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

Name: Dow Chemical Japan Ltd.
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Approved Type 1 Use Regulations

| | |
|---|---|
| Names of Types of Living Modified Organisms | Maize resistant to Lepidoptera and Coleoptera pests, and tolerant to glufosinate, glyphosate and aryloxyalkanoate herbicides (*cry1A.105*, modified *cry2Ab2*, modified *cry1F*, *pat*, modified *cp4 epsps*, modified *cry3Bb1*, *cry34Ab1*, *cry35Ab1*, modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis) (MON89034×*B.t.* Cry1F maize line 1507×MON88017×*B.t.* Cry34/35Ab1 Event DAS-59122-7×DAS40278, OECD UI: MON-89034-3×DAS-01507-1× MON-88017-3× DAS-59122-7×DAS-40278-9) (including the progeny lines which are isolated from the maize lines, MON89034, *B.t.* Cry1F maize line 1507, MON88017, *B.t.* Cry34/35Ab1 Event DAS-59122-7 and DAS40278, that contain a combination of their respective transferred genes (except those already granted an approval regarding Type I Use Regulation)) |
| Content of Type 1 Use of Living Modified Organisms | Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them |
| Method of Type 1 Use of Living Modified Organisms | — |

# Outline of the Biological Diversity Risk Assessment Report

## I. Information collected prior to assessing Adverse Effects on Biological Diversity

### 1. Information concerning preparation of living modified organisms

Maize resistant to Lepidoptera and Coleoptera pests, and tolerant to glufosinate, glyphosate and aryloxyalkanoate herbicides (*cry1A.105*, modified *cry2Ab2*, modified *cry1F*, *pat*, modified *cp4 epsps*, modified *cry3Bb1*, *cry34Ab1*, *cry35Ab1*, modified *add-1*, *Zea mays* subsp. *mays* (L.) Iltis) (MON89034×*B.t.* Cry1F maize line 1507×MON88017×*B.t.* Cry34/35Ab1 Event DAS-59122-7×DAS40278, OECD UI: MON-89Ø34-3×DAS-Ø15Ø7-1× MON-88Ø17-3×DAS-59122-7×DAS-4Ø278-9) (hereinafter referred to as “this stacked maize line”) is the progeny line created by interbreeding the following five modified maize lines using the conventional crossing.

This stacked maize line will be commercialized as a F1 hybrid line (F1). Due to the genetic segregation the seeds harvested from this stacked maize line include the stacked maize line or a combination of the genes transferred in the respective parent lines of this stacked maize line.

- Maize resistant to Lepidoptera pests (*cry1A.105*, modified *cry2Ab2*, *Zea mays* subsp. *mays* (L.) Iltis) (MON89034, OECD UI: MON-89Ø34-3) (hereinafter referred to as “MON89034”)
- Maize resistant to Lepidoptera pests and tolerant to glufosinate herbicide (modified *cry1F*, *pat*, *Zea mays* subsp. *mays* (L.) Iltis) (*B.t.* Cry1F maize line 1507, OECD UI: DAS-Ø15Ø7-1) (hereinafter referred to as “Cry1F line 1507”)
- Maize tolerant to glyphosate herbicide and resistant to Coleoptera pests (modified *cp4 epsps*, modified *cry3Bb1*, *Zea mays* subsp. *mays* (L.) Iltis) (MON88017, OECD UI: MON-88Ø17-3) (hereinafter referred to as “MON88017”)
- Maize resistant to Coleoptera 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) (hereinafter referred to as “Event DAS-59122-7)
- Maize tolerant to aryloxyalkanoate herbicide (modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis) (DAS40278, OECD UI：DAS-4Ø278-9) (hereinafter referred to as “DAS40278”)

The summary of the information concerning preparation of MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, is DAS40278 described below.

## (1) Information concerning donor nucleic acid

### 1) Composition and origins of component elements

The composition of donor nucleic acids and the origins of component elements used for each development of MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, and DAS40278 is shown in Tables 1-5 (p. 4-10).

Table 1. Origins and functions of the component elements of PV-ZMIR245 used for the development of MON89034

