor nucleic acid, including target gene, expression regulation region, localization signal, and selective marker

Functions of component elements of donor nucleic acid are shown in Table 1.

2) Functions of proteins produced by the expression of target gene and selective markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergenicity.

a. Cry1F protein

Cry1F protein is a kind of insecticidal crystal protein (*B.t.* protein) known as δ-endotoxin produced by *Bacillus thuringiensis* (hereinafter referred to as "*B.t.*"), a gram-positive bacterium, universally exists in soil. The *B.t.* proteins are classified according to its insecticidal activity, and the Cry1F protein possesses an insecticidal activity against European corn borer (*Ostrinia nubilalis*) and other insects of order Lepidoptera. The Cry1F protein binds to the specific receptors in the midgut cells of the target pest insects when ingested similarly as other *B.t.* proteins and forms pores in the cells, which leads to the destruction of ion channels and results in the broken midgut cells and successful insecticide activity.

European corn borer (*Ostrinia nubilalis*) is one of the insect pests that cause the most significant insect damage to maize cultivation in the US. The hatched larvae feed the leaves to grow and break into the stem through a joint of leaf. Once they enter the stems, they are less susceptible to typical spray-type control insecticides that are difficult to reach them and thus, they eat away and hollow the tassels from the inside. In addition, the larvae that break into the female flowers feed and damage the growing ears. The annual total cost for control of this insect pest reportedly amounts to about a billion dollar.

Cultivation of Cry1F line 1507 allows effective control of Lepidoptera and then it is expected to provide farmers with a new option for control of Lepidoptera.

To investigate the insecticidal spectrum of Cry1F protein, Cry1F protein produced in the *Pseudomonas fluorescens* was mixed to artificial feeds, which were given to 15 different kinds of insects of the order Lepidoptera which are considered typical insect pests for the farming in the US. Among the 15 kinds of insects of the order Lepidoptera, six (6) are regarded as insect pests for the maize growing in the US, and the rest nine (9) are regarded as insect pests for cotton, soybean, canola, and other crops. Among the 6 kinds of insect pests for the cultivation of maize, LC_{50} (50% lethal concentration) of the Cry1F protein for the target insect pest of Cry1F line 1507, i.e., European corn borer, Fall armyworm and Beet armyworm, was 0.58 μg/g, 2.49 μg/g, and 7.8 μg/g respectively. On the other hand, for the rest 3 kinds of insect pests (Southwestern corn borer, Black cutworm, and Bollworm), LC_{50} was higher than 50 μg/g. Also the test was conducted for the *Danaus plexippus* which is not regarded as insect pests for farming, but even the maximum concentration applied in the test (30 μg/g) did not reach the effect comparable to the $LC_{50.}$ Based on the results, it was found that the Cry1F protein

has highly specific insecticidal spectrum similarly as the other *B.t.* proteins and thus it shows the insecticidal activity against limited insects.

In addition to the insects other than order Lepidoptera, tests were also conducted to the mammals, birds, fishes, and order Coleoptera, Hymenoptera, Neuroptera, Collembola and other insects. As a result, it was confirmed that Cry1F protein exhibits no toxicity against all of the non-target organisms tested.

The Cry1F protein did not share amino acid sequence homology with any of the known allergen proteins.

b. PAT protein

PAT protein (phosphinothricin acetyltransferase) confers the tolerance to glufosinate herbicide. The glufosinate herbicide inhibits the glutamine synthase that synthesizes glutamine from glutamic acid and ammonia, which causes the ammonia to be accumulated in the plant body and resultantly the plant to die. The PAT protein acetylates glufosinate herbicide and transforms it to nontoxic acetylglufosinate, thereby conferring the glufosinate tolerance on plant body (Figure 1). Glufosinate herbicide is a nonselective herbicide, and possesses the control activity against a variety of weeds even with a single agent. Thus it has been safely applied throughout the world including Japan and US. Introduction of *pat* gene has made it possible to apply this herbicide to cornfields for weed control, which would provide farmers with an option for weed control. It is known that the PAT protein is least likely to induce any allergy in the human, and the PAT protein did not share amino acid sequence homology with any of the known allergen proteins.

