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 and glyphosate herbicide (*cry1F*, *pat*, *cp4 epsps*, *Zea mays* subsp. *mays* (L.) Iltis) (1507×NK603, OECD UI：DAS-Ø15Ø7-1×MON-ØØ6Ø3-6)
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

The parent Cry1F line 1507 was developed jointly by the US companies Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc. The NK603 was developed by Monsanto Company in the US. The stack line 1507×NK603 is the hybrid variety raised by conventional cross-breeding between two inbred lines, derived each from conventional F1 hybrids Cry1F 1507 and NK603.
The stack line 1507×NK603 possesses the characteristics given by the *cry1F* gene to confer the resistance to the insects of the order Lepidoptera and the *pat* gene to confer the tolerance to glufosinate herbicide derived from the Cry1F line 1507, and the *cp4 epsps* gene to confer the tolerance to glyphosate herbicide derived from the NK603.

In Japan, in accordance with the “Guideline for the use of recombinant in agriculture, forestry and fisheries” (hereafter referred to as "Guideline"), the intended use of the Cry1F line 1507 in any open system was approved in June 2002. Similarly, the intended use of NK603 in any open system in Japan was also approved in May 2001 as conforming to the Guideline. In addition, for the parent lines Cry1F line 1507 and NK603, applications for approval of Type I Use under the provisions of Article 4 paragraph 2 of the “Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms” carried into effect in February 2004 were already submitted. As a result, these lines were individually approved regarding the Type I Use at the Meeting for Review on the Biological Diversity Risk Assessment based on the judgment that the Type I Use of these lines, in case identical to that stated in the application for approval of this stack line 1507×NK603, could cause no Adverse Effect on Biological Diversity. Consequently, this Outline of the Biological Diversity Risk Assessment Report was developed based on the Biological Diversity Risk Assessment Report submitted for application dated June 11, 2004 for the part describing the Cry1F line 1507, and the published review results and documents, which had been provided by Monsanto Company, for the part describing the NK603.

1. Information concerning donor nucleic acid

(1) Composition and origins of component elements

Composition of the donor nucleic acid that was used for the development of the Cry1F line 1507 and the origins of component elements are shown in Table 1, and composition of donor nucleic that was used for the development of the NK603 and the origins of component elements are shown in Table 2.

Table 1 Composition of donor nucleic acids and the origins of component elements to be used for the development of Cry1F line 1507

Component elements	Size (kbp)	Origin and function
cry1F gene expression cassette		
UBIZM1(2) Promoter	1.98	Ubiquitin promoter derived from maize (including intron and 5' untranslated region). Makes target genes expressed in all the tissues constantly.
cry1F	1.82	A gene that encodes Cry1F protein derived from *Bacillus thuringiensis* var. *aizawai*.
ORF25PolyA Terminator	0.72	A terminator to terminate transcription from *Agrobacterium tumefaciens* pTi5955. Terminates transcription of mRNA and induces polyadenylation.
pat gene expression cassette		
CAMV35S Promoter	0.53	35S promoter region derived from cauliflower mosaic virus (CaMV). Makes target genes expressed in all the tissues constantly.
pat	0.55	A gene that encodes phosphinothricin acetyltransferase (PAT protein), derived from *Streptomyces viridochromogenes*.
CAMV35S Terminator	0.21	35S terminator region derived from cauliflower mosaic virus (CaMV). Terminates transcription of mRNA and induces polyadenylation.

Table 2 Composition of donor nucleic acids and the origins of component elements to be used for the development of NK603

Component elements	Size (kbp)	Origin and function
cp4 epsps gene expression cassette (1)		
P-ract 1	0.9	Promoter region of actin 1 gene derived from rice. It makes target genes expressed.
ract 1 intron	0.5	Intron of rice actin gene. It makes target genes expressed by enhancing splicing.
CTP 2	0.2	N-terminal chloroplast transit peptide sequence derived from the *Arabidopsis epsps* gene. Transfers target proteins from cytoplasm to chloroplast.
cp4 epsps	1.4	5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* CP4.
NOS 3'	0.3	3' untranslated region of nopaline synthase (NOS) gene from T-DNA of *Agrobacterium tumefaciens*. It terminates transcription of mRNA and induces polyadenylation.
cp4 epsps gene cassette (2)		
E35S	0.6	Contains 35S promoter and duplicated enhancer region from cauliflower mosaic virus (CaMV). Makes target genes expressed in all the tissues constantly.
ZmHsp70 intron	0.8	Intron of heat shock protein gene from maize. ZmHsp70 intron is used to enhance the expression of foreign genes in plants.
CTP 2	0.22	N-terminal chloroplast transit peptide sequence derived from the *Arabidopsis epsps* gene. Transfers target proteins from cytoplasm to chloroplast.
cp4 epsps	1.36	5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* CP4.
NOS 3'	0.26	3' untranslated region of nopaline synthase (NOS) gene from T-DNA of *Agrobacterium tumefaciens*. It terminates transcription of mRNA and induces polyadenylation.

