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 Coleoptera and tolerant to glufosinate herbicide（*cry34Ab1*, *cry35Ab1*, *pat*, *Zea mays* subsp. *mays* (L.) Iltis）（*B.t.* Cry34/35Ab1 Event DAS-59122-7, OECD UI：DAS-59122-7）
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

1. Information concerning donor nucleic acid

(1) Composition and origins of component elements

Composition of donor nucleic acid and 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
cry34Ab1 gene expression cassette		
UBI1ZM PRO	1.98	Ubiquitin constitutive promoter[1)] derived from *Zea mays* (including intron and 5' untranslated region).
cry34Ab1	0.37	A gene that encodes Cry34Ab1 protein derived from *Bacillus thuringiensis* PS149B1.
PIN Ⅱ TERM	0.32	Protease inhibitor II terminator to terminate transcription derived from *Solanum tuberosum*
cry35Ab1 gene expression cassette		
TA Peroxidase PRO	1.30	Peroxidase promoter (base sequence 45-1342 of GenBank X53675) derived from *Triticum aestivum* known to express in roots.
cry35Ab1	1.15	A gene that encodes Cry35Ab1 protein derived from *Bacillus thuringiensis* PS149B1.
PIN Ⅱ TERM	0.32	Protease inhibitor II terminator to terminate transcription derived from *Solanum tuberosum*
Pat gene expression cassette		
35S PRO	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*.
35S TERM	0.21	35S terminator to terminate transcription derived 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. Cry34Ab1 protein and Cry35Ab1 protein

Cry34Ab1 protein and Cry35Ab1 protein are a kind of insecticidal crystal protein (*B.t.* protein) known as δ-endotoxin produced by *Bacillus thuringiensis* (hereafter referred to as "*B.t.*"), a gram-posivitive bacterium, universally exists in soil. The *B.t.* proteins exhibit the insecticidal activity only when ingested by insects. In addition, they are classified according to the insecticidal activity, and Cry34Ab1 protein and Cry35Ab1 protein were both discovered newly from *B.t.* PS149B1 strain based on the activity against corn rootworm (*Diabrotica spp.*).

The corn rootworm is one of the most important insect pests to be controlled for maize cultivation in the US. It damages the roots of maize in the larval stage and the silks of maize in the imago stage. Approximately 9.5 million hectare cornfield in the US has been damaged by the corn rootworm, and the sum of costs for extermination and the damages reportedly amounts to about a billion dollar per year.

For extermination of corn rootworm, rotation of maize with soybean and spraying of insecticide have been conventionally considered effective. However, recently, the differentiated type of corn rootworm that could survive even in the soybean fields is found to develop and it is likely to rapidly propagate in the major maize cultivation areas, and the rotation is losing the effectiveness as the means for extermination. In addition, development of the corn rootworm that possesses the resistance to some insecticides has been reported.

Cultivation of this recombinant maize allows effective control of corn rootworm and then it is expected to provide farmers with an additional option for control of the corn rootworm other than the rotation of maize with soybean and spraying of insecticides.

In the tests to investigate the functions of Cry34Ab1 protein and Cry35Ab1 protein, it was suggested that the Cry34Ab1 protein acts as the pore-forming protein against the phospholipid membrane, and the Cry35Ab1 protein enlarges the pores and increases the permeability through the membrane. In the *in vivo* test, it was found that the Cry34Ab1 protein possesses the activity against corn rootworm even by itself, though it exhibits synergistic effects in the presence of the Cry35Ab1 protein. As a result, it was confirmed that coexistence of the both proteins offers a maximum

of about 8-times higher activity compared to single use of the Cry34Ab1 protein. The Cry35Ab1 protein does not possess any activity against corn rootworm by itself.

As a result of immunohistochemical examinations for any morphological change in the midgut tissues of larvae of corn rootworm which were fed with the recombinant maize in which Cry34Ab1 protein and Cry35Ab1 protein are produced, the larvae fed with the non-recombinant exhibited no abnormality, though the larvae fed with the recombinant presented the phenomena including the cell death such as swollen and vacuolated midgut cells and bubble forming and lysis of cell membranes. This result suggests that the Cry34Ab1 protein and the Cry35Ab1 are targeted at the midgut similarly as the other *B.t.* proteins.