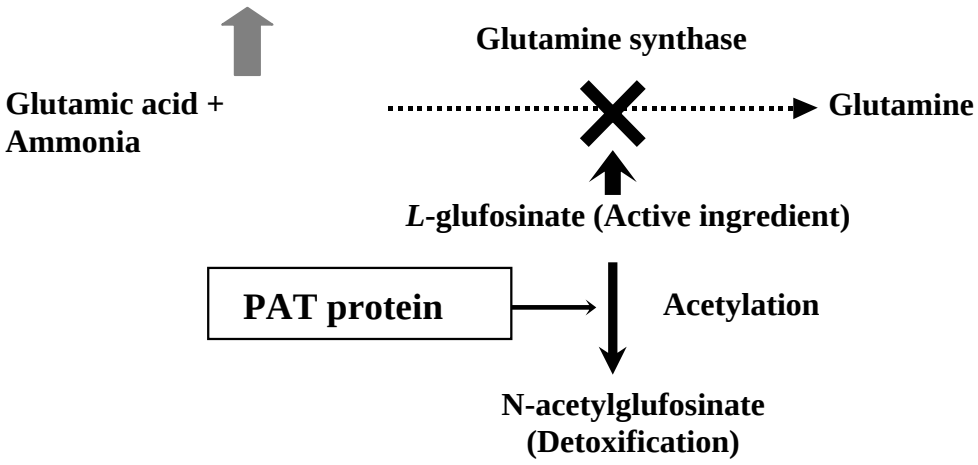

Figure 1 Action mechanism of PAT protein

A plant dies if ammonia accumulates in the plant body due to the inhibition of glutamine synthase caused by the effect of *L*-glufosinate, an active ingredient of glufosinate herbicide. *L*-glufosinate is acetylated and becomes N-acetylglufosinate by work of the PAT protein, and the inhibition of glutamine synthase does not occur. Therefore, ammonia does not accumulate in the plant body, and the plant can grow. It is reported that the PAT protein selects only the *L*-glufosinate for substrate between *D*-glufosinate and *L*-glufosinate.

3) Contents of any change caused to the metabolic system of recipient organism

Similarly to other Cry proteins, it has not been reported that Cry1F protein acts as enzyme in any plant body. It is reported that the PAT protein exhibits very high substrate specificity against *L*-glufosinate, an active ingredient of glufosinate herbicide, and it does not select *D*-glufosinate, an optical isomer of *L*-glufosinate, for substrate. Consequently, neither Cry1F protein nor PAT protein interferes with the metabolic pathway of recipient organism.

2. Information concerning vector

(1) Name and origin

The name and origin of vector used to generate plasmid PHP8999 to which individual gene expression cassette was introduced are as follows.

Name: Plasmid pUC19
Origin: *Escherichia coli* (*E.coli*) K12 strain

(2) Properties

1) The numbers of base pairs and nucleotide sequence of vector

The total number of base pairs of plasmid PHP8999 is 9,504 bp.

2) Types of any nucleotide sequence having specific functions

Vector backborn of plasmid PHP8999 contains antibiotic resistant marker (*nptII* gene), other than the region to which gene is inserted, , to select the microorganisms that contain the transformed plasmid for propagation. The *nptII* gene confers the resistance to antibiotics kanamycin. Upon the introduction of gene into plant cells, plasmid PHP8999 is treated with the restriction enzyme *Pme* I and the resulted linear DNA fragment (PHI8999A) without the region containing the *nptII* gene was used. Therefore, the antibiotic resistant gene is not introduced to the recipient organism.

3) Presence or absence of infectious characteristics of vector

Since plasmid PHP8999 does not posses any sequence that makes the plasmid infectious, the vector offers no infectivity.

3. Method of preparing living modified organisms

(1) Structure of the entire nucleic acid transferred in the recipient organism

The location and orientation of component elements of donor nucleic acid in vector and the section broken by restriction enzyme are shown in Fig.2.

(2) Method of transferring nucleic acid transferred in the recipient organism

The particle gun bombardment method was used to introduce the nucleic acid into the recipient organism. The details are shown in Figure 3.

(3) Processes of rearing of living modified organisms

Cry1F line 1507 was developed jointly by the US companies Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc.

1) Method of selecting the cell into which nucleic acid is transferred

The details are shown in Figure 3.

2) Presence or absence of any residual body cell of *Agrobacterium*

Particle gun bombardment method is used for the introduction of nucleic acid into recipient organism, and thus the *Agrobacterium* was not used.