(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 individual component elements of donor nucleic acid, including target gene, expression regulation region, localization signal, and selective marker, for Cry1F line 1507 and NK603 are shown in Table 1 and Table 2 respectively.

2) Functions of proteins produced by the expression of target genes 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. On the other hand, it was confirmed that the Cry1F protein exhibits no toxicity against all non-target organisms but the insects of order Lepidoptera. The Cry1F protein did not share amino acid sequence homologous with any of the known allergen proteins examined.

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 due to the effect of *L*-glufosinate, an active ingredient of glufosinate herbicide, which causes the ammonia to be accumulated in the plant body and resultantly the plant to die. The PAT protein acetylates the glufosinate herbicide to transform it to nontoxic acetylglufosinate, thereby conferring the glufosinate tolerance on plant body. When the *L*-glufosinate is acetylated and becomes N-acetylglufosinate by work of the PAT protein, the inhibition of glutamine synthase does not occur. As a result, 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, and the PAT protein did not share amino acid sequence homologous with any of the known allergen proteins.

c. CP4 EPSPS protein

The glyphosate herbicide inhibits the activity of 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS), one of the enzymes in the shikimate pathway for aromatic amino acid biosynthesis. As a result, plants treated with glyphosate cannot synthesize aromatic amino acid essential for growth and ultimately die. The CP4 EPSPS protein produced by the *cp4 epsps* gene is not inhibited even in the presence of glyphosate and it properly works as an enzyme in the shikimate pathway, thereby conferring the glyphosate tolerance on plants. In addition, it is suggested that the EPSPS protein is the enzyme which specifically binds to the phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P), and that it is not the rate-determining enzyme in the aromatic amino acid biosynthesis pathway. It is reported that the CP4 EPSPS protein did not share amino acid sequence homologous with any of the known allergen proteins.

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

It is reported that Cry1F protein never acts as enzyme in any plant body similarly as other Cry proteins. The PAT protein exhibits very high substrate specificity against *L*-glufosinate, an active ingredient of glufosinate herbicide, and reportedly it never selects *D* -glufosinate, an optical isomer of *L*-glufosinate, for substrate. On the other hand, CP4 EPSPS protein works as enzyme in the shikimate pathway even in the presence of glyphosate, taking the place of the plant-intrinsic EPSPS, whose activity is inhibited by glyphosate herbicide, thereby conferring the glyphosate tolerance on plants. The EPSPS protein is the enzyme that specifically reacts with phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P), and reportedly it is not the rate-determining enzyme in the aromatic amino acid biosynthesis pathway. As a result, it is considered that EPSPS does not affect the metabolism of plants.

Based on the above understanding that Cry1F protein is considered not to possess any enzyme activity, and that PAT protein and CP4 EPSPS protein possess high substrate specificities and differ from each other in the action mechanism and substrate, it is considered that the introduced genes in the stack line 1507×NK603 do not affect nor interact with the metabolic system of the recipient organism.

2. Information concerning vector

(1) Name and origin

The vector used for the production of Cry1F line 1507 is plasmid PHP8999 derived from the plasmid pUC19 of *Escherichia coli* (Figure 1). The vector used for the production of NK603 is plasmid PV-ZMGT32 derived from the plasmid pUC119 of *Escherichia coli* (Figure 2).

(2) Properties

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

The number of bases in the vector used for the production of Cry1F line 1507 is 9,504bp, and the number of bases in the vector used for the production of NK603 is 9,308bp. The base sequences of the component elements of the both vectors have become clear.

2) Types of any nucleotide sequence having specific functions

For the both vectors, in the region other than the donor nucleic acid, antibiotic (*kanamycin*) resistant marker gene (*nptII* gene) is contained to pick out the microorganisms that contain transformed plasmid while the vector is growing in the microorganisms. In the introduction of gene for the production of Cry1F line 1507, plasmid PHP8999 is treated with the restriction enzyme *Pme*I to use the linear DNA fragment (PHI8999A) excluding the region other than the donor nucleic acid (including *nptII* gene). Similarly, in the introduction of gene for the production of NK603, PV-ZMGT32 is treated with the restriction enzyme *Mlu*I to use the linear DNA fragment (PV-ZMGT32L) excluding the region other than the donor nucleic acid (including *nptII* gene). Therefore, the antibiotic resistant gene is not introduced to both of the recombinant maize Cry1F line 1507 and NK603.

3) Presence or absence of infectivity of vector

The both vectors are known to offer no infectivity.