| Component elements | Origin and function |
|---|---|
| **T-DNA I region** | |
| B[Note 1]-Right Border | DNA fragment containing the right border sequence of nopaline type T-DNA region derived from *Agrobacterium tumefaciens*. The right border sequence is used as the initiation point of T-DNA transfer from *A. tumefaciens* to the plant genome (Depicker *et al.*, 1982; Zambryski *et al.*, 1982). |
| P[Note 2]-*e35S* | Cauliflower mosaic virus (CaMV) 35SRNA (Odell *et al.*, 1985) promoter and 9 bp leader sequence, containing double enhancer regions (Kay *et al.*, 1987). Involved in the constant expression of the target genes in entire tissue of plant body. |
| L[Note 3]-*Cab* | 5'-terminal untranslated leader region of wheat chlorophyll a/b binding protein derived from wheat. It activates the expression of target genes (Lamppa *et al.*, 1985). |
| I[Note 4]-*Ract1* | Intron of the actin gene from rice (*Oryza sativa*) (McElroy *et al.*, 1991). It involves the regulation of expression of target genes. |
| CS[Note 5]-*cry1A.105* | Gene coding for the Cry1A.105 protein. The Cry1A.105 protein encoded by the *cry1A.105* gene, which is used to develop MON89034, is the synthetic Bt protein composed of the domains I and II of the Cry1Ab protein, the domain III of the Cry1F protein, and the C-terminal domain of the Cry1Ac protein. It is developed to enhance insecticidal activity against the target Lepidoptera pests by combination of domains of different Bt proteins. |
| T[Note 6]-*Hsp17* | 3'-terminal untranslated region of the wheat heat shock protein 17.3. It terminates transcription and induces polyadenylation (McElwain and Spiker, 1989). |
| P-*FMV* | 35S promoter derived from the Figwort Mosaic Virus (Rogers, 2000). It induces the constitutive expression of the target genes in all tissues of the plant body. |
| I-*Hsp70* | First intron of the maize heat shock protein 70 gene (Brown and Santino, 1995). It activates the expression of target genes. |
| TS[Note 7]-*Rbcs (Zm)* | Transit peptide of the small subunit of ribulose-1,5-bisphosphate carboxylase of maize. It contains the first intron sequence (Matsuoka *et al.*, 1987). It transfers the proteins connected with the downstream region to the plastids. |
| CS-modified *cry2Ab2* | Gene coding for the modified Cry2Ab2 protein derived from *Bacillus thuringiensis* (Widner and Whiteley, 1989). In order to add a restriction endonuclease cleavage site, which is used in cloning, an aspartic acid is inserted after the methionine at the N-terminal, compared to the wild-type Cry2Ab2 protein. |
| T-*nos* | 3'-terminal untranscribed region of nopaline synthase (*nos*) gene derived from *A.tumefaciens* T-DNA. It terminates transcription of mRNA and induces polyadenylation (Bevan *et al.*, 1983). |
| B-Left Border | DNA fragment containing the left border sequence (25 bp) derived from *A. tumefaciens*. It is the termination point of T-DNA transfer from *A. tumefaciens* to the plant genome (Barker *et al.*, 1983). |

Table 1. Origins and functions of the component elements of PV-ZMIR245 used for the development of MON89034 (continued)

| Component elements | Origin and function |
|---|---|
| **T-DNA II region** | |
| B-Right Border | DNA fragment containing the right border sequence (24 bp) of nopaline type T-DNA region derived from *A. tumefaciens*. The right border sequence is used as the initiation point of T-DNA transfer from *A. tumefaciens* to the plant genome (Depicker *et al*., 1982; Zambryski *et al*., 1982). |
| T-*nos* | 3'-terminal untranscribed region of nopaline synthase (*nos*) gene derived from *A.tumefaciens* T-DNA. It terminates transcription of mRNA and induces polyadenylation (Bevan *et al*., 1983). |
| CS-*nptII* | Gene derived from *Escherichia coli* transposon Tn5 (Beck *et al*., 1982). It codes for the neomycin phosphotransferase II and confers kanamycin tolerance on plants. It is used as a marker to select transgenic plants in transferring genes (Fraley *et al*., 1983). |
| P-*35S* | 35S promoter region of the cauliflower mosaic virus (CaMV) (Odell *et al*., 1985). It induces the constitutive expression of the target genes in all tissues of the plant body. |
| B-Left Border | DNA fragment containing the left border sequence (25 bp) derived from *A. tumefaciens*. It is the termination point of T-DNA transfer from *A. tumefaciens* to the plant genome (Barker *et al*., 1983). |
| **Vector backbone region** | |
| OR Note 8-*ori V* | Replication origin region isolated from the broad-host range plasmid RK2. It allows autonomous replication of vectors in *A. tumefaciens* (Stalker *et al*., 1981). |
| CS-*rop* | Coding sequence for suppression of primer protein to maintain the number of copies of plasmid in *E. coli* (Giza and Huang, 1989). |
| OR-*ori-pBR322* | Replication origin region isolated from the pBR322. It allows autonomous replication of vectors in *E.coli* (Sutcliffe, 1979). |
| *aadA* | Bacteria promoter, code region and terminator for the 3"(9)-*O*-nucleotidyltransferase, the aminoglycoside modified enzyme derived from transposon Tn7 which confers resistance to spectinomycin or streptomycin (Fling *et al*., 1985). |

Note 1 B – Border
Note 2 P – Promoter
Note 3 L – Leader
Note 4 I – Intron
Note 5 CS – Coding sequence
Note 6 T – Transcript termination sequence
Note 7 TS – Targeting sequence
Note 8 OR – Origin of Replication

(All the rights pertinent to the information in the table above and the responsibility for the content remain with Dow Chemical Japan Ltd.)