3) Processes of rearing of living modified organisms and genealogical tree

Cry1F line 1507 and the excellent strain of maize inbreds which is classified as dent type were crossed and selection was performed.

In Japan, in June 2002, based on the “Guidelines for the use of recombinant in agriculture, forestry and fisheries” (hereinafter referred to as “Guideline”), the program for applying Cry1F line 1507 in an open system was confirmed to meet the “Guideline”. In addition, the safety as food was approved in July 2002 and the safety as feed was approved in May 2002.

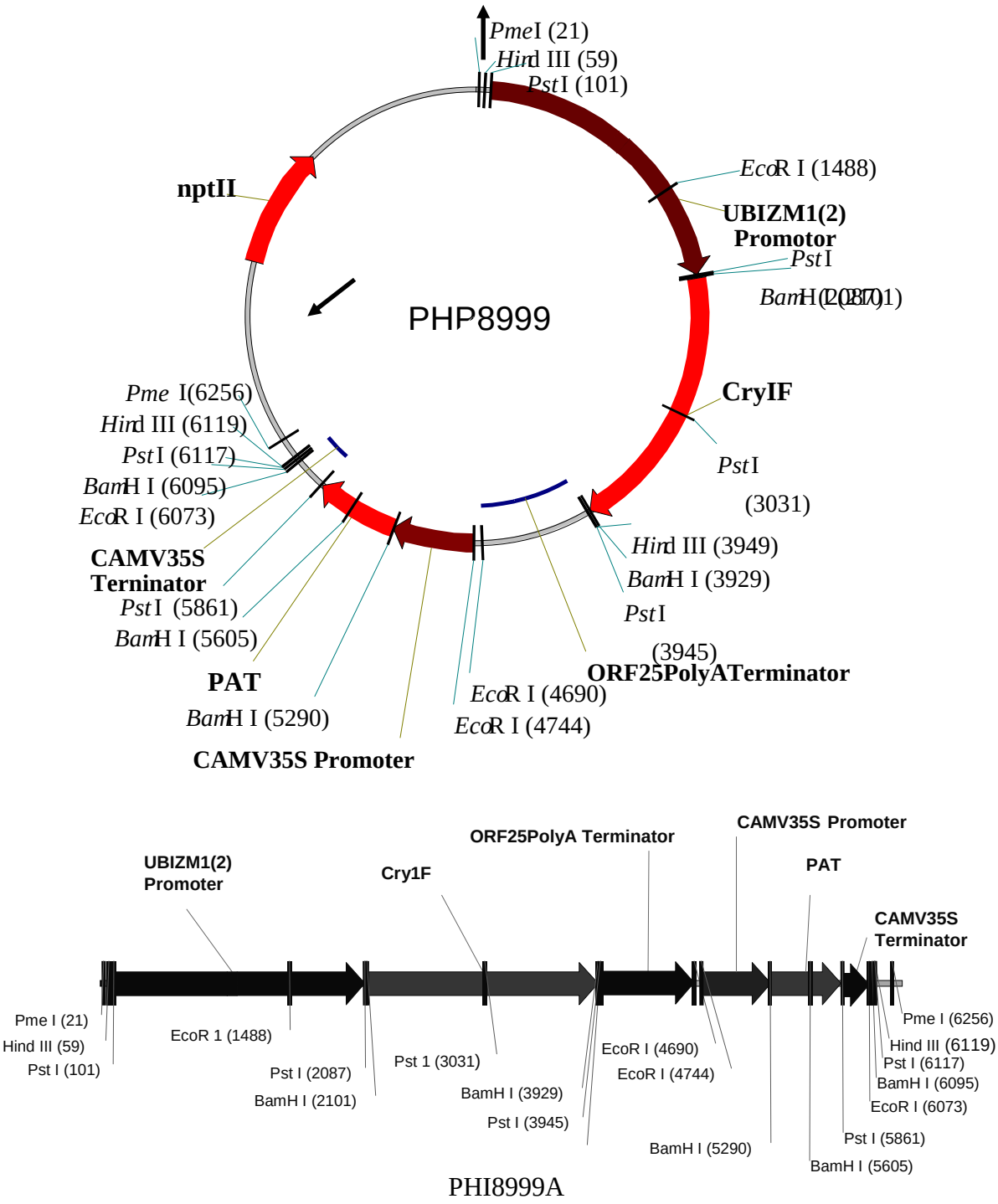

Figure 2 Compositions of plasmid PHP8999 (upper diagram) and inserted DNA region PHI8999A (lower diagram)

Plasmid PHP8999 was treated with the restriction enzyme *Pme* I (broken at the two points indicated by an arrow in the upper diagram) and the resulted linear DNA fragment PHI8999A (lower diagram) was used for introduction of genes into recipient organism.

