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

Name: Syngenta Seeds K.K.
Okifumi Murata, CEO
Address: 401-2, Mukounodai, Takatsuhara,
Tako-machi, Katori-gun,
Chiba Prefecture

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 (modified *cry1Ab*, modified *vip3A*, *cry1F*, *pat*, *mEPSPS*, *Zea mays* subsp. *mays* (L.) Iltis) (Bt11×MIR162×*B.t.* Cry1F maize line 1507×GA21, OECD UI: SYN-BTØ11-1×SYN-IR162-4×DAS-Ø15Ø7-1×MON-ØØØ21-9) (including the progeny lines isolated from the maize lines, Bt11, MIR162, *B.t.* Cry1F maize line 1507 and GA21, that contains a combination of any of the transferred genes in the individual maize lines (except those already granted an approval regarding Type 1 Use Regulation))
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

The Bt11xMIR162xTC1507xGA21 stacked-trait hybrid maize line possesses resistance to Lepidopteran insect pests and tolerance to glufosinate and glyphosate herbicides. These traits were derived from four (4) recombinant maize parent lines. The Bt11xMIR162xTC1507xGA21 stacked-trait hybrid maize will be commercialized as a hybrid variety (F1). The grain harvested from this stacked-trait hybrid maize line contains genes transferred from the constituent parent lines of this stacked-trait hybrid. The information concerning the composition, origins and functions of the constituents Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn are provided in the following sections.

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of donor nucleic acid and the origins of component elements used for the development of Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn are shown individually in Table 1 to Table 4 (pp. 2 - 6).

Table 1 Origins and functions of the component elements of the donor nucleic acid used for the development of Bt11 corn

Gene cassette resistant to Lepidoptera	
Component elements	Origin and function
35S promoter	A promoter obtained as *Dde*I-*Dde*I fragment derived from cauliflower mosaic virus (CaMV) CM1841 strain. This promoter makes the target gene (modified *cry1Ab*) expressed in all the tissues constitutively (Reference 14).
IVS6-ADH1	An intron derived from the alcohol dehydrogenase 1S (Adh1-S) gene of maize (Reference 15). Adh1-S intron was used to enhance the expression of target genes (modified *cry1Ab*) in plants (Reference 16).
Modified *cry1Ab*	A modified version of the full-length *cry1Ab* gene that encodes the Cry1Ab protein of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 strain, by partially deleting the C-terminal code region which is independent from the insecticidal activity of the Cry1Ab protein and modifying some nucleotide sequences to change the contents of GC and enhance its expression level in plants. This modification does not change any amino acid sequences of the core protein of the Cry1Ab protein.

NOS term	The 3' untranslated region of nopaline synthase (NOS) gene of *Agrobacterium tumefaciens*, which contains a transcription terminator and a signal for polyadenylation of mRNA (Reference 17, Reference 18). This sequence terminates transcription of target genes (modified *cry1Ab*).
Gene cassette tolerant to glufosinate herbicide	
Component elements	Origin and function
35S promoter	A promoter obtained as *Alu*I-*Dde*I fragment derived from cauliflower mosaic virus (CaMV) Cabb-s strain. This promoter makes the target gene (*pat*) expressed in all the tissues constitutively (Reference 19).
IVS2-ADH1	An intron derived from the alcohol dehydrogenase 1S (Adh1-S) gene of maize (Reference 15). Adh1-S intron was used to enhance the expression of the target gene (*pat*) in plants (Reference 16).
pat	The gene that encodes the PAT protein of *Streptomyces viridochromogenes*. The PAT protein, that confers glufosinate herbicide tolerance, was used as a selectable marker for modified plants at the time of transferring of genes. The *pat* gene has some nucleotide sequences modified to change the GC contents and enhance its expression level in plants. The amino acid sequence of the PAT protein expressed by the modification remains unchanged (Reference 20).
NOS term	The 3' untranslated region of nopaline synthase (NOS) gene of *Agrobacterium tumefaciens*, which contains a transcription terminator and a signal for polyadenylation of mRNA (Reference 17, Reference 18). This sequence terminates transcription of the target genes (*pat*).
Other regions (hereinafter referred to as “Backbone region”)	
Component elements	Origin and function
ColE1 ori	The replication origin derived from *Escherichia coli* plasmid pUC18 (Reference 21, Reference 22). Permits replication of plasmid in bacteria.
amp^R	Derived from *Escherichia coli*, it has the function to code β-lactamase and confer the tolerance to antibiotic ampicillin (Reference 22).

Table 2 Origins and functions of the component elements of the donor nucleic acid used for the development of MIR162 corn

Component elements	Origin and function
Gene cassette resistant to Lepidoptera	
ZmUbiInt	Promoter region from *Z. mays* polyubiquitin gene which contains the first intron (1,010bp). It provides constitutive expression of target gene in all the tissues of monocots (Reference 23).
Modified *vip3A*	A modified version of the native *vip3A* gene found in the *B. thuringiensis* AB88 strain, a gram-positive bacteria existing normally in soil (Reference 24), to accommodate the preferred codon usage in plants (Reference 25). It encodes the modified Vip3A protein which exhibits the insecticidal activity against Lepidopteran insect pests. In the modified Vip3A protein, the amino acid at position 284th in the amino acid sequence was substituted to glutamine from lysine. In addition, in the modified Vip3A protein expressed in MIR162, 129th methionine was substituted by isoleucine by mutation of forming transformant, as well as 284th amino acid substitution.
iPEPC9	Intron #9 sequence from the phosphoenolpyruvate carboxylase gene from *Z. mays*. Used to enhance the expression of target gene (Reference 26).
35S	Polyadenylation sequence derived from the cauliflower mosaic virus 35S RNA (Reference 27).
Selectable marker gene cassette	
ZmUbiInt	Same as described above
Pmi	A *manA* gene derived from the K-12 strain of *Escherichia coli* (*E. coli*), encoding phosphomannose isomerase (hereinafter referred to as the "PMI protein"); used as a selectable marker for transgenic plants for which genes are transferred (Reference 28).
NOS	Terminator sequence of the nopaline synthase gene of *Agrobacterium tumefaciens* (Reference 29). Its function is to terminate transcription of mRNA by polyadenylation (Reference 30).
Backbone region	
LB	T-DNA left border region (Reference 31) derived from *Agrobacterium tumefaciens* nopaline Ti-plasmid (Reference 29).
Spec	The streptomycin adenyltransferase gene (*aadA*), derived from the transposon Tn7 of *Escherichia coli* (*E. coli*) (Reference 32). Used as a vector selectable marker to confer resistance to erythromycin, streptomycin, and spectinomycin.
Cos	Sticky-end region of linear DNA of lambda phage which is necessary for transferring plasmid to *Escherichia coli* (*E. coli*) and self-replication of plasmid in *Escherichia coli* (*E. coli*) (Reference 33).
ColE1 ori	The replication origin that permits replication of plasmid in bacteria derived from *Escherichia coli* (*E. coli*) (Reference 34).
RB	T-DNA right border region (Reference 35) derived from *Agrobacterium tumefaciens* nopaline Ti-plasmid (Reference 29).