It is generally known that *B.t.* proteins have very highly specific insecticidal spectrum. In fact, as a result of the tests on the insecticidal spectrum of Cry34Ab1 protein and Cry35Ab1 protein against the six (6) different kinds of insect pests concerning the maize cultivation in the US fed with a mixture of the both proteins, the proteins were found to offer the insecticidal activity against limited insects. As Table 2 indicates, among the six (6) kinds of insect pests tested, especially against the larvae of two (2) kinds of insect pests, northern corn rootworm (*Diabrotica barberi*) and western corn rootworm (*Diabrotica virgifera virgifera*) classified as insects of the order Coleoptera, the insecticidal activity worked better (LC_{50} was 5.56 and 44.5 μ g ai/cm^2 respectively). The value of LC_{50} for the southern corn rootworm (*Diabrotica undecimpunctata howardi*) classified as the same family of corn rootworm was 343 μ g ai/cm^2. For the European corn borer, corn earworm, and black cutworm classified as insects of the order Lepidoptera, and the imago of western corn rootworm classified as the insects of the order Coleoptera, no dead individual was observed even at 400 μ g ai/cm^2, the maximum dose applied in the tests.

Table 2 Insecticide activity of a mixture of Cry34Ab1 protein and Cry35Ab1 protein against the insect pests in the maize cultivation

Insect pests in the maize cultivation		LC_{50} (value)[1)]
		(μg ai/cm^2)[2)]
Larvae of northern corn rootworm	[3)]	5.56 (1.76 – 19.6)
Larvae of western corn rootworm	[3)]	44.5 (18.5 – 165)
Larvae of southern corn rootworm	[3)]	343 (190 – 796)
Larvae of European corn borer	[4)]	>400
Larvae of corn earworm	[4)]	>400
Larvae of black cutworm	[4)]	>400
Imagoes of western corn rootworm	[3)]	>400

1) Based on the mortality 3 to 6 days after administration of a mixture of Cr634Ab1 protein and Cry35Ab1 protein (Cry34Ab1 protein: Cry35Ab1 protein = 1:3)

2) Equivalent effective ingredient per 1 cm^2

3) Insects of the order Coleoptera

4) Insects of the order Lepidoptera

To investigate the possible effects on the non-target insects of the order Coleoptera other than corn rootworm, a bioassay to test the two (2) kinds of ladybugs (*Hippodamia convergens* and *Coleomegilla maculata*) was carried out. Of the two (2) kinds of ladybugs tested, *Hippodamia convergens* is the related species of *Hippodamia tredecimpunctata timberlakei* Capra which has ever inhabited in Japan. The *Coleomegilla maculata* is a beneficial insect inhabiting widely in the North America and it is known to feed the pollens of maize, though it is not known that the related species have ever inhabited in Japan.

As a result of the bioassay, even at the maximum dose applied in the assay (160 μg/mL for Cry34Ab1 protein and 120 μg/mL for Cry35Ab1 protein, both in the sugared water), no effect was observed on the imago of *Hippodamia convergens*. For the larvae of *Coleomegilla maculata*, a decrease in the fresh weight was observed, though, even at the maximum dose applied in the assay (900 μg/g for Cry34Ab1 protein and 2 μg/g for Cry35Ab1 protein, both in the artificial feeds), no dead individual was observed.

In addition to the insects of the order Coleoptera, tests were also conducted to the mammals, birds, fishes, Lepidoptera, Hymenoptera, Neuroptera, Hemiptera and other insects. As a result, it was confirmed that Cry34Ab1 protein and Cry35Ab1 protein exhibit no toxicity against all of the non-target organisms tested.

To confirm that the proteins do not share homologous sequences with any of the

known allergen proteins, a custom database was constructed based on the sequence information registered in open databases, and possible sequence homology with a total of 2,228 sequences related to the allergen and gluten-induced enteropathy contained in the constructed database was checked. Cry34Ab1 protein and Cry35Ab1 protein were both found not to share homologous sequences 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 the 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 acide sequence homology with any of the known allergen proteins.