Table 2. Origins and functions of the component elements of PHP8999 used for the development of Cry1F line 1507

| Component elements | Origin and function |
|---|---|
| Modified *cry1F* gene expression cassette | |
| *UBIZM1(2) Promoter* | Ubiquitin constitutive promoter 1) derived from *Z. mays* (including intron and 5’-terminal untranslated region) (Christensen *et al.*, 1992). |
| Modified *cry1F* | Gene coding for the modified Cry1F protein derived from *B. thuringiensis* var. *aizawai*. The nucleotide sequence has been modified to enhance the expression in plants. In the modified Cry1F protein, phenylalanine at position 604 of the amino acid sequence is replaced by leucine. |
| *ORF25PolyA Terminator* | Terminator to terminate transcription derived from *A.tumefaciens* pTi15955 (Barker *et al.*, 1983) |
| *pat* gene expression cassette | |
| *CAMV35S Promoter* | 35S constitutive promoter 1) derived from the cauliflower mosaic virus (CaMV) (Hohn *et al.*, 1982). |
| *pat* | Gene coding for phosphinothricin acetyltransferase (PAT protein) derived from *Streptomyces viridochromogenes*. The nucleotide sequence has been modified to enhance expression in plants, but the amino acid sequence has not been changed by the modification (Eckes *et al.*, 1989). |
| *CAMV35S Terminator* | 35S terminator to terminate transcription derived from the cauliflower mosaic virus (Hohn *et al.*, 1982). |

1) Constitutive promoter: a promoter to express the target genes in the entire plant body.

(All the rights pertinent to the information in the table above and the responsibility for the content remain with Dow Chemical Japan Ltd.)

Table 3. Origins and functions of the component elements of PV- ZMIR39 used for the development of MON88017

| Component elements | Origin and function |
|---|---|
| T-DNA region | |
| B-Left Border | DNA fragment containing the left border sequence (25 bp) derived from *A. tumefaciens*. It is the termination point of T-DNA transfer from *A. tumefaciens* to the plant genome (Barker *et al.*, 1983). |
| P [Note1]-*Ract1* | Promoter region of the actin 1 gene derived from rice (*O. sativa*) (McElroy *et al.*, 1990). It induces the constitutive expression of the target genes in all tissues of the plant body. |
| I [Note 2]-*Ract1* | Intron of the actin gene derived from rice (*O. sativa*) (McElroy *et al.*, 1991). It is involved in the regulation of expression of target genes. |
| TS [Note 3]-*CTP2* | Nucleotide sequence coding for the N-terminal chloroplast transit peptide of the protein EPSPS in the *epsps* gene of thale cress, *Arabidopsis thaliana* (Klee *et al.*, 1987). It transports the target proteins from the cytoplasm to chloroplast. |
| CS [Note 4]-modified *cp4 epsps* | 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium* CP4 strain (Barry *et al.*, 1997; Padgette *et al.*, 1996a). In the modified CP4 EPSPS protein, the second amino acid, serine, from the N-terminal in the wild-type CP4 EPSPS protein is modified to leucine to enhance the level of expression in plants. |
| T [Note 5]-*nos* | 3'-terminal untranslated region of the nopaline synthase (*nos*) gene derived from *A. tumefaciens* T-DNA. It terminates transcription of mRNA and induces polyadenylation (Bevan *et al.*, 1983). |
| P-*e35S* | Cauliflower mosaic virus (CaMV) 35SRNA (Odell *et al.*, 1985) promoter and 9 bp leader sequence, containing double enhancer regions (Kay *et al.*, 1987). Involved in the constant expression of the target genes in entire tissue of plant body. |
| L[Note 6]-*Cab* | 5'-terminal untranslated leader region of the wheat chlorophyll a/b-binding protein derived from wheat. It activates the expression of target genes (Lamppa *et al.*, 1985). |
| I-*Ract1* | Intron of the actin gene derived from rice (*O. sativa*) (McElroy *et al.*, 1991). It involves the regulation of expression of target genes. |
| CS- modified *cry3Bb1* | Gene coding for the Cry3Bb1 protein derived from *B. thuringiensis* (Donovan *et al.*, 1992). In the modified Cry3Bb1 protein, amino acids at six positions are replaced, compared with the wild-type Cry3Bb1 protein. One position of the six and the other five are modified in order to add a restriction endonuclease cleavage site in cloning and enhance insecticidal activity, respectively. |
| T-*Hsp17* | 3'-terminal untranslated region of the wheat heat shock protein 17.3. It terminates transcription and induces polyadenylation (McElwain and Spiker, 1989). |
| B [Note 7]-Right Border | DNA fragment containing the right border sequence of nopaline type T-DNA region derived from *A. tumefaciens*. The right border sequence is used as the initiation point of T-DNA transfer from *A. tumefaciens* to the plant genome (Depicker *et al.*, 1982; Zambryski *et al.*, 1982). |

