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

Name: DuPont Kabushiki Kaisha
Applicant: Yoshiyuki Tanaka, President
Address: 11-1 Nagata-cho 2-chome, Chiyoda-ku, Tokyo

Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Maize resistant to lepidopterous pests and coleopteran pests and tolerant to glufosinate herbicide (Modified *cry1F*, *cry34Ab1*, *cry35Ab1*, *pat*, *Zea mays* subsp. *mays* (L.) Iltis) (4114, OECD UI: DP-ØØ4114-3)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	－

Outline of the Biological Diversity Risk Assessment Report

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

1 Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of the donor nucleic acid and the origins of component elements used for the development of the maize resistant to lepidopterous pests and coleopteran pests and tolerant to glufosinate herbicide (Modified *cry1F*, *cry34Ab1*, *cry35Ab1*, *pat*, *Zea mays* subsp. *mays* [L.] Iltis) (4114, OECD UI: DP-ØØ4114-3) (hereinafter referred to as 'this modified maize') are shown in Table 1 (pages 3 and 4). Also, the base sequence is shown in Attachment 1 (undisclosed due to confidential information).

2) Functions of component elements

(a) Functions of individual components of donor nucleic acid including target genes, range of gene expression regulation, localization signal, and selective marker genes

Functions of individual components of donor nucleic acid are shown in Table 1 (pages 3 and 4).

Table 1 Structure of donor nucleic acid and origins and functions of component elements used for development of this modified maize

Component element		Size (bp)	Origin and function
Right Border (RB)		25	Right border region of T-DNA region of Ti plasmid (pTi) derived from *Agrobacterium tumefaciens*
Expression cassette Modified *cry1F* gene	*ubi*ZM1 promoter	900	Promotor region of the polyubiquitin gene derived from maize (*Z. mays*) (Christensen *et al.*, 1992). Induces constitutive expression in the plant body.
	*ubi*ZM1 5' UTR	83	5' untranslated region of the polyubiquitin gene derived from maize (*Z. mays*) (UTR) (Christensen *et al.*, 1992).
	*ubi*ZM1 intron	1,010	Intron region of the polyubiquitin gene derived from maize (*Z. mays*) (Christensen *et al.*, 1992).
	Modified *cry1F*	1,818	A gene coding the modified Cry 1F protein derived from *Bacillus thuringiensis* var. *aizawai* strain. Base sequence is modified to enhance the expression in plants, and the phenylalanine of the 604th amino acid of the coded protein is replaced with leucine (USDA, 2000).
	ORF25 terminator	714	Terminator region of pTi15955 derived from *A.tumefaciens* strain (Barker *et al.*, 1983). It terminates transcription.
expression cassette *cry34Ab1* gene	*ubi*ZM1 promoter	900	Promotor region of polyubiquitin gene derived from maize (*Z. mays*) (Christensen *et al.*, 1992). Induces constitutive expression in the plant body.
	*ubi*ZM1 5' UTR	83	5' untranslated region of polyubiquitin gene derived from maize (*Z. mays*) (UTR) (Christensen *et al.*, 1992).
	*ubi*ZM1 intron	1,010	Intron region of polyubiquitin gene derived from maize (*Z. mays*) (Christensen *et al.*, 1992).
	cry34Ab1	372	A gene coding Cry34Ab1 protein derived from *B. thuringiensis* PS149B1 strain (Moellenbeck *et al.*, 2001; Ellis *et al.*, 2002; Herman *et al.*, 2002).
	pin II terminator	310	Terminator region of proteinase inhibiter II gene derived from potato (*Solanum tuberosum*) (Keil *et al.*, 1986; An *et al.*, 1989). Terminates transcription.
expression cassette *cry35Ab1* gene	TA Peroxidase promoter	1,298	Peroxidase promoter region derived from wheat (*Triticum aestivum*) (Hertig *et al.*, 1991). Induces constitutive expression in the plant body.
	cry35Ab1	1,152	A gene coding Cry35Ab1 protein derived from *B. thuringiensis* PS149B1 strain (Moellenbeck *et al.*, 2001; Ellis *et al.*, 2002; Herman *et al.*, 2002).
	pin II terminator	310	Terminator region of proteinase inhibitor II gene derived from potato (*S. tuberosum*) (Keil *et al.*, 1986; An *et al.*, 1989). Terminates transcription.

Table 1 Structure of donor nucleic acid and origins and functions of component elements used for development of this modified maize

Component element		Size (bp)	Origin and function
expression cassette *pat* gene	CaMV 35S promoter	530	35S promoter region derived from Cauliflower Mosaic Virus (Franck *et al.*, 1980; Odell *et al.*, 1985; Pietrzak *et al.*, 1986). Induces constitutive expression in the plant body.
	pat	552	A gene coding PAT protein derived from *Streptomyces viridochromogenes*
	CaMV 35S terminator	192	35S terminator region derived from Cauliflower Mosaic Virus (Franck *et al.*, 1980; 1980; Pietrzak *et al.*, 1986). Terminates transcription.
Left Border (LB)		25	Left border region of T-DNA region of pTi derived from *A. tumefaciens*

(b) Functions of the protein produced by the expression of target genes and selective markers, and the fact, if applicable, that the produced protein is homologous with any protein known to possess any allergen

a Functions of proteins produced by the expression of target genes

Bt protein

Pesticidal crystal proteins (Bacillus thuringiensis [Bt] proteins) including the modified Cry1F protein, Cry34Ab1 protein and Cry35Ab1 protein bind in general to the specific receptors located in the midgut cells of pests including lepidopterous and coleopteran pests, form stomas, and exhibit pesticidal activity by destroying the midgut cells (Schnepf *et al*., 1998). Bt proteins have specificity to the insecticide-targeted fauna (Shirai, 2003).

Note that when functions of Cry34Ab1 and Cry35Ab1 proteins are described hereafter, they are referred to as 'Cry34Ab1/Cry35Ab1 proteins'.

Modified Cry1F protein:

The modified *cry1F* gene encodes the modified Cry1F protein (amino acid sequence: USDA, 2012). The modified *cry1F* gene is modified to have increased GC-content in the gene derived from *Bacillus thuringiensis* to enhance the expression in the plant body. And the base sequence is modified to add the restriction enzyme cutting site *Xho* I; the phenylalanine of the 604th amino acid of the coded protein is replaced with leucine (USDA, 2000). This protein targets lepidopterous pests such as European corn borer (*Ostrinia nubilalis*). The LC_{50} value (the lethal concentration to kill 50% of organisms exposed to it) of this protein against European corn borer is 0.58 μg/g (Attachment 2: undisclosed due to confidential information).