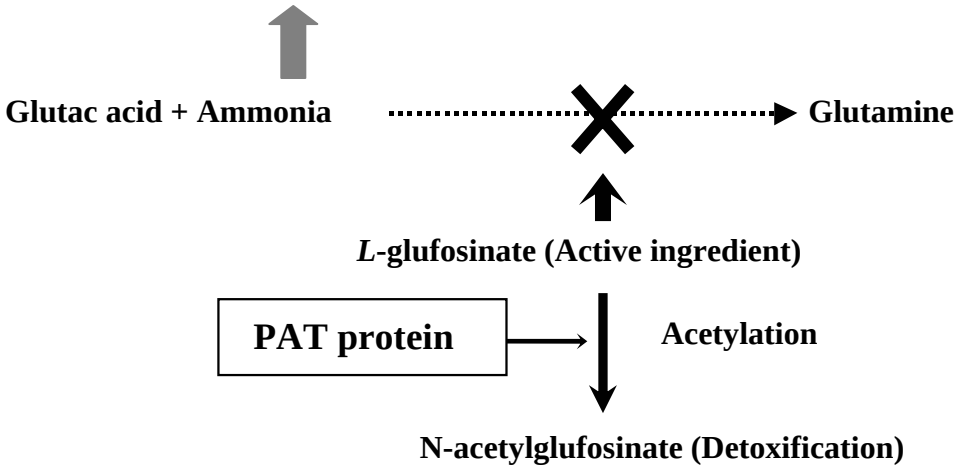

Figure 1 Action mechanism of PAT protein

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

Cry34Ab1 protein, Cry35Ab1 protein, and PAT protein are all found independent from the metabolic system of recipient organism.

2. Information concerning vector

Name and origin

The name and origin of vector used to generate plasmid PHP17662 to which individual gene expression cassette was introduced are as follows.

Name: pSB1
Origin: *Agrobacterium tumefaciens* LBA4404 strain

(2) Properties

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

The total number of base pairs of plasmid PHP17662 is 50,321 bp.

2) Types of any nucleotide sequence having specific functions

Vector backbone of plasmid PHP17662 contains antibiotic resistant markers (*tet* gene and *spc* gene), other than the region to which gene is inserted, to select the microorganisms that contain the transformed plasmid for propagation. The *tet* gene confers the resistance to tetracycline antibiotics, and the *spc* gene confers the resistance to spectinomycin antibiotics. These antibiotic resistant genes are not introduced to the recipient organism.

3) Presence or absence of infectivity of vector

The T-DNA region of pSB1 vector used to generate plasmid PHP17662 has been replaced with three (3) gene expression cassettes shown in Table 1. Therefore, plasmid PHP17662 does not possess any sequence that makes the vector infectious with *Agrobacterium*.

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 Figure 2.

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

Introduction of nucleic acid into recipient organism was based on the *Agrobacterium* method.

(3) Processes of rearing of living modified organisms

Maize resistant to Coleoptera and tolerant to glufosinate herbicide (*cry34Ab1*, *cry35Ab1*, *pat*, *Zea mays* subsp. *mays* (L.) Iltis) (*B.t.* Cry34/35Ab1 Event DAS-59122-7, OECD UI: DAS-59122-7) (hereafter referred to as "Event DAS-59122-7") 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*

Agrobacterium was removed by addition of Carbenicillin, one of the β-lactam antibiotics. The details are shown in Figure 3.

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

Event DAS-59122-7 and the excellent strain of maize inbred which is classified as dent type were crossed and selection was performed.

In Japan, in April 2004, application for approval of the safety as food was submitted to the Ministry of Health, Labour and Welfare, and application for approval of the safety as feed was submitted to the Ministry of Agriculture, Forestry and Fisheries.