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Table 3 Origins and functions of the component elements of the donor nucleic acid used for the development of TC1507 corn

Gene cassette resistant to Lepidoptera	
Component elements	Origin and function
UBIZM1(2) Promoter	Ubiquitin constitutive promoter derived from *Zea mays* [1)] (including intron and 5' untranslated region) (Reference 36).
cry1F	A gene that encodes Cry1F protein derived from *B. thuringiensis* var. *aizawai*. Optimized to activate the expression in plants (GenBank AAA22347).
ORF25PolyA Terminator	A terminator to terminate transcription from *A. tumefaciens* pTi5955 (Reference 37).
Gene cassettes tolerant to glufosinate herbicide	
Component elements	Origin and function
CAMV35S Promoter	35S constitutive promoter [1)] derived from cauliflower mosaic virus (CaMV) (Reference 38).
Pat	A gene that encodes phosphinothricin acetyltransferase (PAT protein), derived from *S. viridochromogenes*. Optimized to activate the expression in plants (Reference 39).
CAMV35S Terminator	35S terminator to terminate transcription from cauliflower mosaic virus (CaMV) (Reference 38).

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

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Table 4 Origins and functions of the component elements of the donor nucleic acid used for the development of GA21 corn

Gene cassette tolerant to glyphosate herbicide	
Component elements	Origin and function
Act promoter ＋intron	A promoter derived from the rice actin 1 gene inducing the initiation of transcription of target gene throughout the entire plant body, including up to the first intron region which functions to enhance the efficiency of transcription (Reference 40).
sssu＋mssu (Hereinafter referred to as "OTP")	The optimized transit peptide (OTP) sequences composed of the ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) gene derived from chloroplast transit peptide sequence (sssu) from sunflowers and the *RuBisCo* gene derived chloroplast transit peptide sequence (mssu) from maize, functions to transport the mEPSPS protein expressed by the target gene *mEPSPS* gene to chloroplasts, where the protein takes action (Reference 41).
mEPSPS	A gene obtained from mutation of the 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) maize gene (Reference 42); It encodes the 5-enol-pyruvyl-shikimate-3-phosphate synthase (mEPSPS), the activity of which is not inhibited by the glyphosate herbicide, with the 102nd amino acid threonine in the wild-type EPSPS amino acid sequence modified to isoleucine, and the 106th proline modified to serine (Reference 1).
NOS	A polyadenylation sequence of the nopaline synthase (NOS) gene from

	Agrobacterium tumefaciens, terminating transcription (Reference 17).
Backbone region	
Component elements	Origin and function
amp	Consists of the lac sequence, composed of partial coding sequence for lacI derived from bacteriophage M13, promoter plac and partial coding sequence for β-galactosidase or lacZ protein (Reference 22), and the β-lactamase gene (*bla*) conferring the ampicillin tolerance derived from plasmid pBR322 of *Escherichia coli* (Reference 43); selects and maintains the *Escherichia coli* which contains the constitutive plasmid by expression of β-lactamase.
ori-puc	The replication origin region derived from the plasmid pUC19 of *Escherichia coli*, conferring the autonomous replication potency of the plasmid in *Escherichia coli* (Reference 35).

2) Function of component elements

(a) Functions of the individual component elements of donor nucleic acid, including target gene, expression regulatory region, localization signal, and selectable marker

Functions of individual component elements of donor nucleic acid used for the development of Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn are individually shown in Table 1 to Table 4 (pp. 2 - 6).

(b) Functions of proteins produced by the expression of target genes and selectable markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergen (except allergenicity as food)

[The insecticidal protein]

Insecticidal proteins isolated from the soil microorganism *Bacillus thuringiensis*, Cry1Ab, Vip3A and Cry1F, exhibit insecticidal activities against specific species of insects. When fed upon by sensitive species of insects, the Bt proteins become active polypeptides (= core protein) via activation of the protein during digestion. The activated proteins selectively bind specific receptors located on the surface of the midgut the brush border of sensitive insects. Binding of the receptors results in cytoclasis destruction of the digestive tract and mortality (Reference 44).

Modified Cry1Ab protein:

Regarding the insecticidal activity of modified Cry1Ab (mCry1Ab) protein which has the same amino acid sequence of Cry1Ab, detailed experimental results are listed in the database operated by the Canadian Government (Reference 45), showing that it exhibits insecticidal activity against European corn borer (*Ostrinia nubilalis*), Corn earworm (*Helicoverpa zea*), Fall armyworm (*Spodoptera frugiperda*) and other Lepidopteran insects which are major pests of maize cultivation. mCry1Ab protein exhibits little to no insecticidal activity against non-Lepidopteran insects.

Modified Vip3A protein:

The modified Vip3A protein exhibits high insecticidal activity against Fall armyworm (*S. frugiperda*), Corn earworm (*H. zea*), Black cutworm (*A. ipsilon*) and other order Lepidopteran insects considered pests of maize cultivation in the US. However, the modified Vip3A protein does not exhibit insecticidal activity against certain Lepidopteran insects including the European corn borer (*O. nubilalis*) and *Danaus plexippus* to which the Cry1Ab protein shows insecticidal activity (Reference 46).