3. Method of preparing living modified organisms

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

The linear DNA fragment (PHI8999A) used for the production of Cry1F line 1507 is composed of [*UBIZM1(2) Promoter*]-[*cry1F*]-[*ORF25PolyA Terminator*]-[*CAMV35S Promoter*]-[*pat*]-[*CAMV35S Terminator*]. On the other hand, the linear DNA fragment (PV-ZMGT32L) used for the production of NK603 is composed of two *cp4 epsps* gene cassettes; ([P-ract1]-[ract1 intron]-[CTP2]-[*cp4 epsps*]-[NOS 3'] and [e35S]-[*Zmhsp70*]-[CTP2]-[*cp4 epsps*]-[NOS 3']).

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

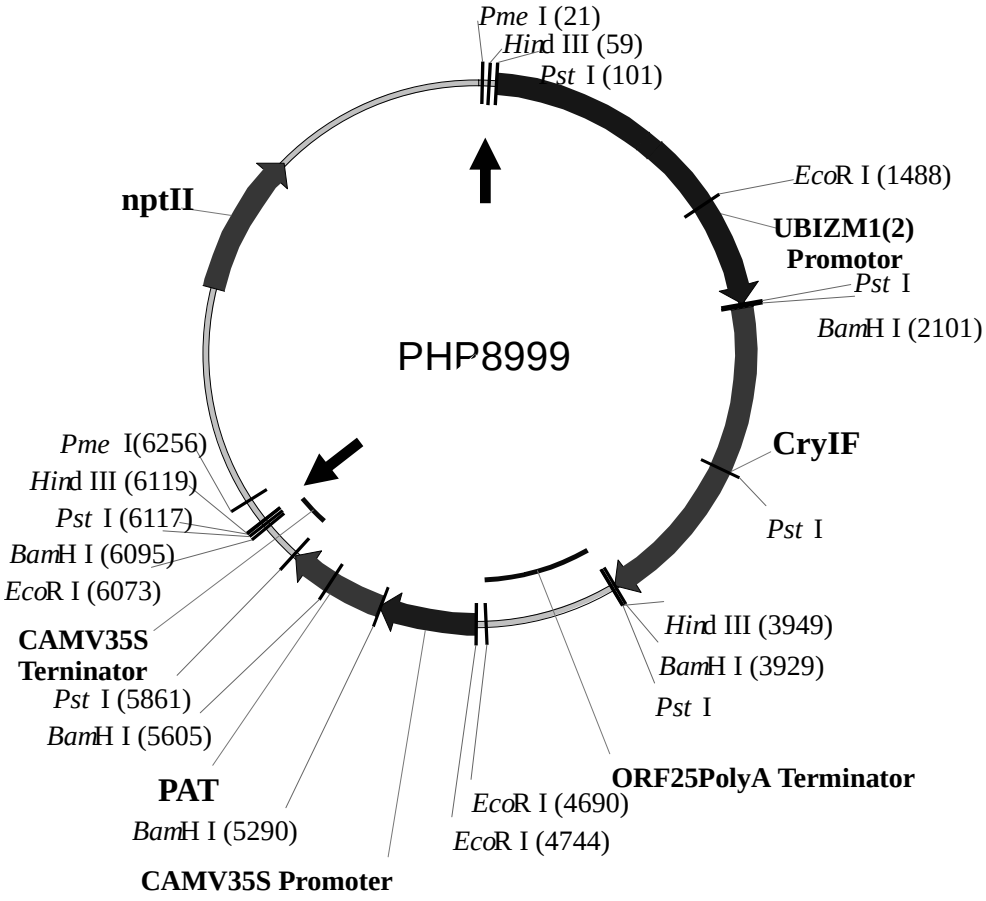
The particle gun bombardment method was used to introduce the nucleic acid into the recipient organism for both Cry1F line 1507 and NK603 as follows. The nucleic acid was introduced into the Hi-II callus of the maize variety A188×B73 for the production of the Cry1F line 1507, and into the maize variety AW×CW for the NK603.