Table 3. Origins and functions of the component elements of PV- ZMIR39 used for the development of MON88017 (continued)

| Component elements | Origin and function |
|---|---|
| Vector backbone region | |
| *aadA* | Bacteria promoter, code region and terminator for the 3”(9)-*O*-nucleotidyltransferase, the aminoglycoside modified enzyme derived from transposon Tn7. They confer resistance to spectinomycin or streptomycin (Fling *et al.*, 1985). |
| OR [Note 8]-*ori*-*pBR322* | Origin of replication isolated from the pBR322. It allows autonomous replication of vectors in *E.coli* (Sutcliffe, 1979). |
| CS-*rop* | Coding sequence for suppression of primer protein to maintain the number of copies of plasmid in *E. coli* (Giza and Huang, 1989). |
| OR-ori-V | Replication origin region isolated from the broad-host range plasmid RK2. It allows autonomous replication of vectors in *A. tumefaciens* (Stalker *et al.*, 1981). |

[Note 1] P – Promoter
[Note 2] I – Intron
[Note 3] TS – Targeting sequence
[Note 4] CS – Coding sequence
[Note 5] T – Transcript termination sequence
[Note 6] L – Leader
[Note 7] B – Border
[Note 8] OR – Origin of replication

(All the rights pertinent to the information in the table above and the responsibility for the content remain with Dow Chemical Japan Ltd.)

Table 4. Origins and functions of the component elements of PHP17662 used for the development of Event DAS-59122-7

| Component elements | Origin and function |
|---|---|
| *cry34Ab1* gene expression cassette | |
| *UBI1ZM PRO* | Ubiquitin constitutive promoter [1] derived from *Z. mays* (including intron and 5’-terminal untranslated region). |
| *cry34Ab1* | Gene coding for the Cry34Ab1 protein derived from *B. thuringiensis* PS149B1 strain. |
| *PINⅡ TERM* | Protease inhibitor II terminator to terminate transcription derived from *Solanum tuberosum*. |
| *cry35Ab1* gene expression cassette | |
| *TA Peroxidase PRO* | Peroxidase promoter derived from *Triticum aestivum*, which is known to express in roots (45-1342 nucleotide sequence of GenBank X53675). |
| *cry35Ab1* | Gene coding for Cry35Ab1 protein derived from *B. thuringiensis* PS149B1 strain. |
| *PINⅡ TERM* | Protease inhibitor II terminator to terminate transcription derived from *S. tuberosum*. |
| *pat* gene expression cassette | |
| *35S PRO* | 35S constitutive promoter [1] derived from the cauliflower mosaic virus. |
| *pat* | Gene coding for the phosphinothricin acetyltransferase (PAT protein) derived from *S. viridochromogenes*. |
| *35S TERM* | 35S terminator to terminate transcription derived from the cauliflower mosaic virus. |

1) Constitutive promoter: a promoter to express the target genes in the entire plant body.

(All the rights pertinent to the information in the table above and the responsibility for the content remain with Dow Chemical Japan Ltd.)

Table 5. Origins and functions of the component elements of pDAS1740 used for the development of DAS40278

| Component elements | Origin and function |
|---|---|
| Modified *aad-1* cassette | |
| *RB7 MAR* | Nuclear matrix attachment region derived from tobacco (Allen *et al.*, 1996). It stabilizes the expression of the modified AAD-1 protein. |
| *ZmUbi1* | Ubiquitin promoter derived from maize and contains the exon and intron regions (Christensen *et al.*, 1992). It initiates the transcription of genes in the entire plant body. |
| Modified *aad-1* | Gene modified from the aryloxyalkanoate dioxygenase gene derived from a gram-negative bacillus, *Sphingobium herbicidovorans*, to have a codon appropriate for expression in plants. It expresses the modified AAD-1 protein. As for the amino-acid sequence of the modified AAD-1 protein, alanine is added in the second position to introduce a cloning site (Dow AgroSciences LLC, 2004). |
| *ZmPer5 3'UTR* | Terminator derived from maize (Dow AgroSciences LLC, 1997). It terminates gene transcription. |
| *RB7 MAR* | Nuclear matrix attachment region derived from tobacco (Allen *et al.*, 1996). It stabilizes the expression of the modified AAD-1 protein. |

(All the rights pertinent to the information in the table above and the responsibility for the content remain with Dow Chemical Japan Ltd.)

2) Function of component elements

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

The functions of the component elements of the donor nucleic acids used for the development of MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, and DAS40278 are shown in Tables 1-5 (p. 4-10).

The modified *aad-1* cassette transferred to DAS40278 contains a nuclear matrix attachment region, the *RB7 MAR* gene**.** A nuclear matrix attachment region is frequently found in genomic DNA sequences and thought to have the function of attaching DNA to the nuclear matrix to form the DNA loop structure. It has been reported that when the nuclear matrix attachment region is adjacent to either end of the transferred gene, the level of expression of the transferred gene increases and gene silencing (which inhibits gene expression) decreases (Allen *et al.*, 2000; Halweg *et al.*, 2005).