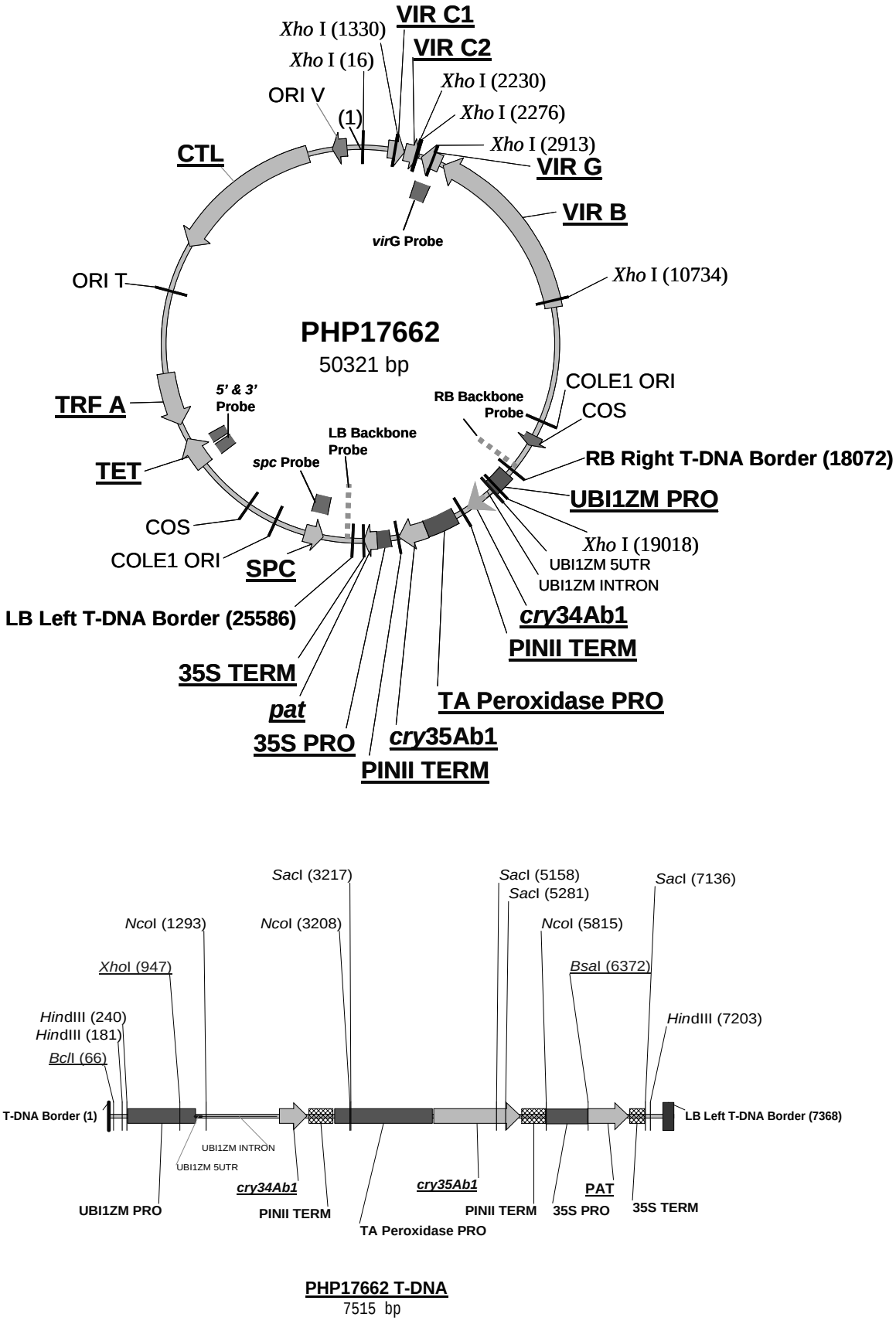

Figure 2 Compositions of plasmid PHP17662 and T-DNA region

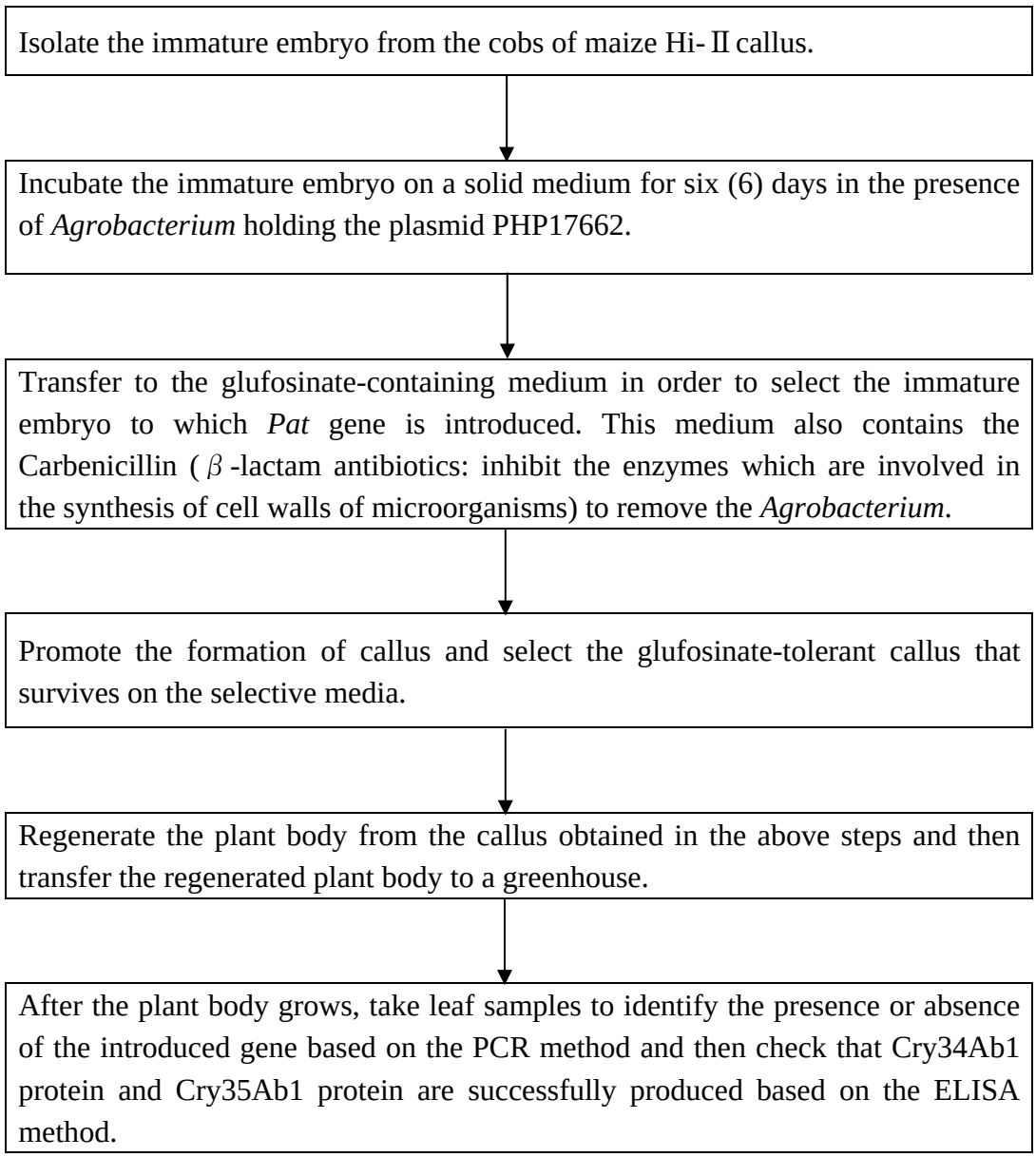

Figure 3 Procedures for introducing the plasmid PHP17662 into recipient organism

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

The transferred nucleic acid is found to exist in the maize genome.

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

The DNA samples extracted from the leaves of Event DAS-59122-7 were analyzed by Southern blotting analysis to examine the number of copies of nucleic acid transferred into Event DAS-59122-7 and their integrity. As a result, it was confirmed that one copy of each of *cry34Ab1* gene expression cassette, *cry35Ab1* 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 Event DAS-59122-7. As a result, it was found that in all the generations, one copy each of *cry34Ab1* gene expression cassette, *cry35Ab1* gene expression cassette, and *pat* gene expression cassette are inserted in the maize genome in the intact form and then it was confirmed that respective genes are stably inherited.

(3) 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 Cry34Ab1 protein, Cry35Ab1 protein, and PAT protein produced in the recombinant maize due to the expression of individual genes introduced into Event DAS-59122-7 are stably produced in the progeny in multiple generations. In the analysis, the samples extracted from the plant body, grains, pollens, leaves, stems, roots and other parts of Event DAS-59122-7 in multiple generations were tested.