As with other Bt proteins, insecticidal effects of the modified Cry1F protein have high specificity; it is efficient only for the targeted lepidopterous pests such as European corn borer. Actually, it does not show pesticidal activity against insects including coleopteran, hymenoptera, neuropteran and collembolan as well as toxicity against non-target organisms including the mammals, birds and fishes (EPA, 2010a).

Cry34Ab1/Cry35Ab1proteins:

The *cry34Ab1*/*cry35Ab1* genes encode the Cry34Ab1/Cry35Ab1 proteins (amino sequence: USDA, 2012). These proteins target coleoptera pests including northern corn rootworm (*Diabrotica barberi*) and western corn rootworm (*D. virgifera virgifera*). Cry34Ab1 protein possesses the pesticidal activity against corn rootworms, but the Cry35Ab1 protein alone does not show pesticidal activity. When both proteins are activated concurrently, the pesticidal activity is increased approximately eightfold at maximum (Herman *et al.*, 2002). The LC_{50} values against northern corn rootworm and western corn rootworm are 5.56 μg/cm^2 and 44.5 μg/cm^2, respectively (for the total of Cry34Ab1/Cry35Ab1 proteins) (Attachment 3: undisclosed due to confidential information).

As with other Bt proteins, the insecticidal effects of the Cry34Ab1/Cry35Ab1 proteins have high specificity; it is efficient only for coleoptera pests such as the targeted corn rootworms. Actually, it does not show pesticidal activity against insects including lepidoptera, hymenoptera, neuropteran and hemipteran as well as toxicity against non-target organisms including mammals, birds and fishes (EPA, 2010b).

PAT protein

The *pat* gene encodes the PAT protein (amino sequence: USDA, 2012).

Glufosinate herbicide inhibits the glutamate synthase activity by the active ingredient, L-glufosinate. Subsequently, ammonia, the substrate, is accumulated in the plant body and the plant would wither and die. The PAT protein acetylates free amino group of L-glufosinate, transforms the free amino group to N-acetylglufosinate and detoxicates it, and thereby confers resistance against glufosinate herbicide to the plant body (OECD, 2002).

b Homology with the protein known to have allergen

Homology search of amino acid sequence[1)] was conducted using the known allergen database of Food Allergy Research and Resource Program (FARRP) (Version of Release 13- February 2013) at Nebraska University. The result did not show any known and expected allergens that display homology with the modified Cry1F protein, Cry34Ab1 protein, Cry35Ab1 protein and PAT protein (Attachments 4, 5 and 6: undisclosed due to confidential information).

[1)] The modified Cry1F protein, Cry34Ab1 protein and Cry35Ab1 protein: searched in March 2013.
The PAT protein: searched in February 2013.

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

Bt protein

The modified Cry1F protein and Cry34Ab1/Cry35Ab1 proteins are both Bt proteins. Bt proteins are considered to show pesticidal activity by binding specifically to the specific receptors located in the midgut cells of the target insect, forming stomas in the cells and destroying the midgut cells (OECD, 2007; Schnepf *et al.*, 1998). However, there has been no report showing Bt proteins have enzymaticactivities.

PAT protein

The PAT protein possesses high substrate specificity and catalyzes acetylating reaction of free amino group of L-glufosinate, the active ingredient of glufosinate herbicide, but does not have other amino acids and D-glufosinate (OECD, 1999).

From the above, it cannot be considered the PAT protein affects the metabolic pathway of the recipient organism.

(2) Information concerning the vector

1) Name and origin

The vector used for the production of this modified maize is the plasmid PHP27118 (Figure 1, page 8) produced from the plasmid pSB1 derived from *Agrobacterium tumefaciens* LBA4404 strain (Komari *et al*., 1996).

2) Properties

(a) The number of base pairs and nucleotide sequence of vector

The number of base pairs of the plasmid PHP27118 is 54,910 bp and the number of base pairs at the T-DNA region is 11,978 bp. The nucleotide sequence is as shown in Attachment 1 (undisclosed due to confidential information).

(b) Presence or absence of nucleotide sequence having specific functions, and the functions, if present

Exoskeleton region of the plasmid PHP27118 contains the spectinomycin resistant gene (*spc*) and the tetracycline resistant gene (*tetA*) as selective marker genes. These genes function as the markers necessary for selecting the microorganisms which contain transforming plasmids when the vector is propagated in microorganisms. However, these antibiotic-resistant genes are not introduced in recipient organisms because they exist not in the T-DNA region which is induced to the recipient organism but in the exoskeleton region. Actually, it has been confirmed by Southern blotting analysis that the exoskeleton region containing antibiotic-resistant genes is not introduced (Attachment 7; undisclosed due to confidential information).

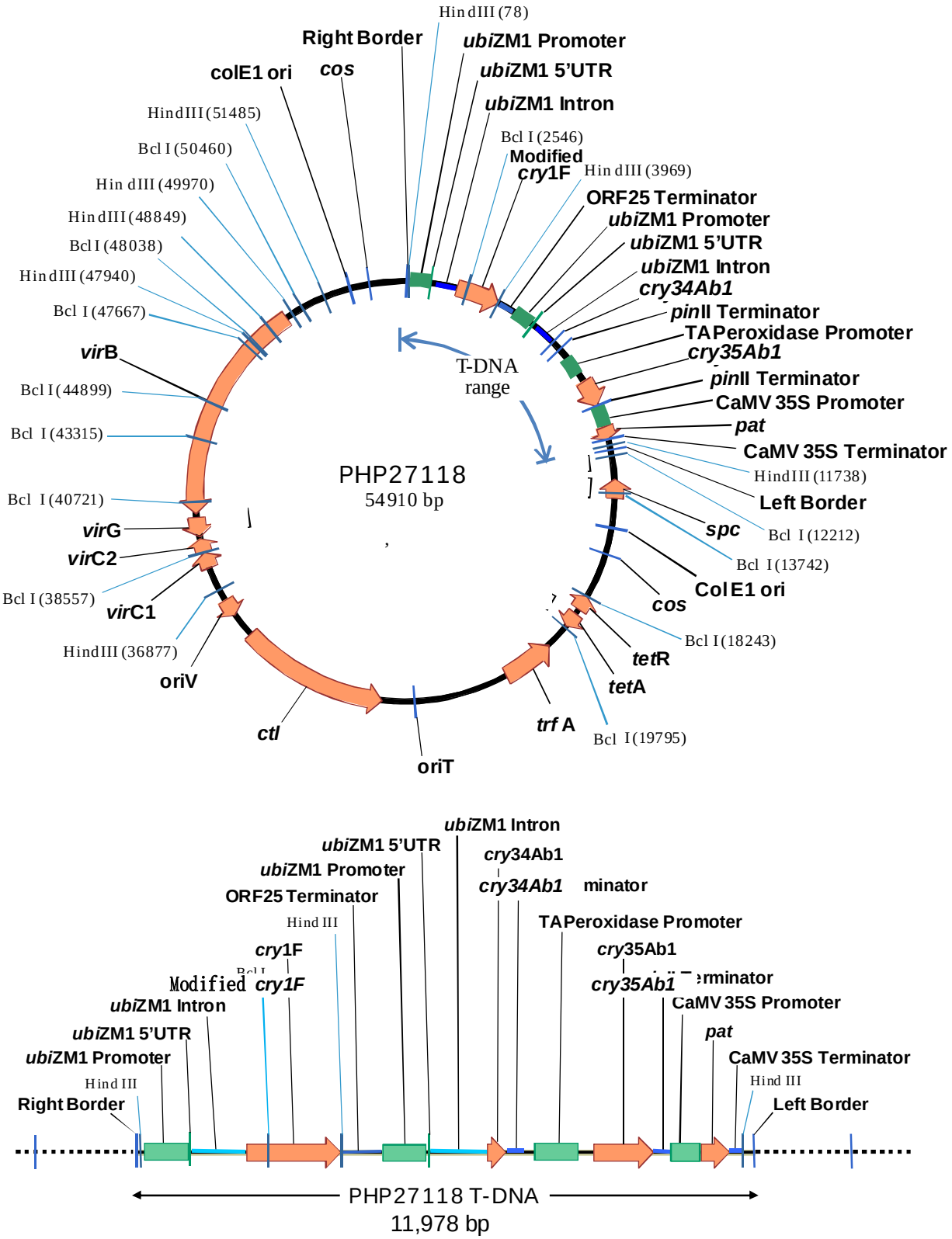
c) Presence or absence of infectivity of vectors and the information concerning the recipient organism range if infectivity is present