In Reference 46 (Lee *et al.*), it was reported that Vip3A protein and the Cry1Ab protein selectively bind receptors located onbrush border membrane vesicles (BBMV) without competing with one another. In addition, the Vip3A protein does not bind to amino peptidase-like molecules and cadherin-like molecules known as receptors of the Cry1Ab protein in the BBMV of Tobacco hornworm (*Manduca sexta*), a sensitive Lepidopteran insect (Reference 46). Consequently, it has been suggested that Vip3A protein acts via a mechanism of action similar to the Cry protein, though Vip3A protein differs from the Cry1Ab protein regarding the receptors involved (Reference 46).

In comparison to the Vip3A protein of *Bacillus thuringiensis* AB88 strain, the modified Vip3A protein presents the following differences: the 284th amino acid sequence (lysine) of the modified Vip3A protein is replaced by glutamine and the 129th methionine is replaced by isoleucine by mutation of forming transformant, as well as 284th amino acid substitution.

Cry1F protein:

In order to investigate the insecticidal spectrum of the Cry1F protein, Cry1F protein produced in the *Pseudomonas fluorescens* was added to artificial feed and administered 15 different Lepidopteran insects considered pests in the US. Of the 15 Lepidopterans selected, six (6) are regarded as pests of maize cultivation in the US and remaining nine (9) are considered pests of cotton, soybean, canola and other crops. Among the six (6) maize pests, Cry1F protein demonstrated greater insecticidal activity against European corn borer (*O. nubilalis*), Fall armyworm (*S. frugiperda*) and Beet armyworm (*Spodoptera exigua*), target insect pests of TC1507 corn. With regard to Southwestern corn borer (*Diatraea grandiosella*), Black cutworm (*Agrotis ipsilon*) and Ballworm, Cry1F demonstrated lower insecticidal activity. Though not regarded as an agricultural insect pest, *Danaus plexippus*, was also evaluated and at the maximum dose obtained in the test, the death rate of *Danaus plexippus* was found to be equivalent to that in the control plot. Based upon on these results, it was determined thatCry1F protein possess a selective insecticidal spectrum (Reference 47) and thus offers insecticidal activity against limited insects.

In addition to the insects of the order Lepidoptera, tests were also conducted on insects of the orders of Coleoptera, Hymenoptera, Neuroptera, and Collembola as well as mammals, birds, and fish. Cry1F protein exhibited no toxicity against any of the non-target organisms tested (Reference 48).

[Herbicide tolerant protein]

PAT protein:

Glufosinate herbicides inhibit glutamine synthase in plants resulting in cellular accumulation of ammonia and mortality. However, the expression of the PAT protein results in acetylation and inactivation of glufosinate. As such, glutamine synthase is not inhibited, thus preventing cellular ammonia accumulation.

mEPSPS protein:

Glyphosate, a non-selective herbicide acting on stems and leaves, inhibits the activity of 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS), one of the enzymes in the shikimate pathway for aromatic amino acid biosynthesis, and interrupts the aromatic amino acid biosynthesis, thereby causing plant mortality (Reference 49). The mEPSPS protein encoded by the *mEPSPS* gene exhibits EPSPS activity even in the presence of glyphosate herbicide, enabling aromatic amino acid biosynthesis, thereby conferring tolerance to glyphosate herbicide.

[Selectable marker]

PAT protein:

Glufosinate herbicides inhibit glutamine synthase in plants resulting in cellular accumulation of ammonia and mortality. However, the expression of the PAT protein results in acetylation and inactivation of glufosinate. As such, glutamine synthase is not inhibited, thus preventing cellular ammonia accumulation.

PMI protein:

The *pmi* gene derived from *Escherichia coli*, encodes the phosphomannose isomerase (PMI) protein. PMI catalyzes the reversible interconversion of mannose-6-phosphate and fructose-6-phosphate. Generally, maize and many other plants cannot utilize mannose as a carbon source. Methodologies involoving transference of the *pmi* gene into plant cells as a selectable marker with the target gene and subsequent incubation of transformed cells in mannose-containing medium can be employed to select for cells containing the target gene (Reference 28). The PMI protein exists widely in nature, including the human digestive system, and soybean and other plants. It has not been identified in maize.

A homology search using the publicly available database (SWISS-PROT, FARRP, etc.) indicated that modified Cry1Ab protein, modified Vip3A protein, Cry1F protein, PAT protein, mEPSPS protein and the PMI protein do not share structurally related homologous sequences with any of the known allergens.

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

There are non data suggesting that modified Cry1Ab protein, mVip3A protein or Cry1F protein possess enzymatic activity. Thus, it is considered very unlikely that these proteins affect the metabolic activities of the recipient organisms.

The PAT protein possesses very high substrate specificity to L-phosphinothricin (glufosinate herbicide) and dimethyl phosphinothricin, and there is no other protein or amino acid reported for the substrate of the PAT protein (Reference 50). Consequently, it is considered very unlikely that the PAT protein affects the metabolic activity of the recipient organism.

The mEPSPS protein is one of the enzymes that catalyze the shikimate pathway (Reference 51), and it is reported to react specifically with phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Reference 52). Consequently, it is considered very unlikely that the mEPSPS protein affects the metabolic activity of the recipient organism.

The PMI protein has the capability of catalyzing the reversible interconversion of mannose-6-phosphate and fructose-6-phosphate. PMI reacts specifically with mannose-6-phosphate and fructose-6-phosphate, and there are no other known natural substrates for the PMI protein (Reference 53). Consequently, it is considered very unlikely that the PMI protein affects the metabolic activity if the recipient organism.

(2) Information concerning vectors

1) Name and origin

The plasmid vectors used for the development of the Bt11 corn, MIR162 corn, TC1507 corn and GA21 cron are as follows.

Bt11 corn:	pZO1502 constructed based on the pUC18 derived from *Escherichia coli* (*E. coli*)
MIR162 corn:	pNOV1300 constructed based on the pSB12 (Reference 54)
TC1507 corn:	PHP8999 constructed based on the pUC19 derived from *Escherichia coli* (*E. coli*)
GA21 corn:	pDPG434 constructed based on the pUC19 derived from *Escherichia coli* (*E. coli*)

2) Properties

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

The total number of base pairs of the plasmids used for the development of Bt11 corn, MIR162 corn, TC1507 corn and GA21 are listed below, and the nucleotide sequences of these plasmids are disclosed.