In all the test samples subjected to analysis, Cry34Ab1 protein and Cry35Ab1 protein were detected. PAT protein was generally low in the yield and it was not detected in some samples from pollens and grains, though no significant difference was observed in the yield among the individual generations tested. Consequently, it was confirmed that the Cry34Ab1 protein, Cry35Ab1 protein, and PAT protein produced in this recombinant maize are stably produced also in the progeny in multiple generations.

Bioassay was conducted to confirm the characteristics conferred by the individual genes produced in Event DAS-59122-7. Cry34Ab1 protein and Cry35Ab1 protein confer the resistance to corn rootworm, one of the insect pests that are targeted for urgent extermination in the maize cultivation in the US. As a result of bioassay conducted in the US to test the seeds of three generations using the western corn rootworm, it was confirmed that, in all of the generations tested, the root of this recombinant maize could survive without being damaged by the corn rootworm and possesses a proper resistance to this pest insect.

On the other hand, PAT protein confers the tolerance to glufosinate herbicide. In the process of rearing Event DAS-59122-7, confirmation was made for tolerance to glufosinate herbicide to select the recombinant (Figure 3). In addition, it was also confirmed based on the glufosinate-spraying test that the progeny also possesses the tolerance to glufosinate herbicide. Based on the above understanding, the tolerance is inherited in multiple generations.