The T-DNA region that is introduced to the recipient organism does not contain any nucleotide sequence having infectious potential; there is no infectivity.

(3) Method of preparing living modified organisms

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

The component elements of the donor nucleic acid used for the production of this modified maize and the location excised by restriction enzyme are shown in Figure 1 (page 8).



The upper figure: Plasmid PHP27118
The lower figure: The schematic picture of the introduced DNA in this modified maize.
Dotted lines show the chromosomal DNA of maize.

Figure 1: Component of the donor nucleic acid and the location excised by the restriction enzyme in the plasmid PHP27118

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

Transferring the nucleic acid into the recipient organism was conducted using the agrobacterium method.

3) Processe of rearing the living modified organism

(a) Mode of selecting the cells containing the transferred nucleic acid

The cells containing the transferred nucleic acid were selected by rearing embryos in the culture medium to which herbicide bialaphos was added. To select the PAT protein-producing cells, either of herbicide bialaphos or glufosinate can be used. However, the target cells can be selected more effectively with herbicide bialaphos (Dennehey *et al.*, 1994).

(b) Presence or absence of remaining fungus body of Agrobacterium in case Agrobacterium method is used for transferring the nucleic acid

Agrobactrium was removed by adding carbenicillin to the culture medium. Furthermore, it was confirmed that the exoskeleton region of the plasmid PHP27118 was not introduced to the genome of this modified maize (Attachment 7; undisclosed due to confidential information). Therefore, no fungus body of Agrobacterium is considered unremoved.

(c) Processes of rearing and pedigree trees of the following lines; cells to which the nucleic acid was transferred, the line in which the state of existence of replication products of the transferred nucleic acid was confirmed, the line subjected to isolated field tests; and the line used for collecting other necessary information for assessment of Adverse Effect on Biological Diversity

Process of rearing of this modified maize is as shown in Figure 2 (page 9; undisclosed due to confidential information). The range of the lines for approval is on and after T1 generation.

(Undisclosed due to confidential information)

Figure 2 Process of rearing this modified maize

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

(a) Place where the replication product of the transferred nucleic acid exists

The transferred nucleic acid is segregated according to the Mendel's law after introduced into a plant chromosome. In order to investigate the segregation ratio of each introduced gene, F1*[1], BC2F1*[1], BC3F1*[1], BC2F1*[2] and BC3F1*[2] generations (Figure 2, page 9; undisclosed due to confidential information) of this modified maize were cultivated in a greenhouse in Iowa, the US in 2010 (Attachment 8; undisclosed due to confidential information). The genome DNA was extracted from leaves of 2-leaf stage, and PCR analysis was conducted for each gene-specific primer of the modified *cry1F* gene, *cry34Ab1* gene, *cry35Ab1* gene and *pat* gene.

As a result, in each primer, the introduced genes were co-segregated.
The results of all primers are summarized in Table 2 (page 10). The segregation ratios of F1*[1], BC2F1*[1], BC3F1*[1] and BC2F1*[2] generations were consistent with the expected ratio of 1:1. Statistically significant differences ($P < 0.05$) were noted in 99 individual samples of BC3F1*[2] generation (Sample A). Therefore, further examinations with 96 individual samples (Sample B) and 73 individual samples from a different lot (Sample C) were conducted in 2011. As a result, no statistically significant differences were noted ($P < 0.05$) in Sample B and Sample C. Therefore, it is considered that the significant difference noted in Sample A occurred because many negative individual samples were contained incidentally in the collected Sample A.

As above, it was confirmed that each introduced gene was inherited according to the Mendel's laws and the replication products of the transferred nucleic acid exist on the maize chromosome.

Table 2 Segregation ratio of the introduced genes based on PCR analysis

Generation	Number of individual samples	Expected value[1)]		Analysis result[2)]		P value
		Positive	Negative	Positive	Negative	
F1*[1]	98	49	49	52	46	0.545
BC2F1*[1]	100	50	50	48	52	0.689
BC3F1*[1]	100	50	50	47	53	0.549
BC2F1*[2]	100	50	50	53	47	0.549
BC3F1*[2]						
Sample A [3)]	99	49.5	49.5	38	61	0.0208 [4)]
Sample B [3)]	96	48	48	49	47	0.838
Sample C [5)]	73	36.5	36.5	39	34	0.558

Statistical analysis: Chi-square test

1) Expected segregation ratio is 1:1.
2) Positive individual samples were positive in all 4 primer pairs, and negative individual samples were negative in all primer pairs.
3) Lot number C10T-31399377.
4) A statistically significant difference was noted ($P < 0.05$).
5) Lot number C11T-39367876.