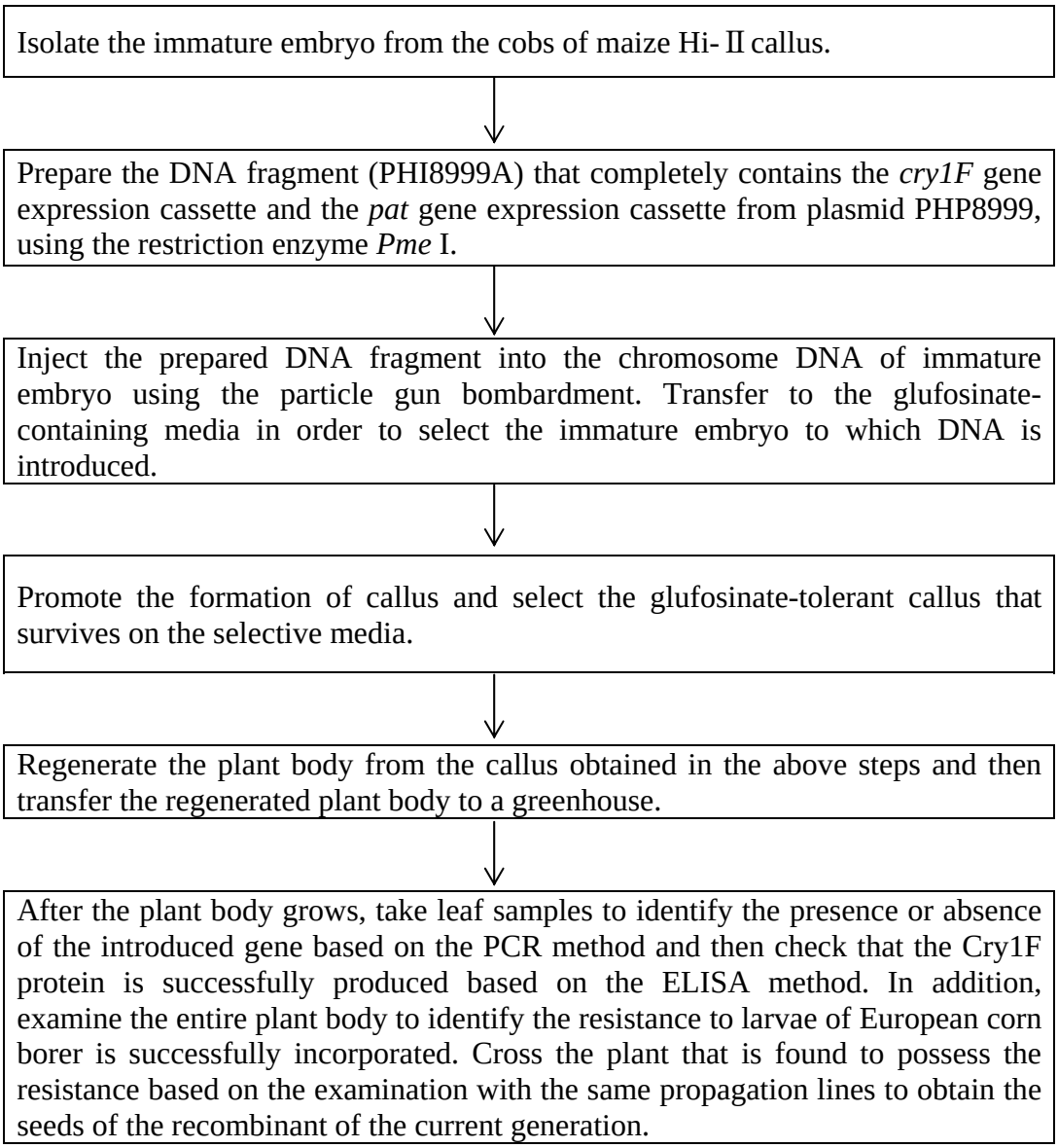

Figure 3 Procedures for introducing the nucleic acid into recipient organism

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

(1) Location of the copy of transferred nucleic acid

Based on the test on the DNA sample extracted from the leaves of T1S1 generation and BC4F1 generation of Cry1F line 1507 by the genome DNA extraction kit, it was confirmed that the copy of transferred nucleic acid is introduced into the maize genome.