Bt11 corn:	A total of 7,240 bp of the plasmid pZO1502
MIR162 corn:	A total of 14,405 bp of the plasmid pNOV1300
TC1507 corn:	A total of 9,504 bp of the plasmid PHP8999
GA21corn:	A total of 6,128bp of the plasmid pDPG434 (Reference 1)

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

The nucleotide sequence having specific functions included in plasmids and used for the development of Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn refers to the following antibiotic resistant marker genes.

However, these antibiotic resistant marker genes are not transferred in the recipient organism.

Bt11 corn:	*amp*^{R} gene, ampicillin resistance
MIR162 corn:	*spec* gene to confer resistance to streptomycin, erythromycin and spectinomycin
TC1507 corn:	*nptII* gene to confer resistance to kanamycin
GA21 corn:	*amp*^{R} gene, ampicillin resistance (Reference 1)

(c) Presence or absence of infectivity of vector and, if present, the information concerning the host range

There are no data suggesting that the plasmids pZO1502, PHP8999 and pDPG434 used for the development of Bt11 corn, TC1507 corn and GA21 corn contain any sequence showing infectivity. In addition, the cos, the sticky-end region derived from lambda phage which permits transferring plasmids to *Escherichia coli* (*E. coli*), exists in the plasmid pNOV1300 used for the development of MIR162 corn; the recipient organism other than *Escherichia coli* (*E. coli*) of lambda phage is not known.

(3) Method of preparing living modified organisms

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

The nucleic acids transferred in the recipient organism of Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn are as follows.

Bt11 corn:	A part obtained by cleaving the plasmid pZO1502 by the restriction enzyme *Not*I and removing the *amp*^{R} gene
MIR162 corn:	Two gene expression cassettes (insect pest-resistant gene cassette and selectable marker gene cassette) between RB and LB of T-DNA region
TC1507 corn:	Two gene expression cassettes (insect pest-resistant gene cassette and herbicide glufosinate-tolerant gene cassette) transferred in the TC1507 corn
GA21 corn:	A DNA fragment composed of the herbicide tolerant gene cassette (Act promoter + intron/OTP/*mEPSPS*/NOS) obtained by cleaving the plasmid pDPG434 by the restriction enzyme *Not*I (Reference 1)

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

The following methods were used to transfer the nucleic acid to the recipient

organisms.

Bt11 corn:	Electroporation method
MIR162 corn:	*Agrobacterium* method
TC1507 corn:	Particle gun bombardment
GA21 corn:	Particle gun bombardment (Reference 1)

3) Processes of rearing of living modified organisms

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

Transformed cells were selected on the medium containing the substances listed below for individual recipient organisms.

Bt11 corn:	Glufosinate
MIR162 corn:	Mannose
TC1507 corn:	Glufosinate
GA21 corn:	Glyphosate (Reference 1)

(b) Presence or absence of remaining *Agrobacterium* when using the *Agrobacterium* method for transferring nucleic acid

For MIR162 corn, after transference of genes, the antibiotic Cefotaxime was added to the culture cell medium to remove any residual *Agrobacterium* used for the transformation. PCR was then carried out for regenerated plants, and the individual plants not containing the antibiotic-resistant marker gene in the backbone of plasmid were selected. As a result of this selection method, there is no remaining *Agrobacterium.*

(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 transferred nucleic acid was confirmed, the line subjected to isolated field tests; and the line used for collection of other necessary information for assessment of Adverse Effect on Biological Diversity

This stacked-trait hybrid maize line was developed by cross-breeding with Bt11 corn (maize resistant to Lepidoptera and tolerant to glufosinate herbicide), MIR162 corn (maize resistant to Lepidoptera), TC1507 corn (maize resistant to Lepidoptera and tolerant to glufosinate herbicide) and GA21 corn (maize tolerant to glyphosate herbicide). The status of approvals and applications for approval of Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn in Japan are listed in Table 5 (p. 13).

Table 5 Status of application for approval of Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn in Japan

	Safety as food	Safety as feed	Environmental safety
Bt11corn	March, 2001: Approved safety of use as food	March, 2003: Approved safety of use as feed	April, 2007: Approved for Type 1 Use Regulation
MIR162 corn	January, 2010: Approved safety of use as food	June, 2010: Approved safety of use as feed	June, 2010: Approved for Type 1 Use Regulation
TC1507 corn	July, 2002: Approved safety of use as food	March, 2003: Approved safety of use as feed	March, 2005: Approved for Type 1 Use Regulation
GA21 corn	March, 2003: Approved safety of use as food	March, 2003: Approved safety of use as feed	November, 2005: Approved for Type 1 Use Regulation
This stacked-trait hybrid maize line	May, 2010: Approved safety of use as food	June, 2010: Approved safety of use as feed	April, 2010: Pending application

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

1) Place where the replication product of transferred nucleic acid exists

It was confirmed that the transferred genes in Bt11 corn, MIR162 corn, TC1507 corn and GA21corn exist on the maize genome.

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

In Bt11 corn, MIR162 corn and TC1507 corn, it was confirmed that one copy of each exists in the chromosome and that the transferred genes were all inherited stably through multiple generations via Southern blot analysis for the number of copies of the transferred gene..

it was confirmed n GA21 corn, via Southern blot analysis for the number of copies of the transferred gene, that transferred genes exist in the chromosome at one site, it consists of six (6) consecutive regions derived from the fragment of the transferred herbicide-tolerant gene cassette (Act promoter + intron/OTP/*mEPSPS*/NOS), and that the transferred genes are all stably inherited through multiple generations.

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

4) Inter-individual or inter-generational expression stability under a natural environment with respect to the characteristics referred to specifically in (6)-1)

The stability of expression was identified as follows.

Bt11 corn:	Confirming the expression of proteins by ELISA method, the bioassay using pest insects of the order Lepidoptera, and glufosinate herbicide-spraying test
MIR162 corn:	Confirming the expression of proteins by ELISA method, and the bioassay using pest insects of the order Lepidoptera
TC1507 corn:	Confirming the expression of proteins by ELISA method, the bioassay using pest insects of the order Lepidoptera, and glufosinate herbicide-spraying test
GA21 corn:	Glyphosate herbicide-spraying test

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

The transferred nucleic acid in Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn do not contain any sequence allowing transmission. Therefore, it is considered unlikely that the nucleic acid transferred to those plants could be transmitted to any other wild animals and wild plants.