(b) The number of copies of the replication product of the transferred nucleic acid and stability of its inheritance in multiple generations

As a result of Southern blotting analysis using leaves of T1, F1*[1], BC3F1*[1], BC2F1*[2], T2 and BC3F1*[3] generations of this modified maize (Figure 2, page 9; undisclosed due to confidential information), it was confirmed that one copy of each gene expression cassette was transferred and inherited to multiple generations stably (Attachments 7 and 9; undisclosed due to confidential information).

(c) The position relationship in the case of multiple copies existing in chromosome

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(d) Inter-individual or inter-generational expression stability under the natural environment with regard to characteristics shown specifically in (6)-(a))

ELISA analyses were conducted using leaves of 9-leaf stage of BC3F1*[1] generation (Figure 2, page 9; undisclosed due to confidential information) of this modified maize cultivated in a greenhouse in Iowa, the US in 2010, leaves and roots of 9-leaf stage, and the pollen at silking stage of F1*[5] generation (Figure 2, page 9; undisclosed due to confidential information) of this modified maize cultivated in 5 locations (2 locations in Iowa, 1 location each in Illinois, Nebraska in the US and Ontario in Canada) in 2010 (Attachments 10 and 11; undisclosed due to confidential information). The result showed that the inter-generation expression stability of the modified Cry1F protein, Cry34Ab1 protein, Cry35Ab1 protein and PAT protein was confirmed with the leaves of 9-leaf stage (Table 3, page 11).

Table 3 Produced quantity of each protein

Mean (minimum – maximum) (ng/mg dry weight)

Generation	Tissue	Modified Cry1F protein	Cry34Ab1 protein	Cry35Ab1 protein	PAT protein
BC3F1*[1] [1)]	Leaf	10 (9 - 11)	31 (26 - 35)	22 (20 - 23)	14 (14 - 14)
F1*[5] [2)]	Leaf	9.7 (5.3 - 14)	26 (22 - 31)	33 (28 - 39)	9.8 (4.8 - 15)
	Root	5.0 (1.3 - 7.5)	21 (13 - 28)	13 (7.8 - 19)	0.65 (0.39 - 0.90)
	Pollen	35 (19 - 49)	9.2 (4.7 - 16)	0.34 (<0.32 [3)] - 0.53)	<0.28 [3)] (<0.28 [3)])

1) The modified maize (Positive individual sample) n = 2

2) The modified maize (Positive individual sample) n = 20

3) Below the lower limit of quantification

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

The transferred nucleic acid does not contain any sequence allowing transmission. Therefore, there is no possibility that the transferred nucleic acid might be transmitted to any wild animals and wild plants.

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

Methods of detection and identification:

The real-time PCR analysis using the primer pairs mentioned below (Attachment 12; undisclosed due to confidential information)

- The modified maize-specific primer pairs: the inserted genes and its 5' border region of the maize are amplified (Table 1 in Attachment 12, page 49; undisclosed due to confidential information)
- Endogenous gene primer pairs (control): the endogenous *hmgA* gene of the maize is amplified (Table 3 in Attachment 12, page 44; undisclosed due to confidential information)

Sizes of the amplified product are 90 bp when specific primer pairs are used and 79 bp when endogenous gene primer pairs are used.

In either of the non-modified maize or modified maize, amplified products can be confirmed with the endogenous gene primer pairs. In contrast, amplified products can be confirmed only in this modified maize when specific primer pairs are used. Therefore, this modified maize can be identified by using both primer pairs.

Sensitivity (Genome DNA of this modified maize/ genome DNA of maize x 100):

- Limit of quantification: 0.08 %
- Limit of detection: 0.04 %

Reliability:

Based on the analyses using this modified maize conducted in 2 institutions (Eurofins GeneScan GmbH, Germany and Pioneer Hi-Bred International, Inc., the US), repeatability was confirmed (Attachment 12, pages 51 to 72; undisclosed due to confidential information).

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

(a) Specific contents of physiological or ecological properties conferred by the expression of the replication product of transferred nucleic acid

Properties conferred to the modified maize are the resistance to lepidopterous pests by the modified *cry1F* gene, the resistance to coleopteran pests by the *cry34Ab1/cry35Ab1* genes and the tolerance to herbicide glufosinate by the *pat* gene.

Concerning the resistance to lepidopterous pests, F1*[6] and F1*[7] generations were cultivated in fields in Nebraska in the US in 2008 and the leaf feeding damage by European corn borer was investigated (Figure 2, page 9; undisclosed due to confidential information). Concerning the resistance to coleopteran pests, F1*[6] and F1*[7] generations were cultivated in fields in Minnesota in the US in 2008 and the level of root feeding damage by western corn rootworm was investigated (Figure 2, page 9; undisclosed due to confidential information). Concerning the tolerance to herbicide glufosinate, BC3F1*[1] generation was cultivated in greenhouses in Iowa in the US in 2010 and the tolerance after spraying the herbicide was investigated (Figure 2, page 9; undisclosed due to confidential information) (Attachment 13; undisclosed due to confidential information).

As a result, the modified maize was confirmed to posse these properties (Table 4, page 13).

Table 4 Investigation result of the properties conferred to the modified maize

Investigated item	Non-modified maize	This modified maize
Resistance to European corn borer (lepidoptera) [1)] (Mean [minimum-maximum])	4.4 (3.0 - 6.0)	9.0 (9.0 - 9.0)
The level of root feeding damage [2)] by western corn rootworm (coleopteran) (Mean [minimum-maximum])	1.1 (0.3 - 2.7)	0.1 (0.0 - 0.6)
Tolerance to herbicide glufosinate [3)] (No. of tolerant individuals/no. of tested individuals)	0 / 194	47 / 47

1) Modified maize (positive individual) n=48, non-modified maize n=48

Test conditions: A total of 300 European corn borer larvae were inoculated per 1 stock at 5-leaf stage (8 stocks per 1generation for 1 repetition; 3 repetitions).

Assessment standard: Visual inspection of feeding damage of leaves approximately 3 weeks after inoculation based on the scale 1 (feeding damage ≥ 2.5 cm in most leaves) to 9 (no feeding damage or pinholes in a few leaves) (Refer to Table 1 in Attachment 13, page 4 [undisclosed due to confidential information]; Guthrie *et al.*, 1960).

2) Modified maize (positive individual) n=30, non-modified maize n=30

Test conditions: Approximately 1,000 eggs of western corn rootworm were inoculated per 1 stock at 2-leaf stage (5 stocks per 1 generation for 1 repetition; 3 repetitions).