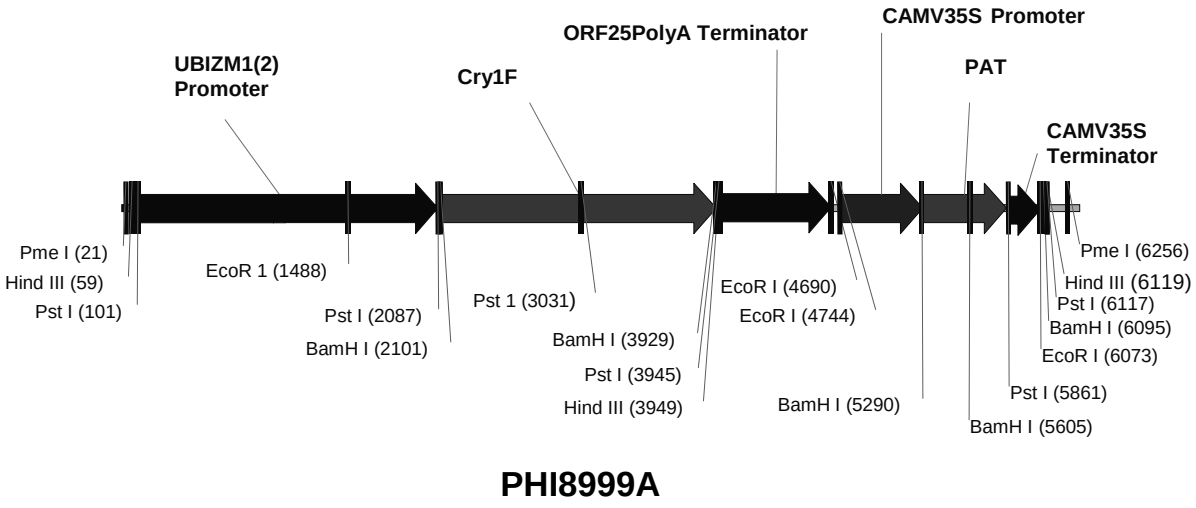
(3) Processes of rearing of living modified organisms

The stack line 1507×NK603 has been raised with the use of traditional cross-breeding method by the US companies Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc.

In Japan, in accordance with the “Guideline for the use of recombinant in agriculture, forestry and fisheries” (hereafter referred to as "Guideline"), the intended use of the Cry1F line 1507 in any open system was approved in June 2002. In addition, the safety of use for food and feed was approved in July 2002 and May 2002 respectively (the safety of use for feed was re-approved in March 2003 when the review was designated as legal obligation). On the other hand, for the NK603, in May 2001, the conformity with the Guideline regarding the intended use in the open system was approved. The safety of use for both food and feed was approved in March 2001 (the safety of use for feed was re-approved in March 2003 when the review was designated as legal obligation). Also the stack line 1507×NK603 obtained the approval for the safety of use for food and feed in March 2004 and July 2003 respectively in Japan.

For the both lines Cry1F line 1507 and NK603, application for approval of Type I Use under the provisions of Article 4 paragraph 2 of the “Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms” carried into effect in February 2004 was already submitted. As a result, these lines were individually approved regarding the Type I Use at the Meeting for Review on the Biological Diversity Risk Assessment based on the judgment that their respective Type I Use, in case identical to that in the application for approval of this stack line 1507×NK603, could cause no Adverse Effect on Biological Diversity.





PHI8999A

Figure 1 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.