(4) Presence or absence, and if present, 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 Event DAS-59122-7, quantitative ELISA analysis kits using the polyclonal antibodies respectively for Cry34Ab1 protein, Cry35Ab1 protein, and PAT protein have been developed. The sensitivity of detecting Cry34Ab1 protein and Cry35Ab1 protein in the grain of this recombinant maize is 0.072 ng/mg tissue dry weight and 0.06 ng/mg tissue dry weight respectively. In addition, the sensitivity of detecting PAT protein is 0.025 ng/mg tissue dry weight. The method for detection and identification based on the quantitative PCR analysis is under development, and it is expected available before the commercialization of Event DAS-59122-7.

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 corn rootworm

It was confirmed that Event DAS-59122-7 is given the resistance to corn rootworm with the production of Cry34Ab1 protein and Cry35Ab1 protein due to the introduction of *cry34Ab1* gene and *cry35Ab1* gene derived from *B.t.* PS149B1 strain (Photo 1).

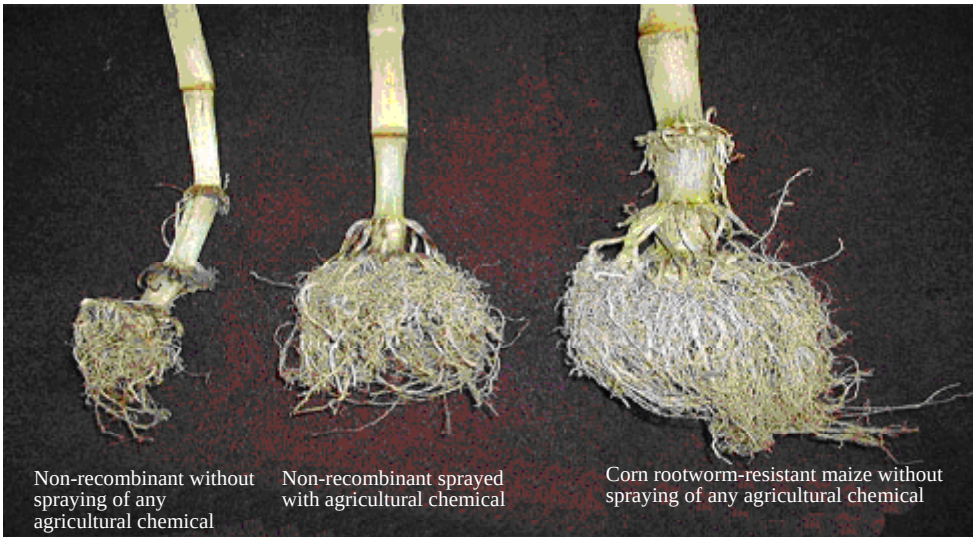

Photo 1 Maize resistant to corn rootworm

Left: [Non-recombinant without spraying of any agricultural chemical] Non-recombinant maize raised without spraying of any agricultural chemical, showing the damage by the corn rootworm.

Center: [Non-recombinant sprayed with agricultural chemical] Non-recombinant maize sprayed with Terbufos (organophosphorus insecticide) to prevent and exterminate the corn rootworm. Damage is not so serious as observed in the fields without spraying of any agricultural chemical, though damage by the corn rootworm could not be completely eliminated.

Right: [Corn rootworm-resistant maize without spraying of any agricultural chemical] Resistant recombinant maize without spraying of any agricultural chemical, showing freedom from any damage by the corn rootworm.

2) Tolerance to glufosinate herbicide

To the Event DAS-59122-7, 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 Event DAS-59122-7 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 Event DAS-59122-7 when it is brought along in the natural conditions in Japan, isolated filed tests were conducted in 2003 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 uniformity of germination, germination rate, time of tassel exsertion, time of silking, maturation time, plant type, tiller number, number of productive ears, grain color and grain shape, culm length, height of ear, fresh weight of above ground part, ear length, ear diameter, shape of flower, time of flower initiation, time of flower completion and flowering period. For the culm length, although statistically significant difference was observed between Event DAS-59122-7 and the non-recombinant ($p=0.04$) in one of the two varieties of Event DAS-59122-7 tested, the difference between the averages is slight (Event DAS-59122-7: 192.0 cm, non-recombinant: 212.3 cm), and in the other variety, no significant difference was observed. In addition, in all evaluation items except the culm length, no difference was observed between Event DAS-59122-7 and the non-recombinant maize.