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

For specific detection of Bt11 and GA21 traits, a method based on the quantitative PCR analysis is available from the European Commission. The detection sensitivity is as follows in terms of the ratio of concentration of genome DNA: 0.08% and over for Bt11 and 0.04% and over for GA21 (Reference 55, Reference 56). In addition, as the detection method for corn event MIR162, the result of Southern blot analysis conducted as follows can be used: the genome DNA 7.5 μg was cut by a restriction enzyme and the modified *vip3A* gene was used as a probe. For the detection and identification of TC1507, a quantitative analysis kit is available from GeneScan Europe AG (Freiberg, Germany) applying an RT (Real Time)-PCR method using the nucleotide sequence specific to TC1507 corn as primers.

In order to detect and identify this stacked-trait hybrid maize line, the above-mentioned methods must be applied to each grain of maize seeds.

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

1) Specific contents of physiological or ecological characteristics that were accompanied by the expression of replication products of transferred nucleic acid

This stacked-trait hybrid maize line is given the traits as described below.

From Bt11 corn: Resistance to Lepidoptera and tolerance to glufosinate herbicide due to the modified Cry1Ab protein and the PAT protein respectively derived from the transferred genes

From MIR162 corn: Resistance to Lepidoptera and a selectable marker due to modified Vip3A protein and PMI protein, respectively, derived from the transferred genes

From TC1507 corn: Resistance to Lepidoptera and tolerance to glufosinate herbicide due to Cry1F protein and the PAT protein, respectively, derived from the transferred genes

From GA21 corn: Tolerance to glyphosate herbicide due to the mEPSPS protein derived from the transferred genes

Modified Cry1Ab protein, modified Vip3A protein and Cry1F protein, when ingested and activated via digesttion by the sensitive species of insects, bind to specific receptors on the cell membranes of midgut epithelium in the target insects. However, it is modified Cry1Ab protein, modified Vip3A protein and Cry1F protein function independently from each other. There are no data to suggest that modified Cry1Ab protein, modified Vip3A protein and Cry1F protein possess any enzymatic activity. Therefore, it is considered unlikely that these proteins would affect the metabolic activity of their recipient organisms. It is considered unlikely that expression of modified Cry1Ab protein, modified Vip3A protein and Cry1F protein in this stacked-trait hybrid maize line would cause emergence of any sensitive species of insects. Furthermore, there are no data suggesting that any stack lines in which multiple insect pest-resistant proteins are expressed exhibit combined effect in terms of resistance to pest insects.

The PAT protein possesses very high substrate specificity to L-phosphinothricin (glufosinate herbicide) and dimethyl phosphinothricin. There are no other proteins or amino acids reported as substrates as of the PAT protein (Reference 50). The mEPSPS protein is an enzymes of the the shikimate pathway (Reference 51), and it is reported to react specifically with phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Reference 52). In addition, the PMI protein is an

enzyme protein that catalyzes the reversible interconversion between mannose-6-phosphate and fructose-6-phosphate. PMI protein reacts selectively with mannose-6-phosphate and fructose-6-phosphate. There is no other natural substrate known for the PMI protein (Reference 53). Consequently, it is considered unlikely that PAT protein, mEPSPS protein or PMI protein would affect the metabolic system of their recipient organisms.

Given that modified Cry1Ab protein, modified Vip3A protein and Cry1F protein expressed in this stacked-trait hybrid maize line differ from each other with regard to specificity are not reported to possess any enzymatic activity; possess PAT protein of extremely high substrate specificity; possess EPSPS protein selective for phosphoenolpyruvic acid (PEP) and shikimate-3-phosphate (S3P), and PMI protein selective for the mannose-6-phosphate and fructose-6-phosphate, it is considered unlikely that these proteins exhibit functional interaction.

In order to confirm that the proteins expressed in this stacked-trait hybrid maize, from the individual parent lines, would interact with each other, the stacked-trait hybrid maize was tested as below. Maize possessing the same genetic background (NP2222×5XH751) as the stacked-trait hybrid maize, was employed as a non-recombinant control maize.

[Bioassay using insects of the order Lepidoptera]

In assessing resistance to Lepidoptera, the severity of insect damage was evaluated using European corn borer and Fall armyworm, target insect pests of Cry1Ab protein and Cry1F protein, and modified Vip3A protein.

For investigating the severity of insect damage by European corn borer, the stacked-trait hybrid maize line, Bt11 corn, MIR162 corn, TC1507 corn, and the non-recombinant control maize were cultivated in greenhouses in three (3) fields in the U.S. in 2009. The first instar larvae of European corn borer were inoculated at the 6th to 8th leaf stage of maize, and on the 15th and 19th day after inoculation, the severity of insect damage was observed visually.

The test site in Illinois, presented a significant difference with regards to (LSD (Leaf Damage Rating) after F-test, $p<0.05$) (Table 6, p. 16) between the stacked-trait hybrid maize and TC1507 corn. However, in Minnesota and Iowa, no significant difference between the stacked-trait hybrid maize, Bt11 corn and TC1507 corn, No consistent compatibility was observed. Therefore, it was concluded that the insecticidal activity of the stacked-trait hybrid maize against Lepidopteran pests (European corn borer) remains virtually unchanged by crossing of parent lines.

Table 6 Severity of damage to plant body by pest insects of the order Lepidoptera (European corn borer) based on bioassay of this stacked-trait hybrid maize line

Location of test	This stacked-trait hybrid maize line		Bt11		MIR162		TC1507 corn		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
Stanton, Minnesota	1.4 c [1]	0.4	1.5 c	0.4	5.5 b	0.3	1.5 c	0.4	7.3 a	0.5
Slater, Iowa	2.0 b	0.0	2.0 b	0.0	7.9 a	0.9	2.0 b	0.0	8.5 a	0.3
Bloomington, Illinois	1.5 c	0.3	1.7 c	0.2	7.4 a	0.1	1.9 b	0.1	7.4 a	0.2

Investigation for severity of insect damage was conducted for 5 plant bodies and 4 repeats.
1: Severity of insect damage was evaluated based on 9-step scales (1 (No damage) to 9 (Seriously damaged)) (Reference 57).
2: Statistical treatment was conducted at each test site, and there is no significant difference in the mean value expressed in the same alphabetical letter (LSD after F-test, $p<0.05$).