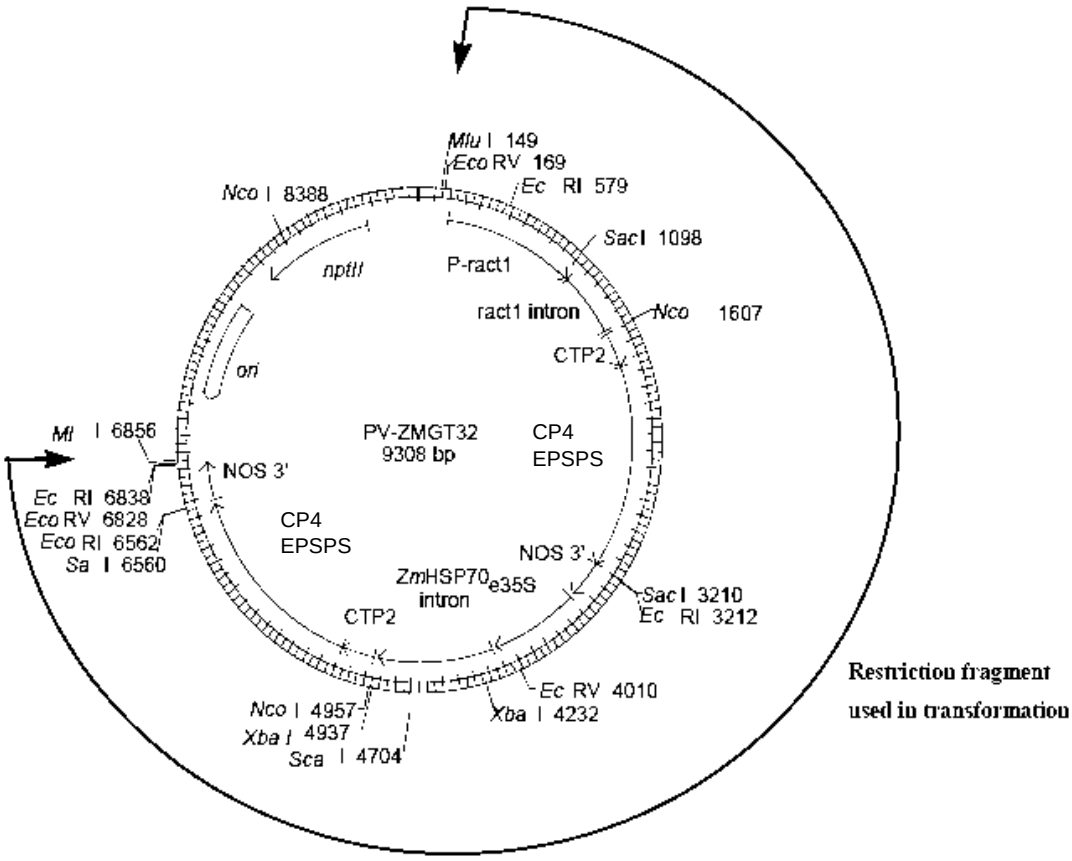


Figure 2 Composition of plasmid PV-ZMGT32

Plasmid PV-ZMGT32 was treated with the restriction enzyme *Mlu*I (broken at the two points indicated by an arrow in the diagram) and the resulted linear DNA fragment PV-ZMGT32L was used for introduction of genes into the 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

It was confirmed that the copy of transferred nucleic acid is introduced into the maize genome for both Cry1F line 1507 and NK603.

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

For Cry1F line 1507, as a result of Southern blotting analysis, it was confirmed that one copy of each of *cry1F* gene expression cassette to confer the resistance to *Ostrinia nubilalis* and other corn borers and *pat* gene expression cassette to confer the tolerance to glufosinate herbicide is inserted in the maize genome in the intact form, and that the introduced genes are all inherited stably in offspring. In addition, as a result of analysis on the base sequence of the introduced DNA, it was also confirmed that the introduced DNA contains a part of *cry1F* gene sequence in the 5'-terminal region, a part of *pat* gene sequence in the 5'- and 3'-terminal regions, and a part of *ORF25PolyA Terminator* sequence in the 3'-terminal region. However, as a result of Northern blotting analysis, it was confirmed that transcription to mRNA is not performed and thus the gene fragments are not functioning.

On the other hand, for NK603, it was confirmed that one copy of linear DNA fragment (PV-ZMGT32L, composed of two *cp4 epsps* gene expression cassettes) to confer the tolerance to glyphosate herbicide is inserted in the maize genome, and that the introduced genes are all inherited stably in offspring. It was found that 217bp fragment of *P-ract1* exists in the reverse direction near the 3'-terminal of the introduced gene, though it was confirmed that this fragment never causes any additional production of protein. In addition, it was also confirmed that the bases of *cp4 epsps* gene induced by *E35S* were changed during production of this recombinant maize and consequently one of the amino acids constituting the CP4 EPSPS protein was changed, though the function of the CP4 EPSPS protein remains unchanged.

As a result of Southern blotting analysis, it was confirmed that the genes derived from Cry1F line 1507 and NK603 are stably inherited in the stack line 1507×NK603. Southern blotting analysis was also conducted on the genome DNAs extracted from the leaves of four individuals for each of the stack line 1507×NK603, Cry1F line 1507, and NK603 raised in a greenhouse which were digested with the restriction enzyme *Eco*R V to provide the respective probes of *cry1F*, *pat* and *cp4 epsps*. As a result, it was proved that this stack line exhibited the same band patterns as those of Cry1F line 1507 and NK603, and the introduced genes in Cry1F line 1507 and NK603 are stably inherited in the stack line 1507×NK603.

In the Southern blotting analysis using the *cry1F* as probe, the stack line 1507×NK603 and the Cry1F line 1507 showed two minor bands in addition to the expected one band. The two minor bands had been anticipated from the analytical results of the introduced genes in the Cry1F line 1507 that the 5'-terminal region of the introduced DNA in the Cry1F line 1507 contains a part of *cry1F* gene sequence and the 5'-terminal region refers to partial digestion of *Eco*R V site due to the highly repetitive sequence and GC-rich sequence.