(2) The number of copies of transferred nucleic acid and stability of its inheritance through multiple generations

The genome DNA sample extracted from the leaves of Cry1F line 1507 was analyzed by Southern blotting to examine the number of copies of nucleic acid transferred into Cry1F line 1507. As a result, it was confirmed that one copy of each of *cry1F* gene expression cassette and *pat* gene expression cassette is inserted in the maize genome in the intact form.

To confirm that the introduced genes are all inherited stably in offspring, Southern blotting analysis was conducted on the DNA samples extracted from the leaves of multiple generations of Cry1F line 1507. As a result, it was confirmed that in all the generations, one copy of each of *cry1F* gene expression cassette and *pat* gene expression cassette is inserted in the maize genome in the intact form and that respective gene expression cassettes are stably inherited.

In addition, as a result of analysis on the nucleotide sequence of the introduced nucleic acid, it was confirmed that the introduced nucleic acid contains a part of *cry1F* gene sequence in the 5'-terminal region, a part of *pat* gene sequence in the 5'-terminal and 3'-terminal regions, and a part of *ORF25PolyA Terminator* sequence in the 3'-terminal region. However, Northern blotting analysis confirmed that transcription to mRNA is not processed and thus these gene fragments are not functioning.

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

This item is not applicable.

(4) The stability of the expression among individuals and generations under natural conditions with respect to the physiological or ecological characteristics that were accompanied by the expression of copies of transferred nucleic acid

It was confirmed based on the ELISA analysis that the Cry1F protein and PAT protein produced in the recombinant maize due to the expression of individual genes introduced into Cry1F line 1507 are stably produced in the progeny in multiple generations. In the analysis, leaves, the entire plant body, and samples extracted from grains of Cry1F line 1507 in multiple generations were tested.

In all the test samples subjected to analysis, Cry1F protein was detected. Based on the results, it was confirmed that the Cry1F protein produced in Cry1F line 1507 is stably

produced in the progeny in multiple generations. The production of PAT protein was generally low.

Cry1F protein confers the resistance to Lepidoptera. In the process of rearing the Cry1F line 1507, confirmation was made for the presence of resistance to Lepidoptera to select the recombinant (See Figure 3). In addition, biological examination was conducted using Lepidoptera to confirm that the progeny possesses the resistance to Lepidoptera. Based on the above understanding, in multiple generations, production of Cry1F protein provides the Cry1F line 1507 with resistance to Lepidoptera.

PAT protein confers the tolerance to glufosinate herbicide. In the process of rearing Cry1F line 1507, confirmation was made for the presence of tolerance to glufosinate herbicide to select the recombinant (Figure 3). In addition, glufosinate spraying test was conducted to confirm that the progeny possesses the tolerance to glufosinate herbicide. Based on the above understanding, in multiple generations, production of PAT protein provides the recombinant maize with tolerance to glufosinate herbicide.

(5) Presence or absence, and degree of transmission of nucleic acid transferred through virus infection and/or other routes to wild animals and wild plants

Transmission of this item is absence, because the transferred nucleic acid does not contain any sequence allowing transmission.

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

For the detection and identification of Cry1F line 1507, a quantitative analysis kit from GeneScan Europe AG (Freiburg, Germany) (Cat. No.: 512 12023 10) is commercially available for RT (Real Time)-PCR whicih uses the nucleotide sequence specific to Cry1F line 1507 as primers. The detection sensitivity (allowable lower detection limit) of this quantitative analysis kit is 40 PCR-amplified copies. In addition, the Cry1F protein detection kit from Strategic Diagnostics Inc. (Newark, DE, USA) (Cat. No.: 7000018), and the PAT protein detection kit from EnviroLogix (Portland, ME, USA) (Cat. No.: AP 014) are also commercially available. The Cry1F protein detection kit can detect one Cry1F protein-containing grain per 600 maize grains. The PAT protein detection kit can detect one PAT protein-containing grain per 500 maize grains.