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

a. Functions of proteins produced by target gene expression

-- Pest resistance protein--

The insecticidal proteins (Bt proteins) are derived from crystalline inclusion bodies which are produced by a gram-positive bacterium, *B. thuringiensis,* which is generally present in soil. It is known that Bt proteins are associated with a specific receptors in the midgut epithelium of the target insect to form cation selective pores, resulting in inhibiting the digestion process to exhibit insecticidal activity (Hofmann *et al.*, 1988; Slaney *et al.*, 1992; VanRie *et al.*, 1990). Previous studies also revealed that the Bt proteins consist of multiple domains and what function each domain has. For example, it is known that the Bt protein consists of domains I, II, and III and a C-terminal domain: the domain I forms cation selective pores to inhibit the digestion process, the domain II recognizes specific receptors, the domain III is associated with the receptors, and the C-terminal is involved in the crystal structure of the Bt protein (de Maagd *et al.*, 2001; Masson *et al.*, 2002).

< Lepidoptera pest resistance protein >

**【Cry1A.105 Protein】**

The Cry1A.105 protein, which is expressed in MON89034, is a synthetic Bt protein consisting of domains I and II of the Cry1Ab protein, domain III of the Cry1F protein, and C-terminal domain of the Cry1Ac protein. This synthetic protein was developed in order to enhance the insecticidal activity against target Lepidoptera pests by combining it with different Bt proteins.

The insecticidal spectrum of the Cry1A.105 protein was examined by dietary administration of the Cry1A.105 protein to 15 insect species, including five Lepidoptera insects. The results showed that the Cry1A.105 protein exhibits insecticidal activity against larvae of major Lepidoptera pests of maize including; corn earworm (*Helicoverpa zea*) (MacRae *et al.*, 2005), black cutworm (Tamayanaga) (*Agrotis* *ipsilon*) (MacRae, 2005), fall armyworm (Tsumajirokusayoto) (*Spodoptera frugiperda*) (MacRae, 2005), southwestern corn borer (*Diatraea grandiosella*) (MacRae, 2005), and European corn borer (Yoroppa awanomeiga) (*Ostrinia nubilalis*) (MacRae *et al.*, 2006a). The Cry1A.105 protein is not active against beneficial insects other than Lepidoptera insects, such as honeybees (Richards, 2006a; Richards, 2006b) and ladybird beetles (Paradise, 2006a).

Based on the above observations, it was confirmed that the Cry1A.105 protein exhibits selective insecticidal activity against only Lepidoptera pests, but not against other insects like its component elements, the Cry1Ab, Cry1F, and Cry1Ac proteins, which only exhibit insecticidal activity against Lepidoptera pests.

### 【Modified Cry2Ab2 protein】

The insecticidal spectrum of the modified Cry2Ab2 protein, which is expressed in MON89034, was examined by dietary administration of the Cry2Ab2 protein mixed with artificial feed to 15 insect species including four Lepidoptera insects. The results showed that the modified Cry2Ab2 protein was active against larval corn earworm (MacRae *et al.*, 2006a), fall armyworm (MacRae *et al.*, 2006b) and European corn borer (MacRae *et al.*, 2006a) out of the four major Lepidoptera pests,. The modified Cry2Ab2 protein exhibited no insecticidal activity against black cutworm (MacRae *et al.*, 2006b). Moreover, it did not exhibit insecticidal activity against beneficial insects other than Lepidoptera pests, including honeybees (Maggi, 2000a; Maggi, 2000b) and ladybird beetles (Paradise, 2006b). Therefore, it was confirmed that the modified Cry2Ab2 protein exhibited selective insecticidal activity against certain *Lepidoptera* pests, but not against other insect species.

The modified Cry2Ab2 protein encoded by the modified *cry2Ab2* gene has an aspartic acid inserted after the methionine at the N-terminal, compared to the wild-type Cry2Ab2 protein, in order to add a restriction endonuclease cleavage site, which is used in cloning.

### 【Modified Cry1F protein】

The modified Cry1F protein, which is expressed in Cry1F line 1507, exhibits high insecticidal activity against larvae of several Lepidoptera pests, including European corn borer, fall armyworm, and beet armyworm (Shiroichimonjoto) (*Spodoptera exigua*), but the toxicity of the protein has not been observed against insects other than Lepidoptera insects, including Coleoptera, Hymenoptera, Neuroptera and Collembola, and other non-target organisms, including mammals, birds, and fish (EPA, 2005a).

In addition, in the modified Cry1F protein, the phenylalanine at position 604 in the native amino acid sequence is substituted by leucine.

< Coleoptera pest resistance protein >

### 【Modified Cry3Bb1 protein】

The modified Cry3Bb1 protein, which is expressed in MON88017, exhibits insecticidal activity against larval corn rootworms (*Diabrotica* sp.), which are major Coleoptera pests that damage roots of maize cultivated in the US.