Assessment standard: Visually inspection of roots at the grain filling stage (the period when grain is whitish and swelling). Counted the total number of roots and the number of roots feeding-damaged at each section, and calculated the feeding-damage score (the number of roots feeding-damaged/ total number of roots). Roots which became approximately < 5 cm due to feeding damage were judged as feeding-damaged. For roots feeding-damaged at multiple sections, scores were added. The score without feeding damage is 0.00. When 1, 2, 3 or more sections of roots are feeding-damaged, scores are 1.00, 2.00 and 3.00 (the upper limit) (Refer to Table 2 in Attachment 13, page 5; [undisclosed due to confidential information]; Oleson *et al.*, 2005).

3) Modified maize (positive individual) n=47, non-modified maize n=194

Assessment standard: Herbicide glufosinate (0.45 kg active ingredient/ha [usual quantity]) was sprayed 13 days after inoculation. The presence or absence of tolerance was visually inspected 7 days after spraying.

(b) Concerning physiological or ecological characteristics listed below, the presence or absence of differences between genetically modified agricultural products and taxonomic species to which the recipient organism belongs, and degree of difference, if present

Investigation was carried out at the isolation field of Utsunomiya Office of DuPont Kabushiki Kaisha in 2011 and 2012 to know whether differences were noted between the modified maize and the taxonomic species to which the recipient organism belonged based on the indexes of a to g listed below (Attachment 14; undisclosed due to confidential information). F1*[5] generation (Figure 2, page 9; undisclosed due to confidential information) was used as the modified maize and PHNAR x PHTFE lines that possesses the genomic background identical to those of the modified maize were used as the non-modified maize.

a Morphological and growth characteristics

The germination rate, uniformity of germination, time of tasseling, time of silking, attitude of leaf in relation to main stem [2)], tiller number, number of ears, height of ear, culm length (the length to tassel neck), above-ground weight, ear length, ear diameter, grain shape and grain color were investigated (Attachment 14, pages 9 to 11; undisclosed due to confidential information).

As a result, uniformity of germination was observed one day earlier than the non-modified maize. A statistically significant difference was noted in culm length between the modified and non-modified maizes ($P < 0.05$). No other differences were noted in the investigated items between the modified maize and the non-modified maize (Table 5, page 14).

Table 5 Morphological and growth characteristics

Item	Non-modified maize		Modified maize		P value
	Mean	95% confidence interval	Mean	95% confidence interval	
Germination rate [1)]	98.6	—	99.3	—	0.6859
Uniformity of germination	May 16	—	May 15	—	—
Time of tasseling[2)]	July 16	—	July 16	—	—
Time of silking[2)]	July 16	—	July 16	—	—
Attitude of leaf in relation to main stem [2)]	3.1	—	3.1	—	—
Tiller number [2)]	0.9	0.3 - 1.5	0.9	0.3 - 1.5	0.9295
Number of ears [3)]	1.7	—	1.8	—	0.5850
Height of ear (cm)[2)]	142.7	134.2 - 151.1	151.9	143.5 - 160.3	0.1040
Culm length (cm) [2)]	288.2	281.1 - 295.3	298.2	291.2 - 305.3	0.0493 [4)]
Above-ground weight (kg) [2)]	1.728	1.639 - 1.817	1.687	1.598 - 1.777	0.4017
Ear length (cm) [2)]	22.82	22.13 - 23.51	22.57	21.88 - 23.26	0.5406
Ear diameter (cm) [2)]	5.13	5.05 - 5.21	5.06	4.98 - 5.13	0.1405
Grain shape [2)]	Middle	—	Middle	—	—
Grain color [2)]	Yellow and white	—	Yellow and white	—	—

1) A total of 288 grains inoculated in each line. Statistical analysis: Fisher's exact test。
2) A total of 32 stocks investigated in each line. Statistical analysis: Linear mixed model。
3) A total of 32 stocks investigated in each line. Statistical analysis: Fisher's exact test。
4) A statistically significant difference was noted ($P<0.05$).

[2)] Angle of leaf between blade and stem (3 categories: 3 =±25°, 5=±50°, 7=±75°)

b Chilling tolerance and heat tolerance at the early stage of growth

Seeds of each line were sown in the pots on November 25, 2011, and cultivated for 2 weeks in the greenhouse. The pots were moved to outdoors (December 9; at 2-leaf stage). When observed 13 days after outdoor cultivation (December 22), both the modified maize and the non-modified maize were found to be dead (Attachment 14, page 12; undisclosed due to confidential information).

c Overwintering ability of the matured plant

The modified maize and the non-modified maize which were planted in May were observed after they matured on October 18. Both were found to be dead (Attachment 14, pages 9 to 11; undisclosed due to confidential information).

d Fertility and size of the pollen

The adequacy of the pollen (Lugol solution staining rate) and major axis were investigated; no statistically significant differences were noted in either item from the non-modified maize ($P < 0.05$) (Table 6, page 15; Attachment 14, page 16; undisclosed due to confidential information).

Table 6 Results of pollen investigation

Item	Non-modified maize		Modified maize		P value
	Mean	95% confidence interval	Mean	95% confidence interval	
Adequacy (%) [1)]	99.8	—	99.8	—	1.0000
Major diameter (μm) [2)]	96.95	91.12 - 102.79	97.07	91.24 - 102.91	0.9704

1) A total of 400 grains were observed in each line. Statistical analysis：Fisher's exact test.

2) A total of 32 grains were observed in each line. Statistical analysis：Linear mixed model.

e Production, shedding habit, dormancy and germination rate of the seeds

Seed production:

The number of rows of the ear, and the number of grains per row and 100-kernel weight were investigated. As a result no statistically significant differences were noted from non-modified maize ($P < 0.05$) Table 7, page 15; Attachment 14, pages 9 to 11 [undisclosed due to confidential information]).

Table 7 Seed production

Item	Non-modified maize		Modified maize		P value
	Mean	95% confidence interval	Mean	95% confidence interval	
Number of rows of an ear	15.8	14.9 - 16.6	15.4	14.5 - 16.2	0.4540
Number of grains per a row of an ear	47.3	46.2 - 48.5	46.9	45.8 - 48.0	0.5468
100-kernel weight (g)	39.66	38.63 - 40.69	39.16	38.13 - 40.19	0.4180

A total of 32 stocks were observed in each line. Statistical analysis: Linear mixed model.

Shedding habit:

No shedding of the seeds was noted at the harvesting time as in the case of the non-modified maize (Attachment 14, pages 9 to 11; undisclosed due to confidential information).

Dormancy and germination rate of the seeds:

Seeds on the harvest day were sown and the germination rate was investigated. The germination rate was high and no statistically significant differences were noted from non-modified maize ($P < 0.05$) (Table 8, page 16; Attachment 14, page 12 [undisclosed due to confidential information]).