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

(1) Specific contents of physiological or ecological characteristics that were accompanied by the expression of copies of transferred nucleic acids

1) Resistance to Lepidoptera

With the production of Cry1F protein due to the introduction of *cry1F* gene derived from *Bacillus thuringiensis* (hereinafter referred to as "*B.t.*") var. *aizawai*, resistance to European corn borer (*Ostrinia nubilalis*) and other Lepidoptera insects that feed and damage maize is conferred to Cry1F line 1507.

2) Tolerance to glufosinate herbicide

To the Cry1F line 1507, tolerance to glufosinate herbicide is also conferred with the introduction of *pat* gene derived from *Streptomyces viridochromogenes*. The PAT protein produced by the expression of *pat* gene acetylates the glufosinate herbicide and transforms it to nontoxic acetylglufosinate, thereby conferring on the plant body the tolerance to glufosinate (Figure 1). In the actual isolated field tests, it was confirmed that the Cry1F line 1507 exhibited the glufosinate tolerance while the non-recombinant maize sprayed with glufosinate herbicide died completely.

(2) Presence or absence of difference between recombinant plant and the species to which recipient organism belongs, and the degree of difference, if any

1) Morphological and growth characteristics

In order to evaluate the characteristics of Cry1F line 1507 when it is brought along in the natural conditions in Japan, isolated filed tests were conducted in 2001 by the National Institute for Agro-Environmental Sciences (NIAES) (City of Tsukuba, Ibaraki Prefecture) for comparison with non-recombinant maize.

For the morphological and growth characteristics, evaluation was conducted regarding the germination rate, uniformity of germination, time of tassel exertion, time of silking, time of flower initiation, time of flower completion, flowering period, maturation time, plant type, tiller number, total number of ears, number of productive ears, grain color and grain shape, culm length, height of ear, ear length, ear diameter, and fresh weight of above ground part at harvesting time. For the germination rate, in one of the two hybrid varieties tested, statistically significant difference was observed between the Cry1F line 1507 and the non-recombinant maize ($p=0.033$), though the germination rate was 96.7% and 92.8% respectively, exceeding 90%, the germination rate of typical varieties of maize. In the other hybrid variety, no significant difference was observed. In addition, in the F2 hybrid seeds, no difference was observed in germination rate between the recombinant and the non-recombinant. For the ear diameter, in one variety, statistically significant difference was observed ($p=0.033$), though the difference between the averages is slight (Cry1F line 1507: 4.60 cm, the non-recombinant: 4.32 cm), and in the other hybrid, no significant difference was observed.

In all evaluation items except the germination rate and ear diameter, no difference was observed between Cry1F line 1507 and the non-recombinant maize.

2) Chilling-tolerance at the early stage of growth

The plant body near the third leaf stage was placed in a growth cabinet set at temperatures between 12 and 14°C for 12 hours under daylight illumination, and 2°C for 12 hours under dark condition to observe the growing process. About three weeks after placement, all the opened leaves lost chlorophyll totally and withered. In the withering, no difference was observed between Cry1F line 1507 and the non-recombinant maize.

3) 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 is not known that it can overwinter. In fact, it was confirmed that, in the field in the US where cultivation test of Cry1F line 1507 was conducted in the previous year, there was no plant body observed which could survive to the next year.

4) Fertility and size of the pollen

To examine the fertility and size of the pollens, pollens were sampled during the flowering period in the process of isolated field test, and stained with neutral red solution or potassium iodine solution and observed under a microscope. As a result of staining, it was confirmed that the protoplasm was stained, showing the maturity of the pollens of both Cry1F line 1507 and the non-recombinant maize. Also regarding size of pollen, no difference was observed between Cry1F line 1507 and the non-recombinant maize.

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

The row number per ear, grain number per row and 100-kernel weight were examined during the isolated field test as the characteristics referring to the production of seeds. As a result, no difference was observed between Cry1F line 1507 and the non-recombinant maize in all of the characteristics examined. In addition, for both of Cry1F line 1507 and the non-recombinant maize, the germination rate of F2 hybrid seeds was found satisfactory (94% or more for all of the varieties tested) and then it was concluded that there is no dormancy of the seeds. Also for the germination rate and shedding habit of F2 seeds, no difference was observed between Cry1F line 1507 and the non-recombinant maize.

6) Crossability

Crossability test was not performed since there are no wild relatives growing in Japan that can be crossed with recipient organism maize.