(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

The traits inherited in the stack line 1507×NK603 from Cry1F line 1507 and NK603 were checked based on the ELISA analysis. The levels of expression of Cry1F protein, PAT protein and CP4 EPSPS protein in this stack line were identified based on the ELISA analysis on the stem and leaf samples taken in the R4 stage (period of flowering and pollination) from the 6 fields in Chile where the glyphosate herbicide (glyphosate of 1.14 kg per hectare, Product name: Roundup-ultra Max) was sprayed in the V4 stage (time of 4 foliage leaves exsertion) and glufosinate herbicide (glufosinate of 0.51 kg per hectare, Product name: Basta) was sprayed in the V7 stage (time of 7 foliage leaves exsertion) in accordance with the directions for use specified for the individual herbicides by the US company Pioneer Hi-Bred International, Inc. from 2002 to 2003. As a result of observation after spraying of the herbicides, there was no plant body that died or suffered any damage from the herbicides and it was confirmed that the stack line 1507×NK603 is comparable to the parent lines in possession of the tolerance to the both herbicides glyphosate and glufosinate. As a result of ELISA analysis, the mean expression level of Cry1F protein in stem and leaf was found 5.57ng/mg dry weight (Min. value - Max. value of analytical results: 4.31 - 6.77ng/mg dry weight) for the stack line, 4.14ng/mg dry weight (3.22 - 4.94ng/mg dry weight) for the parent line Cry1F line 1507, and the mean expression level of PAT protein was found 0.58ng/mg dry weight (0.48 - 0.64ng/mg dry weight) for the stack line, 0.17ng/mg dry weight (<0.045 - 0.44ng/mg dry weight) for Cry1F line 1507. In addition, the mean expression level of CP4 EPSPS protein was found 62.0ng/mg dry weight (49.9 - 71.6ng/mg dry weight) for the stack line, 76.5ng/mg dry weight (54.0 - 93.6ng/mg dry weight) for the parent line NK603. The expression levels of Cry1F protein, CP4 EPSPS protein and PAT protein in the stack line 1507 ×NK603 were found almost equivalent to those in the parent lines.

In addition, in order to confirm that the stack line 1507×NK603 is comparable to the parent line Cry1F line 1507 and the parent line NK603 in the level of tolerance to herbicides, greenhouse test was conducted in which herbicides were sprayed in higher dose than approved in the registration of agricultural chemicals. In the Cry1F line 1507 and the stack line 1507×NK603 sprayed with glufosinate herbicide, no significant chemical injury was observed compared to the non-sprayed control field even in the spraying test of 16-times higher dose than normal. In the Cry1F line 1507 sprayed with 32-times higher dose than normal, statistically significant chemical injury (22.0%) was observed compared to the non-sprayed control field. The similar result was obtained in the stack line 1507×NK603, in

which the plant body sprayed with 32-times higher dose suffered statistically significant chemical injury (26.5%) compared to the non-sprayed control field. In the degree of chemical injury between the Cry1F line 1507 and the stack line 1507×NK603 sprayed with 32-times higher dose, no statistically significant difference was observed.

On the other hand, in the glyphosate-spraying test, both NK603 and the stack line 1507×NK603 suffered no significant chemical injury compared to the non-sprayed control field in all the spray doses. In the spraying of 32-times higher dose than normal, the both lines suffered chemical injury of around 5%.

In actuality, normal dose assures satisfactory weed-killing effects and thus the actual farming fields never be exposed to such high doses of glyphosate herbicide or glufosinate herbicide as applied in the tests which were sprayed specifically for the purpose.

Based on the above understanding, it was confirmed that the obtained traits derived from Cry1F line 1507 and NK603 are stably expressed and the glufosinate tolerance and glyphosate tolerance are independent from each other in the stack line 1507×NK603.

(4) 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

ELISA analysis kits using the polyclonal antibodies respectively for Cry1F protein, PAT protein and CP4 EPSPS protein are 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. The CP4 EPSPS protein detection kit can detect one CP4 EPSPS protein-containing grain per 800 maize grains.

The detection kits have been all certified as reliable enough by a variety of tests.

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

The stack line 1507×NK603 is given the resistance to European corn borer (*Ostrinia nubilalis*) and other Lepidoptera insects that feed and damage maize with the production of Cry1F protein due to the introduction of *cry1F* gene derived from *Bacillus thuringiensis* (hereafter referred to as "*B.t.*") var. *aizawai* introduced in Cry1F line 1507. The European corn borer (*Ostrinia nubilalis*) is one of the insect pests that cause the most significant insect damage to the maize cultivation in 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 exterminating 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 this insect pest control reportedly amounts to about a billion dollar per year.

2) Tolerance to glufosinate herbicide

To the stack line 1507×NK603, tolerance to glufosinate herbicide is also conferred with the introduction of *pat* gene derived from *Streptomyces viridochromogenes* introduced in Cry1F line 1507. The PAT protein produced by the expression of *pat* gene acetylates the glufosinate herbicide and transforms it to nontoxic acetylglufosinate, thereby conferring the glufosinate tolerance on plant body.

3) Tolerance to glyphosate herbicide

To the stack line 1507×NK603, tolerance to glyphosate herbicide is also conferred with the introduction of *cp4 epsps* gene derived from *Agrobacterium* CP4 strain introduced in NK603. The glyphosate herbicide inhibits the activity of 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway in the process of aromatic amino acid biosynthesis. As a result, plants treated with glyphosate cannot synthesize amino acid essential for growth and ultimately die. The CP4 EPSPS protein produced by *cp4 epsps* gene is not inhibited even in the presence of glyphosate and it properly works as an enzyme in the shikimate pathway, thereby conferring the glyphosate tolerance on plant body.