Table 8 Germination rate of seeds immediately after harvest

Item	Non-modified maize	Modified maize	P value
Germination rate (%)	97.8	97.8	1.0000

A total of 400 grains were sown in each line.
Statistical analysis: Fisher's exact test.

f Crossability

No related wild species which are able to cross with maize has been reported in Japan. Therefore, the crossing rate was not investigated.

g Productivity of harmful substances

Succeeding crop tests, plow-in tests and soil microflora tests were conducted to compare the productivity of harmful substances by the modified maize with that by the non-modified maize.

Succeeding crop tests:

Radish, the testing crop, was cultivated in the soil where the modified maize and non-modified maize had been cultivated, and the germination rate and dry weight were investigated (Attachment 14, page 13; undisclosed due to confidential information).

As a result, no statistically significant differences were noted in either item between the soil where the modified maize was cultivated and the soil where non-modified maize was cultivated ($P < 0.05$) (Table 9, page 16).

Table 9 Germination rate and dry weight of radish in succeeding test

Item	Non-modified maize post-cultivation soil		Modified maize post-cultivation soil		P value
	Mean	95% confidence interval	Mean	95% confidence interval	
Germination rate (%)[1)]	99.0	—	99.0	—	1.0000
Dry weight (mg)[2)]	407.0	175.2 - 638.8	369.5	137.7 - 601.3	0.5750

1) A total of 96 grains were sown in each line. Statistical analysis: Fisher's exact test.
2) A total of 32 plants were observed in each line. Statistical analysis: Linear mixed model.

Plow-in tests:

Radish, the testing crop, was cultivated in the soil in which leaf blades and leaf sheaths of the modified and non-modified maizes were plowed, and the germination rate and dry weight were investigated (Attachment 14, page 14; undisclosed due to confidential information).

As a result, no statistically significant differences were noted in either item between the soil where the modified maize was plowed and the soil where non-modified maize was plowed ($P < 0.05$) (Table 10, page 17).

Table 10 Germination rate and dry weight of radish in plow-in tests

Item	Non-modified maize plowed soil		Modified maize plowed soil		P value
	Mean	95% confidence interval	Mean	95% confidence interval	
Germination rate (%) [1)]	94.8	—	92.7	—	0.7670
Dry weight (mg) [2)]	178.2	147.9 - 208.4	172.2	141.9 - 202.4	0.7337

1) A total of 96 grains were sown in each line. Statistical analysis: Fisher's exact test.

2) A total of 32 plants were observed in each line. Statistical analysis: Linear mixed model.

Soil microflora tests:

The number of microorganisms (the numbers of bacteria, actinomycetes and filamentous fungi) were measured in the soil where the modified maize was cultivated and the soil where the non-modified maize was cultivated (Attachment 14, page 15; undisclosed due to confidential information).

As a result, a statistically significant difference was noted in the number of actinomycetes in the soil where the modified maize was cultivated and the soil where the non-modified maize was cultivated ($P < 0.05$) (Table 11, page 18). However, both the minimum and maximum values were within the range of fluctuation of the number of actinomycetes when the usual manuring practice was conducted in the cultivated field (Table 12, page 18). Also, no effects on the soil microflora have been noted in the lines[3)] already approved to which similar genes were transferred.

Based on the above, it was considered that, in the number of actinomycetes after the modified and non-modified maizes were cultivated, a statistically significant difference occurred because the fluctuation in the number of actinomycetes in the collected samples was incidentally small.

[3)] ・Maize resistant to lepidopterous pests and tolerant to glufosinate herbicide (*cry1F*, *pat*, *Zea mays* subsp *mays* (L.) Iltis) (*B.t.* Cry1F maize line 1507, OECD UI: DAS-Ø15Ø7-1) (Approved on March 2, 2005)

・Maize resistant to coleopteran pests 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) (Approved on April 10, 2006)

Table 11 Number of bacteria in soil microflora tests

Item	Non-modified maize post-cultivation soil		Modified maize post-cultivation soil		P value
	Mean	Minimum-Maximum	Mean	Minimum-Maximum	
Number of bacteria ($x10^5$)	969	658 - 1,252	714	346 - 992	0.1111
Number of actinomycetes ($x10^4$)	276	246 - 317	238	209 - 260	0.0320 *
Number of filamentous fungi ($x10^3$)	130	94 - 145	125	101 - 158	0.7858

4 repetitions, mean of 5 petri dish per 1 repetition n=20.
The number of bacteria: cfu /1g dry soil
Statistical analysis: Linear mixed model
* A statistically significant difference was noted ($P < 0.05$).

Table 12 The number of actinomycetes in the soil of the same isolated field in the past

Cultivation year	Minimum-Maximum
2007	388 - 717
2011	547 - 1,047
2012	12 - 64

The number of bacteria: x 10^4 cfu /1g dry soil
Measured before starting cultivation each year

3 Information concerning use of living modified organisms

(1) Contents of use

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

(2) Method of use

—

(3) Method of information collection by a person who intends to obtain approval after initiation of Type 1 Use

—

(4) Measueres to prevent Adverse Effect on Biological Diversity, if it may occur

See the Emergency Measures Plan.

(5) Result of the use in laboratories or the use in an environment similar to that where Type 1 use is planned

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(6) Information concerning the use in foreign countries

The status of application for approval of the modified maize in Japan and in foreign countries are as shown in Table 13 and Table 14 (page 20).

Table 13 Status of application for approval in foreign countries

Application		Dates of application and approval	Purposes
US	United States Department of Agriculture (USDA)	Approved in June 2013	Cultivation
	United States Food and Drug Administration (FDA)	Confirmation completed in March 2013	Provision for food and feed
	United States Environmental Protection Agency (EPA)	Approved in June 2012	Exemption of setting acceptable values for the expression protein
Canada	Canadian Food Inspection Agency (CFIA)	Approved in June 2013	Environmental safety, use for feed
	Health Canada (HC)	Approved in June 2013	Use for food
South Korea	Rural Development Administration (RDA)	Applied in November 2012	Use for feed

As of August 2013

Table 14 Status of application for approval in Japan

Application	Dates of application and approval	Purposes
Ministry of Agriculture, Forestry and Fisheries, Ministry of Environment	Approved in September 2011	Type 1 use (cultivation in isolated cultivated field, storage, transportation, disposal and acts incidental to them [1)]
Ministry of Health, Labour and Welfare	Applied in July 2013	Use for food [2)]
Ministry of Agriculture, Forestry and Fisheries	Applied in July 2013	Use for feed [3)]

As of August 2013

1) Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Law No. 97 of 2003)
2) Food Sanitation Law (Law No. 233 of 1947)
3) Act on Safety Assurance and Quality Improvement of Feeds (Act No. 35 of 1953)