(All the rights pertinent to the information in the table above and the responsibility for the content rest upon Syngenta Seeds K.K.)

In assessing the severity of insect damage by Fall armyworm, the stacked-trait hybrid maize, Bt11 corn, MIR162 corn, TC1507 corn and their non-recombinant maizecontrol were cultivated in greenhouses in three (3) fields in the U.S. in 2009. The first instar larvae of Fall armyworm were inoculated at the 5th to 7th leaf stage of maize, and on the 10th to 11th day after inoculation, the severity of insect damage was observed visually.

A significant difference was observed between the stacked-trait hybrid maize and MIR162 at the test site in Minnesota.. However, the Iowa and Illinois test sites presented no significant differences the stacked-trait hybrid maize and MIR162 corn (LSD after F-test, $p<0.05$) (Table 7, p. 18), and no consistent compatibility was observed. Therefore, it is concluded that the insecticidal activity of this stacked-trait hybrid maize line against pest insects of the order Lepidoptera (Fall armyworm) remains virtually unchanged by crossing of parent lines.

Table 7 Severity of insect damage to this stacked-trait hybrid maize line by pest insects of the order Lepidoptera (Fall Armyworm)

Location of test	This stacked-trait hybrid maize line		Bt11		MIR162		TC1507 corn		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
Stanton, Minnesota	1.2 e [1]	0.2	5.2 b	0.3	2.2 d	0.9	3.5 c	0.4	8.7 a	0.4
Slater, Iowa	1.0 d	0.0	6.1 b	0.4	1.0 d	0.0	4.4 c	0.3	9.0 a	0.1
Bloomington, Illinois	1.9 d	0.2	6.2 b	0.5	2.0 d	0.1	2.9 c	0.1	8.3 a	0.2

Investigation for severity of insect damage was conducted for 5 plant bodies and 4 repeats.
1: Severity of insect damage was evaluated based on 9-step scales (1 (No damage) to 9 (Seriously damaged)) (Reference 58).
2: Statistical treatment was conducted at each test site, and there is no significant difference in the mean value expressed in the same alphabetical letter (LSD after F-test, $p<0.05$).

[Bioassay using glufosinate herbicide]

Regarding tolerance to glufosinate, the stacked-trait hybrid maize , Bt11 corn, TC1507 corn and the non-recombinant control maize were cultivated in a greenhouse in the U.S. in 2008, and the severity of injury incurred by the spraying of herbicide was assessed. At the 2nd leaf stage of maize maize plants were sprayed with glufosinate (Product name: Liberty™) at concentrations of 467g active ingredient (a.i.)/ha (normal dosage), 1,868g a.i./ha (4-times higher dosage) and 3,736g a.i./ha (8-times higher dosage).On the 10th day after spraying herbicide glufosinate, the severity of injury was observed visually.

The severity of insect damage to the stacked-trait hybrid maize line was significantly lower when compared to Bt11 corn, though the severity was similar to that of TC1507 corn (Table 8, p. 19). Therefore, it was concluded that the tolerance of this stacked-trait hybrid maize line to herbicide glufosinate remains unchanged by crossing of parent lines.

Table 8 Investigation result of the severity of injury by spraying of herbicide glufosinate to this stacked-trait hybrid maize line

Concentration of herbicide (g.a.i/ha)	Levels of herbicide injury (%) [1]							
	This stacked-trait hybrid maize line		Bt11		TC1507 corn		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
467	0.17 g [2]	0.9	0.47 g	1.3	0.0 g	0.0	76.33 b	5.1
1868	5.0 f	0.0	9.93 e	2.0	5.47 f	1.3	100.0 a	0.0
3736	11.63 d	2.3	19.43 c	3.0	11.4 d	2.3	100.0 a	0.0

Investigation for severity of herbicide injury was conducted for 10 plant bodies and 3 repeats.

1: For each maize line, a non-sprayed plot was prepared. The level of herbicide injury in the non-sprayed plot is set as 0% (intact) for comparison. Then the levels of herbicide injury were evaluated based on the scale from 0% (intact) to 100% (complete death) in the herbicide sprayed plots.

2: There is no significant difference in the mean value expressed in the same alphabetical letter (Student-Newman-Keuls test, $p<0.05$).

[Bioassay using glyphosate herbicide]

Regarding tolerance to glyphosate, the stacked-trait hybrid maize, GA21 corn and the non-recombinant control maize were cultivated in a greenhouse in the U.S. in 2008, and the severity of injury by spraying of herbicide was investigated. At the 2nd leaf stage, glyphosate (Product name: Touchdown Total™) was sprayed at concentrations of 840 g acid equivalent (a.i.)/ha (normal dosage), 3,360 g a.e./ha (4-times higher dosage) and 6,720 g a.e./ha (8-times higher dosage). On the 19th day after spraying herbicide glyphosate, the severity of injury was observed visually.

No significant difference between this stacked-trait hybrid maize line and GA21 was observed in the severity of herbicide injury (Table 9, p. 20). Therefore, it was concluded that tolerance of this stacked-trait hybrid maize line to herbicide glyphosate remains unchanged by crossing of parent lines.

Table 9 Investigation result of the severity of injury by spraying of herbicide glyphosate to this stacked-trait hybrid maize line

Concentration of herbicide (g.a.e/ha)	Levels of herbicide injury (%) [1]					
	This stacked-trait hybrid maize line		GA21		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
840	0.57 d [2]	1.2	0.57 d	1.1	100.0 a	0.0
3360	18.8 c	8.7	17.2 c	10.1	100.0 a	0.0
6720	29.0 b	8.1	28.0 b	7.1	100.0 a	0.0

Investigation for severity of herbicide injury was conducted for 10 plant bodies and 3 repeats.