2) Cold-tolerance at the early stage of growth

In the isolated field test, the plant bodies in the fourth leaf stage having a plant height of 16 – 19 cm were left out in the open in the winter season at a minimum temperature below 10°C to observe the growing condition. When the minimum temperature dropped down to 1.5°C, all the plant bodies tested died, and no difference was observed in the sensitivity to low temperatures between Event DAS-59122-7 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 Event DAS-59122-7 was conducted in the previous year, there was no plant body observed in the following year, which could survive the winter.

4) Fertility and size of the pollen

In the isolated field test, pollens were sampled during the flowering period to evaluate the shape and size, fertility and yield of pollens. In all evaluation items, no difference was observed between Event DAS-59122-7 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 Event DAS-59122-7 and the non-recombinant maize in all of the characteristics examined. In addition, for both Event DAS-59122-7 and the non-recombinant maize, no dormancy of the seeds was observed. Also in the germination rate and shedding habit of second generation hybrid (F2) seeds, no difference was observed between Event DAS-59122-7 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 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 Event DAS-59122-7, Cry34Ab1 protein and Cry35Ab1 protein are newly produced, though there is no report that the proteins work as enzyme in plant body similarly as other Cry proteins in *B.t.* In Event DAS-59122-7, PAT protein is also produced, though there is no report that the protein has an adverse effect on the growth of plants. In addition, the PAT protein reportedly possesses high substrate specificity. Consequently, it was considered unlikely that these proteins could newly produce any unexpected harmful substance which affects the metabolic pathway of the maize of recipient organism. In fact, as a result of isolated field test to examine the morphological, growth and propagation characteristics and the analysis of major and trace constituents, in all of the items examined, no unexpected significant difference was observed between Event DAS-59122-7 and the non-recombinant maize, and there was no implication showing a possibility that the introduction of the genes and the expressed proteins 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 check for production of any new allelochemicals in Event DAS-59122-7 which are secreted from the roots and have an adverse effect on the surrounding plants, analysis was made on the weeds growing in the areas for isolated field test for composition of species, total number of individuals, and dry weight. As a result, in all of the items analyzed, no difference was observed between Event DAS-59122-7 and the non-recombinant maize.

To confirm that there is no new allelochemicals produced in Event DAS-59122-7 which are secreted from the roots and have an adverse effect on microorganisms in soil, Event DAS-59122-7 and the non-recombinant maize were cultivated in a screen house. Crops were harvested before ear emergence, and then the soil used for the cultivation was examined for the number of bacteria, actinomyces, and filamentous fungi. For all of the microbes examined, no statistically significant difference was observed between DAS-59122-7 and the non-recombinant maize. In addition, as a result of the similar test conducted in the State of Hawaii in the US, for all of the number of bacteria, actinomyces, and filamentous fungi, no statistically significant difference was observed between DAS-59122-7 and the non-recombinant maize. In relation to the examination items, during the field experiment in the US, soil was sampled from the test field to identify the organisms inhabiting in the soil. Microorganisms in soil degrade the residues of plants and animals in the soil and contribute to provide the nutrition to the insects and other invertebrates inhabiting in the soil. This suggests that the organisms inhabiting in soil can be directly influenced by any change in the biota of soil microorganisms. As a result of the examination, Collembola, Staphylinid, Carabid, Milliped, Arachnid, Centipede, Cryptostigmata and other insects were discovered. As a result of statistical analysis based on ANOVA (analysis of variance), no difference was observed between the soils taken from the fields for Event DAS-59122-7 and the non-recombinant maize in the composition of species of organisms inhabiting in the soils.

To confirm that Event DAS-59122-7 does not involve production of any substances in the plant body that can affect the growth of other plants after dying, radish was cultivated in the soil to which the residues of Event DAS-59122-7 or the non-recombinant maize raised in a screen house were plowed back. As a result, regarding all of the items examined, germination rate, plant height, fresh weight, and dry weight, no statistically significant difference was observed between Event DAS-59122-7 and the non-recombinant maize. In addition, for the Event DAS-59122-7, a lot of field experiments were conducted in the US, and as a result of visual observation by researchers on the fields in the year following the cultivation, there was no apparent effect of the cultivation of this recombinant maize on the succeeding crops observed in any of the fields where Event DAS-59122-7 or the non-recombinant maize were cultivated.