Maize 1507, maize 59122 and maize 1507 x 59122[4)] which possess the introduced genes of the modified maize have already been approved for Type 1 Use (Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them)

[4)] • Maize resistant to lepidopterous pests and tolerant to glufosinate herbicide (*cry1F*, *pat*, *Zea mays* subsp. *mays* (L.) Iltis) (*B.t.* Cry1F maize line 1507, OECD UI: DAS-Ø15Ø7-1) (Approved on March 2, 2005)
• Maize resistant to coleopteran pests 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) (Approved on April 10, 2006)
• Maize resistant to lepidopterous pests and coleopteran pests and tolerant to glufosinate herbicide (*cry1F*, *cry34Ab1*, *cry35Ab1*, *pat*, *Zea mays* subsp.*mays* (L.) Iltis) (1507×59122, OECD UI: DAS-Ø15Ø7-1×DAS-59122-7)((Approved on April 10, 2006)

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

Maize, the recipient organism, has been used for many years in Japan. Therefore, in this assessment of Adverse Effect on Biological Diversity, the possibility that some effect may occur was evaluated by comparing the modified maize with the non-modified maize based on Appendix III in Operating Procedures for assessment of Adverse Effect on Biological Diversity.

1 Competitiveness

(1) Identification of wild animals and plants that may be affected

Natural growth of maize has not been reported in Japan.

Various traits of the modified maize regarding the competitiveness (morphological and growth characteristics, cold tolerance at the early stage of growth, wintering ability of the mature plant, production, shedding, dormancy and germination rate of seeds) were investigated in the isolated field. As a result, no differences were noted between the modified and non-modified maizes except uniformity of germination and culm length (I-2-(6)-(b)), page 14). A difference in uniformity of germination was noted in the non-modified maize, and a statistically significant difference was noted in culm length between the modified and non-modified maizes. However, the difference in uniformity of germination was 1 day and the difference in culm length was 10 cm (3% as a ratio). It is unlikely these have any impact on enabling natural growth of the modified maize.

Resistance to lepidopterous pests and coleopteran pests is conferred to the modified maize. However, the feeding damage caused by these insects is not the main factor making it difficult for maize to grow in the natural environment. Consequently, it is considered unlikely that conferring these characteristics will be the factor to make the modified maize grow in the natural environment. Tolerance to herbicide glufosinate was also conferred. However, this herbicide is unlikely to be sprayed in the natural environment.

Therefore, it is considered unlikely that the competitiveness of the modified maize will be enhanced because of the conferred characteristics.

Based on the above, no wild animals and plants that might be affected by the competitiveness were identified.

(2) Assessment of concrete contents of adverse effects

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(3) Assessment of the possibility that adverse effects may occur

—

(4) Judgment of the possibility that Adverse Effect on Biological Diversity may occur

Based on the above, it was considered that there is no possibility that Adverse Effect on Biological Diversity will occur resulting from the competitiveness of the modified maize.

2 Productivity of harmful substances

(1) Identification of wild animals and plants that may be affected

There have been no reports presenting that maize produces any harmful substances that may affect the living and growing of wild animals and plants.

Succeeding crop tests, plow-in tests, and soil microflora tests were conducted in the isolated field (I-2-(6)-(b)-g, page 16). As a result, no statistically significant differences were noted either in succeeding crop tests or plow-in tests. In soil microflora tests, no statistically significant differences were noted in the numbers of filamentous fungi and bacteria. However, a statistically significant difference was noted in the number of actinomycetes from the non-modified maize. Both the minimum and maximum measured values of the number of actinomycetes were within the range of fluctuation of the number of actinomycetes when the usual manuring practice was conducted in the isolated field. In any lines already approved to which similar genes are introduced, no effects have been noted on the soil microflora. Based on the above, statistically significant differences in the number of actinomycetes post-cultivation between the modified and non-modified maizes were considered occurring because the fluctuation of the number of actinomycetes in the collected samples was incidentally small. Therefore, it is considered unlikely that the productivity of harmful substance is enhanced in the modified maize.

In the modified maize, the modified Cry1F protein, Cry34Ab1 protein, Cry35Ab1 protein and PAT protein are produced. No homology of amino acid sequence has been noted between these proteins and the known allergens (I-2-(1)-2)-(b)-b), page 5).

There has been no report describing that PAT proteins are hazardous against wild animals and plants (ILSI, 2011; OECD, 1999). The PAT protein in the modified maize produces *N-acetylglufosinate* when herbicide glufosinate is sprayed. However, the toxicity of *N-acetylglufosinate* against animals has been confirmed to be lower than that of glufosinate (Food Safety Commission, 2012). Furthermore, *N-acetylglufosinate* is categorized as one of the compounds of glufosinate that should be analyzed under the Agricultural Chemicals Control Act; the pesticide residue limits in food are regulated as glufosinate in maize. Safety of the PAT protein has already been verified (The Japan Food Chemical Research Foundation, 2013).

Each of the modified Cry1F protein and Cry34Ab1/Cry35Ab1 proteins has insecticide activity against lepidopterous pests and coleopteran pests, but does not have toxicity against other animal species (I-2-(1)-2)-(b)-a, page 4).

Regarding lepidoptera insects, 196 species are listed as endangered species and near threatened species in the 4th red list of the Ministry of Environment (2012). In order to identify the species that can be affected by eating the pollen of the modified maize at larval stage, the species that eats plants at the larval stage around the agricultural zone and whose active period at larval stage overlaps the flowering time of the maize (from late May to late October) were investigated referring to the assessment method by Yamamoto, et al. (2003). As a result, 30 species were identified as lepidoptera insects meeting these conditions. Regarding 69 species among the remaining 166 species, sufficient information was not obtained concerning the habitat or active period at the larval stage. Consequently, in total, 99 species were identified as lepidoptera insects that may be affected (Attachment 15).

Regarding coleoptera insects, 4 species were identified as the species that may inhabit near the agricultural zone and eat the pollen of maize or the plants plowed in among the 275 endangered and near threatened species listed in the red list referring to the assessment method by Yamamoto, et al. (2003) (Attachment 16).

(2) Assessment of concrete contents of adverse effects

The LC_{50} value (lethal concentration for killing 50%) against European corn borer, the target insect of the modified Cry1F protein, is 0.58 μg/g (Attachment 2: undisclosed due to confidential information).

LC_{50} value against Northern corn rootworm and Western corn rootworm, the target insect of the Cry34Ab1/Cry35Ab1 protein, is 5.56 μg/cm^2 and 44.5 μg/cm^2, respectively (Attachment 3: undisclosed due to confidential information).

(3) Assessment of the possibility that adverse effects may occur

The possibility was assessed that 99 species of lepidoptera insects and 4 species of coleoptera insects identified in (1) will be affected when they eat the pollen or the plant body of the maize.