7) Productivity of harmful substances

It is not known that maize secretes any harmful substances from the roots that could have adverse effects on the surrounding plants and/or microorganisms in soil. Also it is not known that maize produces any allelochemicals after dying that could affect other plants. In the Cry1F line 1507, Cry1F protein and PAT protein are newly produced due to the introduction of *cry1F* gene and *pat* gene. It is reported that Cry1F protein does not work as enzyme in plant body similarly as other Cry proteins in *B.t.* and also that PAT protein possesses very high substrate specificity. In addition, as a result of the field experiment to examine the morphological, growth and propagation characteristics and the analysis of major constituents or trace constituents, in all of the items examined, no unexpected significant difference was observed between Cry1F line 1507 and the non-recombinant maize, and there was no implication showing a possibility that the introduction of the gene and the protein can affect the metabolic pathway of the maize of recipient organism and cause any unexpected changes.

To make sure of the above, with the intention to confirm for possible production of any new allelochemicals in the Cry1F line 1507 which are secreted from the roots and have an adverse effect on the surrounding plants, lettuce was cultivated in the residual soil used in the isolated field test for cultivation of Cry1F line 1507 and the non-recombinant maize, and the germination rate and growth were examined. As a result, for the germination rate, in all of the two hybrids examined, no statistically significant difference was observed between plots of soils planted with the recombinant and the non-recombinant. For the fresh weight of lettuce, in one variety, significant difference was observed ($p = 0.033$) (the recombinant plot: 0.63g, the non-recombinant plot: 0.43g). However, based on the facts that no significant difference was observed for the germination rate, growth of the lettuce on the recombinant plot was not necessarily slow or insufficient, and no significant difference was observed in the other variety, it was considered that the introduced gene does not cause productivity of any unexpected harmful substance. For confirmation, examination was conducted based on the Sandwich method to identify the effects of the roots of Cry1F line 1507 and the non-recombinant maize on the germination rate, length of radicle, and length of hypocotyl of lettuce. As a result, in all of the items examined, no statistically significant difference was observed between the recombinant and the non-recombinant.

Based on the results described above, it was confirmed that Cry1F line 1507 does not involve productivity of any allelochemicals in the plant body that are secreted from the roots and can affect the surrounding plants.

As a result of examination on the number of filamentous fungi, number of bacteria, and number of actinomyces in the soil used for the cultivation of Cry1F line 1507 and the non-recombinant maize, no difference was observed between Cry1F line 1507 and the non-recombinant maize. Based on this result, it was confirmed that Cry1F line 1507 does not involve productivity of any harmful substances in the plant body that are secreted from the roots and can affect the microorganisms in soil.

Possible effects of died maize on other plants were examined based on the results of isolated field tests conducted in Japan, the tests using the Sandwich method, and a total of 46 field experiments in US. In the isolated field tests, using the soil prepared by adding the residues of Cry1F line 1507 or plant body of the non-recombinant maize to the residual soil used for cultivation of Cry1F line 1507 or the non-recombinant maize, lettuce was raised to examine the germination rate and growth. As a result, for the germination rate, in both of the two varieties of hybrids tested, no statistically significant difference was observed between the recombinant and the non-recombinant. For the fresh weight of lettuce, in one variety, significant different was observed ($p = 0.033$) (the recombinant: 0.77g, the non-recombinant: 0.43g). However, based on the facts that no significant difference was observed for the germination rate, the growth of lettuce in the soil mixed with residues of recombinant was not necessarily poor, and no significant difference was observed in the other variety, it was considered that the introduction of gene does not cause productivity of any unexpected harmful substances. For confirmation, examination was conducted based on the Sandwich method to identify possible effects of leaves and stems of Cry1F line 1507 and the non-recombinant maize on the germination rate, length of radicle, and length of hypocotyls of lettuce. As a result, in all of the

items examined, no statistically significant difference was observed between the recombinant and the non-recombinant. In addition, in the 46 field experiments conducted in US, breeders visited the fields in the following year of cultivation for visual observation. As a result of the visual observation, in all of the fields used for cultivation of Cry1F line 1507, there was no apparent effect observed in the growth of succeeding crops that might be attributed to the cultivation of the recombinant maize.

Based on the above understanding, it was confirmed that Cry1F line 1507 does not involve productivity of any unexpected harmful substance in the plant body that could affect other plants after dying.

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

Regarding the maize (*Zea mays* subsp. *mays* (L.) Iltis.), to which the recipient organism belongs, cultivation has long been conducted in Japan, though there is no report that it has grown naturally in Japan.