The half-life of activity of Cry34Ab1 protein and Cry35Ab1 protein in soil was investigated based on the activity against southern corn rootworm. A mixture of these proteins was added to the field soil (fine soil containing montmorillonite) in the maize cultivation district in the Midwestern part of the US at the initial concentration of 5 mg in terms of active ingredient per 1g of soil for the both proteins, and the soil suspension was mixed with the feeds and given to the southern corn rootworm. As a result, the half-life of activity of the proteins was found to be 3.2 days. This result implies that the remaining amount of these proteins in soil would decrease to 1/1000 of the initial concentration in a month, and 1/10,000 of the initial concentration in two months if these proteins were released in soil.

Based on the results described above, it was confirmed that, in the productivity of harmful substance, there is no significant difference between Event DAS-59122-7 and the non-recombinant maize.

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 Coleoptera and tolerant to glufosinate due to the transferred *cry34Ab1* gene, *cry35Ab1* gene and *pat* gene respectively. However, the insect damage by Coleoptera 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 field in Japan, it was confirmed that there is no significant difference with regard to various traits relating to competitiveness except that a slight difference was observed in the culm length in one of the two varieties of Event DAS-59122-7 tested, compared to the non-recombinant maize.

Based on the above understanding, there is no wild animals and plants identified which may be subjected to the effects attributable to competitiveness and thus, the conclusion 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 Cry34Ab1 protein and Cry35Ab1 protein that possesses the insecticidal activity against insects of the order Coleoptera, the insects of the order Coleoptera living in Japan are specified as possibly affected wild animals/plants.

(b) Evaluation of the specific contents of the effects

The insecticidal activity against the three (3) kinds of corn rootworms (northern corn rootworm (*Diabrotica barberi*), southern corn rootworm (*Diabrotica undecimpunctata howardi*) and western corn rootworm (*Diabrotica virgifera virgifera*)), which are the major pest insects of the order Coleoptera for the maize cultivation in the US, was investigated. As a result of the examination on the mortality rate three (3) to six (6) days after giving the artificial feeds containing Cry34Ab1 protein and Cry35Ab1 protein, it was confirmed that the northern corn rootworm exhibited the highest sensitivity, and the mortality rate exceeded 50% on the 4th day on which accumulated pollen protein reached 5.56 μ g/cm^2.

(c) Evaluation of susceptibility to the effects

Possible route of exposure of Cry34Ab1 protein and Cry35Ab1 protein to larvae of insects of the order Coleoptera other than insect pests for farming includes ingestion of pollens dispersed from this recombinant maize under cultivation and/or during transportation together with feed plants, and ingestion of humus of recombinant maize plowed back in the soil.

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 density of pollen deposit was 81.7 particles/cm^2 within the field, 136.5 particles/cm^2 at a point 1m distant from the filed, and 33.5 particles/cm^2 at a point 2m distant from the field. At the point 1m distant from the field where the density of maize pollen deposit was found highest, the concentration of protein converted from the pollen deposit is estimated 0.006851 μ g/cm^2. Since it is confirmed that there is no difference in the characteristics of pollen dispersion between this recombinant maize and the non-recombinant maize, similar deposit of pollens is expected around the cultivation fields of this recombinant maize.

However, it is considered unlikely that any individuals of insects of the order Coleoptera, which has similar level of high sensitivity to Cry34Ab1 protein and Cry35Ab1 protein as the species described above, could be affected by such level of pollen protein even if they stay in the area within 1m from the field for 4 days or more. Thus, it is considered that there exists no such insect of the order Coleoptera which answers the above requirements at the level of species or individual population.

Should the seeds of this 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 Coleoptera to be possibly affected similarly as in the case of cultivation.

The above conclusion may not be affected even if the distance is increased from the field where the death of insects of the order Coleoptera is foreseen due to possibly varying amounts of pollen dispersion of this recombinant maize depending on the characteristics of different lines.

In addition, it is considered that, in view of food habit, habitat, behavioral characteristic, distribution range, and other characteristics, insects of the order Coleoptera are less susceptible to the effects due to feeding of humus of the recombinant maize plowed back into the soils in the cultivation fields of this recombinant maize and the surroundings.

(d) Judgment of existence of Adverse Effect on Biological Diversity

Based on the above understanding, it is considered that the Cry34Ab1 protein and Cry35Ab1 protein produced by this recombinant maize do not disturb the maintenance of species or individual population of insects of the order Coleoptera 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 Event DAS-59122-7 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.