1: For each maize line, a non-sprayed plot was prepared. The level of herbicide injury in the non-sprayed plot is set as 0% (intact) for comparison. Then the levels of herbicide injury were evaluated based on the scale from 0% (intact) to 100% (complete death) in the herbicide sprayed plots.

2: There is no significant difference in the mean value expressed in the same alphabetical letter (Student-Newman-Keuls test, $p<0.05$).

(All the rights pertinent to the information in the table above and the responsibility for the content rest upon Syngenta Seeds K.K.)

Based on the results presented in table 9, it was concluded that the individual proteins expressed in the relevant parental lines do not interact with each other and that the traits obtained from the transferred genes remained unchanged in the stacked-trait hybrid maize.

Regarding the differences in physiological or ecological characteristics between the stacked-trait hybrid maize and the taxonomic species to which the recipient organism belongs, evaluation was conducted based on the results of individual examinations on the parent lines Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn.

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

(a) Morphological and growth characteristics

For the morphological and growth characteristics of Bt11 corn, MIR162 corn, TC1507 corn, GA21 corn and their non-recombinant control maize, an assessment was conducted for the items listed in Table 10 (p. 21) in an isolated field in Japan. In all items examined except culm length of MIR162 corn and germination rate and ear diameter of TC1507 corn, no significant difference was observed, or comparable results presented. For TC1507 corn, a significant difference was observed from its non-recombinant control maize, though no consistent trend was identified between the two species examined (Annex 1, 2, 3 and 4; Confidential: Not disclosed to unauthorized person).

Table 10 Investigation results of morphological and growth characteristics of Bt11 corn, MIR162 corn, TC1507 corn and GA21corn

	Bt11	MIR162	TC1507 corn	GA21
Start of germination	-	○	-	○
Uniformity of germination	○	○	○	○
Germination rate	○	○	○	○
Time of tasseling	○	○	○	○
Time of silking	○	○	○	○
Time of flower initiation	○	-	-	○
Time of flower completion	○	-	-	○
Flowering period	○	-	-	-
Culm length	○	○	○	○
Plant shape	○	○	○	○
Tiller number	○	○	○	○
Height of ear	○	○	○	○
Maturation time	○	○	○	○
Number of ears (Total number of ears)	○	-	○	○
Number of productive ears	○	○	○	○
Ear length	○	○	○	○
Ear diameter	○	○	○	○
Row number per ear	○	○	○	○
Grain number per row	○	○	○	○
Grain color	○	○	○	○
100-kernel weight	○	○	○	○
Grain shape	○	○	○	○
Fresh weight of above-ground parts at the harvest time	○	○	○	-
Plant weight at the harvest time (Total plant weight)	-	-	-	○

○: Examined

-: Notexamined

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

Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn withered or died due to the low temperatures at the early stage of growth similarly to their non-recombinant control maize (Annex 1, 2, 3 and 4; Confidential: Not disclosed to unauthorized person).

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

Maize is a summer type annual plant, and after ripening the matured plant body usually withers and dies out. In fact, there is no report that, maize has further propagated by vegetative parts, set seeds again, or produced seeds after maturity. Actually, at the end of isolated field tests, withering had begun and death after ripening was observed.

(d) Fertility and size of the pollen

Per observation under a microscope, no difference was observed in fertility (maturity of the pollen due to staining), shape and size of the pollen between Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn, and their non-recombinant control maize (Annex 1, 2, 3, 4; Confidential: Not disclosed to unauthorized person).

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

Regarding seed production, comparisons were conducted between Bt11 corn, MIR162 corn, TC1507 corn, GA21 corn and their non-recombinant control maize for the characteristics referring to the production of seeds. A significant difference was observed between TC1507 corn and its non-recombinant control maize regarding ear diameter. For TC1507 corn, a significant difference was observed from its non-recombinant control maize, though no consistent trend was observed between the two species examined (Annex 1, 2, 3, 4; Confidential: Not disclosed to unauthorized person).

Regarding shedding of seed, maize seed never shed spontaneously, they adheres to ears and the ears are covered with husks (Reference 3). Thus, as observed in the non-recombinant maize, the ears of Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn, of were found covered with husk at harvest time.

Regarding germination rate of harvested seeds, Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn all showed comparable results to those of their non-recombinant control maize (Annex 1, 2, 3, 4; Confidential: Not disclosed to unauthorized person). Consequently, it was considered unlikely

that the dormancy of Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn would differ significantly from that of their non-recombinant control maize.

(f) Crossability

Crossability test was not performed for the parent lines Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn since there is no report that any wild relatives that can be crossed with maize are growing voluntarily in Japan.

(g) Productivity of harmful substances

Plow-in tests, succeeding crop tests and soil microflora tests conducted for Bt11 corn, MIR162 corn, TC1507 corn and GA21 corn, presented no significant difference from their non-recombinant control maize. A significant difference was observed in the fresh weight of lettuce in the succeeding crop tests and plow-in tests for TC1507 corn and its non-recombinant control maize. However, no consistent trend was observed between the two species examined (Annex 1, 2, 3, 4; Confidential: Not disclosed to unauthorized person).

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.

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

This stacked-trait hybrid maize line was developed by crossing maize resistant to Lepidoptera and tolerant to glufosinate herbicide (Bt11), maize resistant to Lepidoptera (MIR162), maize resistant to Lepidoptera and tolerant to glufosinate herbicide (TC1507) and maize tolerant to glyphosate herbicide (GA21). These parent lines were individually judged at the Committee for Review on the Biological Diversity Risk Assessment as causing no Adverse Effect on Biological Diversity when used in line with Type 1 Use described in the application for this stacked-trait hybrid maize line.

It was considered that the specificity of the Bt proteins might be governed by the structure of the proteins and the proteins would bind to different receptors in the midgut cell of pest insects. In addition, there are no data demonstrating that the Bt proteins have exhibited synergetic activity. Consequently, it was considered unlikely that the constituent Bt proteins of this stacked-trait hybrid maize (modified Cry1Ab protein, modified Vip3A protein and Cry1F protein) would interact with each other in this stacked-trait hybrid maize line and affect the specificity of the constituent Bt proteins. Furthermore, the PAT protein, the mEPSPS protein and the PMI protein differ from each other with regard to their substrates, mechanism of action, and their involved metabolic pathways. There are no data to suggest that the Bt proteins possesses any enzymatic activity. Therefore, it was considered unlikely that these proteins, even if expressed in this stacked-trait hybrid maize line, would interact with each other to affect the metabolic activity of their recipient organisms and produce any unexpected metabolites. Consequently, it was considered unlikely that the proteins expressed in this stacked-trait hybrid maize line would exhibit functional interaction with each other.