Regarding the effects the pollen of the insect-resistant maize may cause to the larvae of insects (lepidoptera monarch butterfly: *Danaus plexippus*), some reports show that effects such as death may occur at the accumulation level of the pollen concentration as high as that in maize cultivated field. Other reports show that the effects on an individual group level are negligible (Attachments 17 and 18).

The conditions that the exposure to the pollen adversely affects larvae of lepidoptera insects and coleoptera insects may include that the pollen of the modified maize is scattered and adhered to the host plants growing around the cultivated field and the larvae eat the plants.

The quantity of pollen accumulation around the maize field is reported to decrease in the area 10 m away from the cultivated field (≤ 10 grains/cm^2; Shirai and Takahashi, 2005; Hansen-Jesse and Obrycki, 2000). Meanwhile, the plant body plowed into the soil after cultivation is decomposed in the soil. Therefore, the exposure to the pollen or plant body of the modified maize is limited in the area around the cultivated field.

Regarding Crambiinae and Acentropinae, small moths, among the identified 99 species of lepidoptera insects, no species inhabit primarily in the limited environment of maize-cultivated field. On the other hand, Aloa lactinea, a large moth, eats maize. Although its population is increasing, increased damage by them is not reported. Taking that into account, Aloa lactinea does not seem to eat maize preferentially. Therefore, the possibility to be affected is limited. It is considered unlikely that the habitat and edible grass of other lepidoptera insects are limited to around the cultivated filed of maize.

Among the identified 4 species of coleoptera insect, the habitat environment of Donacia frontalis, Donacia hirtihumeralis and Donacia japana is in marshy grounds and at the edge of ponds, and no record shows that Diboma costata has been caught from Poaceae plants other than bamboo. Consequently, none of them is considered to inhabit primarily in the limited environment of the cultivated filed of maize.

Based on the above, it is considered unlikely that the identified species of lepidoptera insects and coleoptera insects inhabit locally around the cultivated field of maize and that they will be adversely affected by the modified maize at the individual group level.

(4) Judgment whether Adverse Effect on Biological Diversity may occur or not

Based on the above, it was considered there is no possibility that Adverse Effect on Biological Diversity will occur resulting from the productivity of harmful substances of the modified maize.

3 Crossability

(1) Identification of wild animals and plants that may be affected

No case has been noted in Japan that the maize, the recipient organism, has become wild in Japan. Also, no literature has reported that teosinte and Tripsacum genus which are related wild species and able to cross breed grow naturally. Consequently, no wild animals and plants which may be affected by crossability were identified.

(2) Assessment of concrete contents of adverse effects

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(3) Assessment of the possibility that adverse effects may occur

—

(4) Judgment whether Adverse Effect on Biological Diversity may occur or not

Based on the above, it was considered that there is no possibility that Adverse Effect on Biological Diversity will occur resulting from the crossability of the modified maize.

5 4 Other properties

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III. Overall assessment of Adverse Effect on Biological Diversity

Maize has been cultivated for many years in Japan. No literature has reported that maize became wild and affected inhabiting or growing of wild animals and plants.

Characteristics of the modified maize relating to the competitiveness (morphological and growth characteristics, chilling tolerance at the early stage of growth, overwintering ability of the mature plant, production, shedding, dormancy and germination rate of seeds) were investigated. As a result, uniformity of germination was earlier than that of non-modified maize and a statistical difference was noted in culm length between the modified and non-modified maizes. However, there were no differences in other investigated items including seed production and dormancy. Differences in uniformity of germination and clum length are considered unlikely to become the factors to make the modified maize grow naturally.

Resistance to lepidopterous pests and coleopteran pests is conferred to the modified maize. However, the feeding damage caused by these insects is not the primary factor that makes it difficult for maize to grow in the natural environment in Japan. Consequently, it is considered unlikely that conferring the properties to the modified maize will become the factor to make the modified maize grow in the natural environment. Tolerance to herbicide glufosinate is also conferred. It is also considered unlikely that the herbicide is sprayed in the natural environment.

Therefore, it was considered that there is no possibility that Adverse Effect on Biological Diversity will occur resulting from the competitiveness of the modified maize.

There has been no literature reporting that maize produces harmful substances that may influence inhabiting and growing of wild animals and plants.

In succeeding crop tests and plow-in tests, no statistically significant differences were noted. In the number of actinomycetes measured in soil microflora tests, a statistically significant difference was noted. However, both the minimum and maximum values were within the range of fluctuation of the number of actinomycetes when the usual manuring practice was conducted in the cultivated field. Also, in the lines already approved to which similar genes were introduced, no effects have been noted on soil microflora. Based on the above, a statistically significant difference in the number of actinomycetes in the collected samples appeared to occur between the modified maize and the non-modified maize post-cultivation because fluctuation in the numbers of the collected samples was incidentally small. Consequently, it is considered very unlikely that productivity of harmful substances in the modified maize is enhanced.

The modified Cry1F protein and Cry34Ab1/Cry35Ab1 proteins which are produced in the modified maize have insecticide activity against lepidopterous pests and coleopteran pests. However, they have no toxicity against other animal species. No hazardous properties of PAT proteins against wild animals and plants have been reported.

A total of 99 species in lepidoptera insects and 4 species in coleoptera insects were identified as wild animals and plants that may be influenced by eating the pollen or plow-in plant body of the modified maize. The pollen quantity accumulating around the cultivated field of maize remarkably decreases in the area 10 m away from the cultivated field ($\leq$ 10 grans/cm^2), and the

plant body plowed into the soil after cultivation is decomposed in the cultivated field and in the soil around the field. Therefore, exposure to the pollen or plant body of the modified maize is limited to the area around the cultivated field. The possibility that the identified species of insects inhabit locally around the cultivated field of maize is considered low. Therefore, it is considered unlikely that the insects will be affected by the modified maize at the individual group level.

Based on the above, it was considered that there is no possibility that Adverse Effect on Biological Diversity occurs resulting from the productivity of harmful substances of the modified maize.

There are no wild plants that are able to cross-bleed with maize in Japan. Therefore, it was considered that there is no possibility that Adverse Effect on Biological Diversity will occur resulting from the crossability of the modified maize.

As the overall assessment, it is concluded that there is no possibility that Adverse Effect on Biological Diversity will occur when the modified maize is used according to the regulation of Type 1 Use.

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Attachment List

Undisclosed due to confidential information

1. to 14. (undisclosed due to confidential information)

15. Lepidoptera insects categorized as endangered species and near threatened species which may be influenced

16. Coleoptera insects categorized as endangered species and near threatened species which may be influenced

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