It was confirmed that the insecticidal spectrum of the Cry3Bb1 protein was extremely narrow and the protein exhibited high insecticidal activity against larvae of the Colorado potato beetles (Kororado hamushi) (*Leptinotarsa decemlineata*) and corn rootworms, which are both classified into two genera (*Leptinotarsa*, *Diabrotica*) of the family *Chrysomelidae*. (Head *et al.*, 2001). It has never been reported that species related to the genera *Leptinotarsa and Diabrotica* inhabit Japan (Nakane *et al.*, 1963).

In the modified Cry3B1 protein, amino acids in six positions are replaced, compared with the wild type Cry3Bb1 protein. One of the positions was modified to add a restriction endonuclease cleavage site in cloning and the other 5 positions were modified to enhance insecticidal activity.

### 【Cry34Ab1 protein + Cry35Ab1 protein】

The Cry34Ab1 and Cry35Ab1 proteins are both expressed in Event DAS-59122-7. The Cry34Ab1 protein alone exhibits the activity against corn rootworms, but the Cry35Ab1 protein does not. When both the proteins act simultaneously, the insecticidal effect is increased approximately eight times relative to the Cry34Ab1 protein alone. As for the mechanism of action, it is considered that the Cry34Ab1 protein acts as a pore-forming protein in the phospholipid membrane, while the Cry35Ab1 protein enlarges pores and increases membrane permeability (Herman *et al.*, 2002).

The Cry34Ab1/Cry35Ab1 proteins exhibit insecticidal activity against larvae of two Coleoptera pests, northern corn rootworm (*Diabrotica barberi*) and western corn rootworm (*D. virgifera virgifera*). No toxicity has been observed against non Coleoptera insects, such as insects of Lepidoptera, Hymenoptera, Neuroptera, and Collembola, or other non-target organisms, including mammals, birds, and fishes (EPA 2005b).

-- Herbicide tolerant proteins --

### 【PAT protein】

The PAT protein (phosphinothricin acetyltransferase), which is expressed in Cry1F line 1507 and Event DAS-59122-7, confers tolerance to the herbicide, glufosinate. Glufosinate inhibits glutamine synthetase, which synthesizes glutamine from glutamic acid and ammonia, and the resulting accumulation of ammonia in the plant causes plant death. The PAT protein acetylates glufosinate to transform it into nonherbicidal acetyl glufosinate, resulting in conferring the tolerance to glufosinate on the plant.

### 【Modified CP4 EPSPS protein】

The modified CP4 EPSPS protein, which is expressed in MON88017, exhibits tolerance to the herbicide, glyphosate. Plants treated with glyphosate die because 5-enolpyruvylshikimate 3-phosphate synthetase (enzyme number: E.C.2.5.1.19, hereinafter referred to as “EPSPS protein”) is inhibited which inhibits the synthesis of aromatic amino acids essential to protein synthesis. The activity of the modified CP4 EPSPS protein is not inhibited even in the presence of glyphosate, and therefore the recombinant plants expressing this protein can grow by the normal synthesis of shikimate.

Moreover, for the modified *cp4 epsps* gene, the nucleotide sequence of the wild-type *cp4 epsps* gene is modified in order to improve the level of expression in plants without changing the functional activity of the wild-type CP4 EPSPS protein. As for the amino acid sequence of the modified CP4 EPSPS protein, only serine at the second position from the N-terminal is substituted by leucine.

### 【Modified AAD-1 protein】

The modified AAD-1 protein expressed in DAS40278 is an enzyme, which transforms a compound into one without herbicidal activity by catalyzing the reaction of oxygen introduction to aryloxyalkanoate herbicides. For example, the modified AAD-1 protein catalyzes the reaction of oxygen introduction to 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide to transform it into 2,4-dichlorophenol (2,4-DCP), which has no herbicidal activity, and glyoxylic acid (Dow AgroSciences LLC, 2004).

In addition, the modified *aad-1* gene has codons modified to optimize expression in plants and in its amino acid sequence alanine is added in the second position in order to introduce a cloning site.

b. Homology to known allergen proteins

The amino acid sequences of Cry1A.105, modified Cry2Ab2, modified Cry1F, modified Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, modified CP4 EPSPS, and modified AAD-1 proteins were examined for sharing the functionally important with the known allergens, using the following databases. The results showed that structurally similar sequences of those proteins were not shared with the known allergens.

AD11: Cry1A.105, modified Cry2Ab2, and modified CP4 EPSPS proteins
AD8：Modified Cry3Bb1
FARRP Allergen Database version 11: Modified Cry1F, Cry34Ab1, Cry35Ab1, PAT, and modified AAD-1 proteins

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

The Cry1A.105, modified Cry2Ab2, modified Cry1F, modified Cry3Bb1, Cry34Ab1, and Cry35Ab1 proteins are all the Bt proteins. A number of studies have been performed on the mechanism of the insecticidal activity of these Bt proteins (OECD, 2007), and so far it has not been reported that the Bt proteins have other functions. Therefore, it is believed that these Bt proteins have no enzymatic activities and do not change the metabolic system of recipient organisms.