In addition, resistance to Lepidoptera, tolerance to glufosinate herbicide, and tolerance to glyphosate herbicide expressed in this stacked-trait hybrid maize line were found to be expressed at similar levels by the individual parent lines. Thus, it is considered unlikely that the proteins expressed in this stacked-trait hybrid maize line from individual parent lines would cause functional interaction in the plant body of

this stacked-trait hybrid maize line, and it is considered unlikely that any notable changes in traits have occurred in this stacked-trait hybrid maize line except for the traits that it received from the parent lines.

(1) Competitiveness

Maize (*Zea mays* subsp. *mays* (L.) Iltis), the taxonomical species to which the recipient organism belongs, has been long used in Japan, though there is no report that it has become self-seeding in the natural environment in Japan.

An assessment of various characteristics referring to the competitiveness of Bt11 corn, MIR162corn, TC1507, GA21 corn, and the parent lines of this stacked-trait hybrid maize, presented a significant difference between the stacked-trait hybrid maize line and its non-recombinant maize regarding some items examined. However, the differences were judged not to be so large as to enhance the competitiveness of this stacked-trait hybrid maize line.

This stacked-trait hybrid maize line is given traits to be resistant to the insects of order Lepidoptera. However, insect damage by Lepidopteran insects is it is not generally considered a major cause in making maize difficult to grow in the natural environment in Japan. Consequently, it is considered unlikely that this trait causes maize to become self-seeding in the natural environment and enhance the competitiveness. This stacked-trait hybrid maize line is given traits to be tolerant to glufosinate and glyphosate herbicides, however, it is unlikely that glufosinate and glyphosate herbicides are sprayed in the natural environment in Japan. Consequently, it is considered that this trait does not increase the competitiveness of this stacked-trait hybrid maize line. In addition, this stacked-trait hybrid maize line is also given traits to produce the PMI protein in which mannose can be a carbon source. However, it is not expected that this stacked-trait hybrid maize line uses the mannose as a carbon source in the natural environment in Japan. Therefore, it is considered unlikely that this trait enhances the competitiveness of this stacked-trait hybrid maize line.

Based on the above understanding, it was determined that the following conclusion made by the applicant is valid: This stacked-trait hybrid maize line and the progeny lines of stacked-trait hybrid maize line isolated from the parent lines of this stacked-trait hybrid maize line, Bt11, MIR162, Cry1F maize line 1507 and GA21, that contain a combination of any of the transferred genes in the individual parent lines, would pose no risk of Adverse Effect on Biological Diversity that is attributable to competitiveness.

(2) Productivity of harmful substances

Maize (*Zea mays* subsp. *mays* (L.) Iltis), the taxonomical species to which the recipient organism belongs, has been long used in Japan, though it is not generally known that the maize produces any harmful substances that could affect wild animals and wild plants.

It has been confirmed that the modified Cry1Ab protein, the modified Vip3A protein, the Cry1F protein, the PAT protein, the mEPSPS protein and the PMI protein expressed in this stacked-trait hybrid maize line do not have any homology with any of the known allergens.

In addition, the modified Cry1Ab protein, the modified Vip3A protein, the Cry1F protein, the PAT protein, the mEPSPS protein and the PMI protein expressed in this stacked-trait hybrid maize line are considered unlikely to exhibit any functional interaction with each other. Therefore, it is considered unlikely that these proteins would cause production of any harmful substances in this stacked-trait hybrid maize line. In practice, as a result of plow-in tests, succeeding crop tests and soil microflora tests conducted to examine the ability of the parent lines of this stacked-trait hybrid maize line, Bt11, MIR162, Cry1F maize line 1507 and GA21, to produce any harmful substances (the substances secreted from the roots which can affect other plants and microorganisms, the substances existing in the plant body which can affect other plants after dying), there was no difference from the non-recombinant control plants observed in all tests, suggesting that the productivity of harmful substances of the parent lines might have increased.

On the other hand, it has been found that the modified Cry1Ab protein, the modified Vip3A protein and the Cry1F protein expressed in this stacked-trait hybrid maize line exhibit the insecticidal activity against the insects of the order Lepidoptera. For these findings, there is a concern about possible effects on the non-target species of Lepidopteran insects which could eat directly this stacked-trait hybrid maize line or eat pollens dispersed from this stacked-trait hybrid maize line by eating with dietary plants. However, it is considered unlikely that these non-target insects inhabit locally near the fields for cultivation of this stacked-trait hybrid maize line and then, it is considered extremely low that they could be affected in the level of population.

Based on the above understanding, it was judged that the following conclusion made by the applicant is valid: This stacked-trait hybrid maize line and the progeny lines of stacked-trait hybrid maize line isolated from the parent lines of this stacked-trait hybrid maize line, Bt11, MIR162, Cry1F maize line 1507 and GA21, that contain a combination of any of the transferred genes in the individual parent lines, would pose no risk of Adverse Effect on Biological Diversity that is attributable to the production of harmful substances.

(3) Crossability

In the Japanese natural environment, there are no wild plants which can cross with maize. Therefore, it was judged that there are no specific wild plants that are possibly affected by this recombinant maize, and that the use of such maize poses no risk of Adverse Effect on Biological Diversity that is attributable to crossability. It was judged that the conclusion above made by applicant is valid.

2. Conclusion based on the Biological Diversity Risk Assessment Report

Based on the above understanding, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this stacked-trait hybrid maize line and the progeny lines of stacked-trait hybrid maize line isolated from the parent lines of this stacked-trait hybrid maize line, Bt11, MIR162, Cry1F maize line 1507 and GA21, that contain a combination of any of the transferred genes in the individual parent lines, in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity in Japan. It was judged that the conclusion above made by the applicant is reasonable.