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

The stack line 1507×NK603 is a hybrid obtained by traditional breeding of Cry1F line 1507 and NK603. Therefore, it is expected that such crossbreeding of the two lines may result in hybrid vigor. Based on the findings that Cry1F protein is considered not to possess any enzyme activity, and both PAT protein and CP4 EPSPS protein possess high substrate specificities and differ from each other in the action mechanism and substrate, it is considered that the introduced genes in this stack line do not affect nor interact with the metabolic system of the recipient organism. In fact, as a result of ELISA analysis, it was found that the levels of

expression of Cry1F protein, CP4 EPSPS protein and PAT protein are comparable to those in the parent lines. Comprehensively, it is judged that possible effects of hybrid vigor produced in this stack line on the characteristics do not exceed the range of variations generated in the hybrid varieties obtained by crossbreeding of traditional non-recombinant maize lines or recombinant and non-recombinant maize lines.
Based on the above understanding, the difference between this stack line and the taxonomic species of maize to which the recipient organism belongs was described with the use of the results of individual examinations for the various characteristics conducted in isolated fields in Japan in 2001 for the Cry1F line 1507 and in 2000 for the NK603.

1) Morphological and growth characteristics

For the morphological and growth characteristics of Cry1F line 1507, 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. In all evaluation items except germination rate and ear diameter, no difference was observed between the Cry1F line 1507 and the non-recombinant control maize. Also for the germination rate and ear diameter for which significant difference was observed between the recombinant and the non-recombinant, the significant difference was observed only in one of the two recombinant varieties tested and the difference from the average was slight.

For the morphological and growth characteristics of NK603, evaluation was also conducted regarding the uniformity of germination, germination rate, time of tassel exertion, time of silking, culm length, plant shape or plant type, tiller number, height of ear, maturation time, number of ears, and weight of plant at harvesting time. As a result, in all evaluation items, no statistically significant difference was observed between the recombinant and the non-recombinant control maize.

As a result of germination test conducted actually in the US on this stack line, the germination rate of this stack line was 98%, which was equivalent to 98% in the case of Cry1F line 1507, 98% in NK603, and 97% in the non-recombinant maize.

Consequently, it is considered that there is no difference in morphological and growth characteristics between this stack line and the taxonomic species of maize to which the recipient organism belongs.

2) Chilling-tolerance at the early stage of growth

Chilling-tolerance of the seedlings of Cry1F line 1507 was evaluated. As a result, all of the opened leaves lost chlorophyll totally and withered about three weeks after exposure to low temperature. In the withering, no difference was observed between the Cry1F line 1507 and the non-recombinant control maize.

Also for NK603, chilling-tolerance of the seedlings was evaluated. As a result, almost all the seedlings died on the 14th day after exposure to low temperature, and no difference was observed between the NK603 and the non-recombinant control maize.

Consequently, regarding chilling-tolerance of this stack line 1507 × NK603, it is considered that there is no difference between this stack maize and the taxonomic species of maize to which the recipient organism belongs.

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 does not regrow, propagate vegetatively, or produce seeds. In fact, it was confirmed that, in the US field where cultivation test of Cry1F line 1507 was conducted in the previous year, there was no plant body observed which remained alive through the winter. It was also confirmed at the end of the isolated field test for NK603 that plant bodies started withering after ripening. Based on the above, an overwintering test for the matured plant of Cry1F line 1507 and NK603 was not carried out.

4) Fertility and size of the pollen

As a result of examination on the fertility and size of the pollens of Cry1F line 1507 sampled during the flowering period, no difference was observed in the evaluation items between the Cry1F line 1507 and the non-recombinant control maize.

Also for the NK603, in the fertility and size of the pollens, no difference was observed between the NK603 and the non-recombinant control maize.

Consequently, regarding fertility and size of pollen, it is considered that there is no difference between this stack 1507×NK603 and the taxonomic species of maize to which the recipient organism belongs.

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

The row number per ear, grain number per row, 100-kernel weight, and the others of Cry1F line 1507 were examined as characteristics referring to the production of seeds, and no difference was observed between the Cry1F line 1507 and the non-recombinant maize in all of the characteristics examined. In addition, for both of the Cry1F line 1507

and the non-recombinant maize, the germination rate of harvested seeds (F2) was found satisfactory and then no dormancy of the seeds was observed.