The PAT protein acetylates L-phosphinothricin (classified as an L-amino acids), an active ingredient of glufosinate herbicide, but does not acetylate other L-amino acids. The PAT protein has little affinity for glutamic acid, the structure of which is especially similar to L-phosphinothricin (Thompson *et al*., 1987). Furthermore, it has been reported that even in the excessive presence of various amino acids, the transacetylation reaction of glufosinate by the PAT protein is not inhibited and PAT has extremely high substrate specificity to glufosinate (OECD, 1999). Therefore, considering their high substrate specificity, the PAT protein is not thought to change the metabolic system of recipient organisms.

The EPSPS protein, functionally identical to the modified CP4 EPSPS protein, is an enzyme protein, which catalyzes the shikimate pathway for biosynthesis of aromatic amino acids. However, it is not a rate-deterrmining enzyme in the pathway, and therefore it has been considered that the levels of the aromatic amino acids, the end products of this pathway, do not increase even with the increased activities of the EPSPS proteins (Padgette *et al.*, 1996b; Ridley *et al*., 2002). In addition, it has been identified that the EPSPS protein specifically reacts with the substrates, phosphoenolpyruvate (hereinafter referred to as “PEP”) and shikimate-3-phosphate (hereinafter referred to as “S3P”) (Gruys *et al.*, 1992). Other than those substrates, only shikimic acid, an analog of S3P, is known to react with the EPSPS protein. However, the comparison of the reaction of the EPSPS proteins with shikimate and S3P by the specificity constant ($k_{cat}/K_m$), which represents the degree of occurrence of reaction, showed that the reaction specificity between the EPSPS protein and shikimate is one-two millionth of that between the EPSPS protein and S3P (Gruys *et al.*, 1992), and shikimate is unlikely to react as a substrate of the EPSPS protein. Therefore it is not thought that the modified CP4 EPSPS protein changes the metabolic system of recipient organisms.

The modified AAD-1 protein is an enzyme to catalyze the reactions when oxygen is specifically introduced to the compounds with aryloxyalkanoate group, especially R-enantiomers of chiral as well as achiral compounds. Endogenous plant compounds that share structural and physiological similarities to the compounds with an aryloxyalkanoate group were examined for the activities of the modified AAD-1 protein, and the impact on the metabolic pathways were discussed. As a substrate, plant hormones (indole-3-acetic acid, abscisic acid, gibberellic acid and aminocyclopropane 1-carboxylic acid) and phenylpropanoid intermediates (trans-cinnamic acid, coumaric acid and sinapinic acid) were examined. Twenty kinds of L-amino acids were also examined.

For the 20 L-amino acids, no reactions were observed at a concentration of 1 μM modified AAD-1 protein. Among the plant hormones and phenylpropanoid intermediates treated with 1 μM modified AAD-1 protein, abscisic acid, gibberellic acid, trans-cinnamic acid, and coumaric acid showed slight reactions. Treatment with 5 μM modified AAD-1 protein produced a slight reaction with aminocyclopropane 1-carboxylic acid and at 10 μM modified AAD-1 protein, a slight reaction was observed for indole-3-acetic acid. Since no correlation was observed between the concentrations of the modified AAD-1 protein and enzyme activity, oxides were then measured by Fourier transform mass spectrometry (FT/MS). The results showed that oxides of indole-3-acetic acid and trans-cinnamic acid were detected by the treatment with the modified AAD-1 protein at 10 μM. However, reaction rates were very slow and the parameters, *Km* and *Vmax*, of the Michaelis-Menten equation could not be obtained. Since oxides were detected only when highly sensitive Fourier transform mass spectrometry was performed on the compounds treated with high levels of the modified AAD-1 protein and since the reaction rates were extremely slow, the oxidative reactions observed are unlikely to affect the metabolic pathways of plants (Cicchillo *et al.*, 2010).

In addition, since no compounds of the aryloxyalkanoate group have been yet identified in plant bodies, the modified AAD-1 protein is not thought to change any other metabolic pathways in plant bodies.

(2) Information concerning vectors

1) Name and origin

The plasmid vectors used for the development of the parent lines are as follows.

MON89034: PV-ZMIR245 constructed from the vector pBR322 derived from *E. coli*
Cry1F line 1507: PHP8999 constructed from the vector pUC19 derived from *E. coli*
MON88017: PV- ZMIR39 constructed from the vector pBR322 derived from *E. coli*
Event DAS-59122-7: PHP17662 constructed from the vector pSB1 derived from *A. tumefaciens*
DAS40278: pDAS1740 constructed from the plasmid pUC19 derived from *E. coli*

2) Properties

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

The numbers of base pairs in the plasmid vectors used for the development of parent lines are as follows.

MON89034: PV-ZMIR245; 17,600 bp
Cry1F line 1507: PHP8999; 9,504 bp
MON88017: PV- ZMIR39; 12,368 bp
Event DAS-59122-7: PHP17662; 50,311 bp
DAS40278: pDAS1740; 8,512 bp, linear DNA used for transfection; 6,236 bp

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

The antibiotic resistant genes used as selective markers are as follows. None of these antibiotic resistant genes have been transferred in the recipient organism.