This recombinant maize is given a trait to be resistant to insects of the order Lepidoptera and tolerant to glufosinate due to the transferred *cry1F* gene and *pat* gene respectively. However, the insect damage by Lepidoptera is not the major cause to make the maize difficult to grow in the natural environment in Japan. In addition, it is not generally considered that the glufosinate exerts pressure for selection under a natural environment. Based on these characteristics, it is not considered that the recombinant maize becomes self-seeding.

In addition, as a result of examination in the isolated fields in Japan, it was confirmed that there is no significant difference between the recombinant maize and the non-recombinant maize with regard to various traits relating to competitiveness except that a slight difference was observed in the germination rate and ear diameter between them.

Based on the above understanding, there are no wild animals and plants identified which may be subjected to the effects attributable to competitiveness and thus, 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

(a) Identification of any wild animals and plants which may be subjected to the effects attributable to the productivity of harmful substances

For the maize, the biological species to which the recipient organism belongs, there is no report that it possesses productivity of any harmful substances that could affect wild animals and wild plants.

The recombinant maize produces the phosphinothricin acetyltransferase (PAT protein) that inactivates the glufosinate, though it is reported that the protein possesses very high substrate specificity and thus has no adverse effect on the growth of plants and that it offers no toxicity to any animals.

In addition, as a result of examination on the productivity of harmful substances of the recombinant maize (the effects of the secretion from roots on other plants, the effects of the secretion from roots on the microorganisms in soil, and the effects of the possession in the plant body on other plants), no significant difference from the non-recombinant maize was observed.

However, since the recombinant maize produces the Cry1F protein that possesses the insecticidal activity against insects of the order Lepidoptera, the insects of the order Lepidoptera living in Japan are specified as possibly affected wild animals/plants.

(b) Evaluation of the specific contents of the effects

As a result of examination on the mortality of the 1st instar larvae within 12 hours after hatching of *Zizeeria maha* subsp. *argia* Menetries, which features high sensitivity to the pollen of BT protein and ease of collection and raising for successive generations, fed with the pollens from the recombinant maize placed on a piece of leaf, it was confirmed that the mortality began increasing on the 3rd day from the start of pollen feeding at a pollen density of 100 particles/cm^2 and it exceeded 50% on the 5th day.

(c) Evaluation of susceptibility to the effects

Possible route of exposure of Cry1F protein to larvae of insects of the order Lepidoptera other than insect pests for farming includes ingestion of pollens dispersed from the recombinant maize under cultivation and/or from unintentionally established plants started from seeds spilled during transportation, together with feed plants.

As a result of experiments on the density of maize pollens deposited on the surface of leaf of sunflower (*Helianthus annuus*) in the vicinity of cornfields, the pollen density of maize deposit was 81.7 particles/cm^2 within the field and 33.5 particles/cm^2 at a point 2m distant from the filed. Since it is confirmed that there is no difference in the characteristics of pollen dispersion between the recombinant maize and the non-recombinant maize, similar deposit of pollens is expected around the cultivation fields of the recombinant maize.

This suggests that any individuals of insects of the order Lepidoptera, which has similar level of high sensitivity to Cry1F protein as *Zizeeria maha* subsp. *argia* Menetries, may be possibly affected if they stay in the area within 2m from the field for 3 days or more. However, it is considered that there exists no such insect of the order Lepidoptera which answers the above requirements at the level of species or individual population.

In the case if the seeds of the recombinant maize drop out during transportation and grow, the number of the individuals is very small compared to the case of cultivation and the density of pollen deposit in the surroundings is also estimated very small and thus, it is considered that there exists no insect of the order Lepidoptera to be possibly affected similarly as in the case of cultivation.

The above conclusion may not be affected even if the distance from the recombinant maize field to the presumable site where the death of insects of the order Lepidoptera is concerned is extended to some extent, due to possible dispersion of increased amount of pollens of the recombinant maize depending on the varied pollen productivity of different lines.

Based on the above understanding, it is considered that the Cry1F protein produced by the recombinant maize does not disturb the maintenance of species or individual population of insects of the order Lepidoptera living in Japan and thus, 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 species that can be crossed with maize in natural environment has not been reported.

Based on the above understanding, no wild species can be specified as having some effects, 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 Cry1F line 1507 in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion above made by the applicant is valid.