Also for NK603, examination was conducted on row number per ear, grain number per row, 100-kenrl weight and other characteristics. As a result, in the 100-kernel weight, statistically significant difference was observed between one sample, NK603-B, of the two recombinant maize NK603 samples examined and the non-recombinant control maize, though, in the other characteristics examined, no difference was observed between the NK603 and the non-recombinant maize. The difference observed in the 100-kernel weight was slight, and the other sample NK603-A exhibited no difference in the 100-kernel weight. For both of the NK603 and the non-recombinant maize, the germination rate of harvested seeds (F2) was found satisfactory and then no dormancy of the seeds was observed.

The ears of Cry1F line 1507 and NK603, and the non-recombinant control maize were covered with bracts at the time of harvesting and thus, shedding habit was not observed in the natural condition.

Based on the above understanding, regarding production of the seed and other characteristics of this stack line, it is considered that there is no difference between this stack maize and the taxonomic species of maize to which the recipient organism belongs.

6) Crossability

Crossability test was not performed since there are no wild relatives (teosinte) 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 alleochemicals 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.

For the Cry1F line 1507, succeeding crop test, soil microflora tests and plow-in test were performed in the isolated filed. In addition, in the US, evaluation on the productivity of harmful substance was conducted based on the Sandwich method using the roots, leaves and stems of the Cry1F line 1507 and the non-recombinant, along with visual observation on possible effects on the growth of succeeding crops in the 46 field experiments. Based on the above results, it was concluded that there was no difference between the

recombinant maize examined and the non-recombinant control maize in the productivity of harmful substances.

In the NK603, CP4 EPSPS protein is newly produced due to the introduction of *cp4 epsps* gene. CP4 EPSPS protein possesses high substrate specificity similarly as the PAT protein and thus it is considered not to affect the metabolism of plants.

Also for the NK603, succeeding crop test, soil microflora tests and plow-in test were performed in the isolated field test. As a result, no statistically significant difference was observed between the recombinant maize examined and the non-recombinant control maize in all evaluation items.

Consequently, regarding the productivity of harmful substances, it is also considered that there is no difference between the stack line 1507×NK603 and the taxonomic species of maize to which the recipient organism belong.

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 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.

This stack line maize was produced by traditional crossbreeding of maize resistant to Lepidoptera and tolerant to glufosinate herbicide (DAS-01507-1) and maize tolerant to glyphosate herbicide (MON-00603-6), and the parent lines were individually judged at the Meeting for Review on the Biological Diversity Risk Assessment as causing no Adverse Effect on Biological Diversity when applied for the Type I Use same as the stack line maize.

It is reported that the Cry1F protein which is encoded by *cry1F* (gene resistant to Lepidoptera) derived from DAS-01507-1 does not possess enzyme activity, and that the PAT protein which is encoded by *pat* (gene tolerant to glufosinate) derived from DAS-01507-1 possesses high substrate specificity. In addition, it is suggested that CP4 EPSPS protein which is encoded by *cp4 epsps* (gene tolerant to glyphosate) derived from MON-00603-6 possesses high substrate specificity. Therefore, it is considered that the characteristics conferred by Cry1F protein and CP4 EPSPS protein, and PAT protein and CP4 EPSPS protein do not interact with each other.

It was confirmed based on the ELISA analysis on proteins that the levels of expression of Cry1F protein, PAT protein and CP4 EPSPS protein in this stack line maize are equivalent to those of the respective proteins in DAS-01507-1 and MON-00603-6. In addition, it was also confirmed based on the herbicide-spraying tests that the tolerance of this stack line maize to glufosinate and glyphosate is equivalent to that of DAS-01507-1 and MON-00603-6.

Based on the above understanding, it is considered that there is no specific change in the characteristics in this stack line maize except it possesses the same characteristics as the parent lines do.

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

(1) Competitiveness

This stack line maize possesses the Lepidoptera resistance and glufosinate tolerance derived from DAS-01507-1 and the glyphosate tolerance derived from MON-00603-6. However, it is not generally considered that the glufosinate or glyphosate exerts pressure for selection under a natural environment. In addition, the insect damage by Lepidoptera is not the major cause to make the maize difficult to grow in the natural environment in Japan. Consequently, it is considered that these characteristics do not increase the competitiveness and thus this stack line maize is not predominant over the parent lines in the competitiveness. Based on the above understanding, it is judged that 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

This stack line maize possesses the productivity of Cry1F protein and PAT protein derived from DAS-01507-1 and the productivity of CP4 EPSPS protein derived from MON-00603-6. It is confirmed that the Cry1F protein possesses the insecticidal activity against insects of the order Lepidoptera, though PAT protein and CP4 EPSPS protein are not harmful substances to animals and plants. Thus, it is considered that the productivity of harmful substances of this stack maize would not become higher than that of parent lines. Based on the above understanding, the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to the productivity of harmful substances is judged 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 being affected. Therefore, 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 stack line maize 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.