MON89034: *aadA* gene conferring the resistance to spectinomycin and streptomycin and *nptII* gene conferring the resistance to kanamycin
Cry1F line 1507: *nptII* gene conferring the resistance to kanamycin
MON88017: *aadA* gene conferring the resistance to spectinomycin and streptomycin
Event DAS-59122-7: *tet* gene conferring the resistance to tetracycline and *spc* gene conferring the resistance to spectinomycin

DAS40278: $ap^r$ gene conferring the resistance to ampicillin

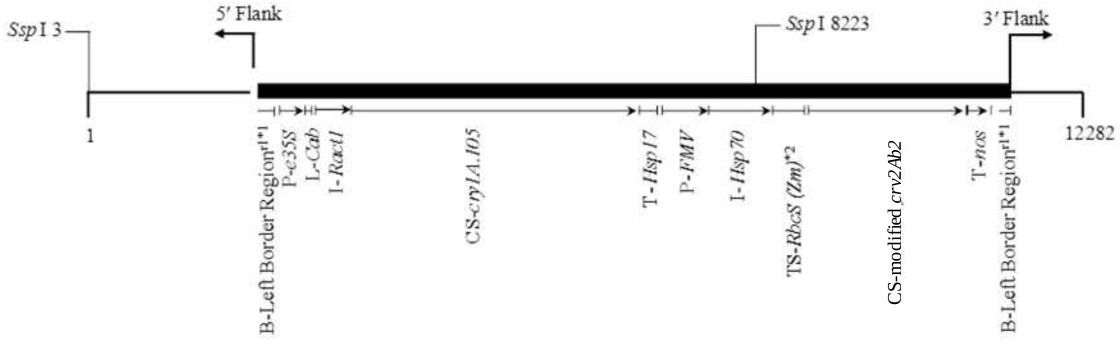
(c) Presence or absence of infectious characteristics of vector and, if present, the information concerning the host range

The infectivity of PV-ZMIR245, PHP8999, PV- ZMIR39, PHP17662, and pDAS1740 is not known.

### (3) Method of preparing living modified organisms

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

The structure diagrams of the entire nucleic acid transferred to the MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, and DAS40278 are shown in Figure 1 to 5 (p. 18-19).



[*1] r1 means that the B-Left Border Region in the MON89034 is shorter than that before the transfection.

[*2] TS-R*bcS (Zm)* was considered as "TS-*SSU-CTP*" in the Biological Diversity Risk Assessment Report of MON89034.

Figure 1. Structure diagram of the entire nucleic acid transferred to MON89034

The angled arrows in the structure diagram show the 5'- and 3'-terminal regions of the transferred genes and the subsequent adjacent endogenous sequences of maize. The positions of the component elements and restriction endonuclease cleavage sites are shown as estimated approximate positions.

(All the rights pertinent to the information in this figure and the responsibility for the content remain with Dow Chemical Japan Ltd.)

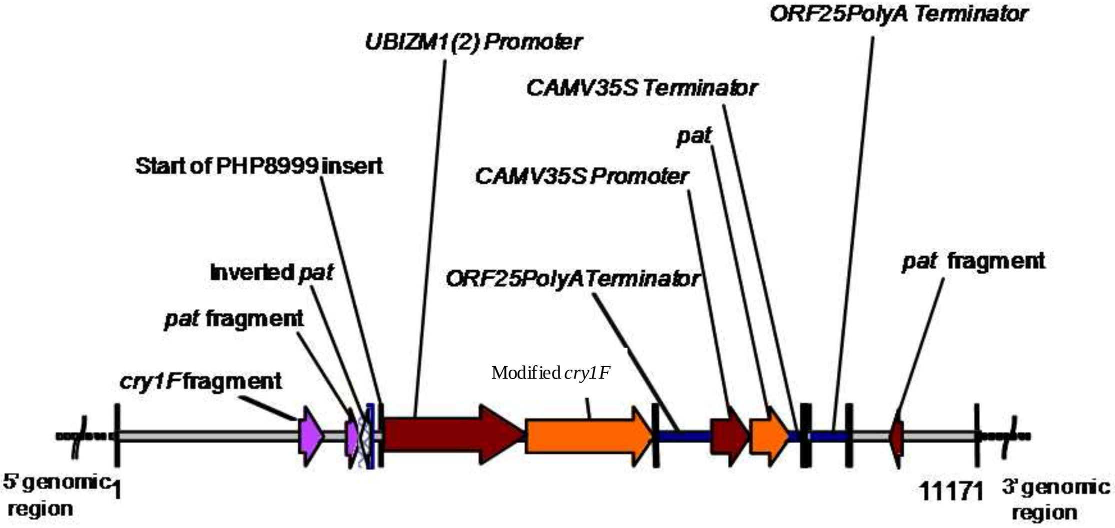
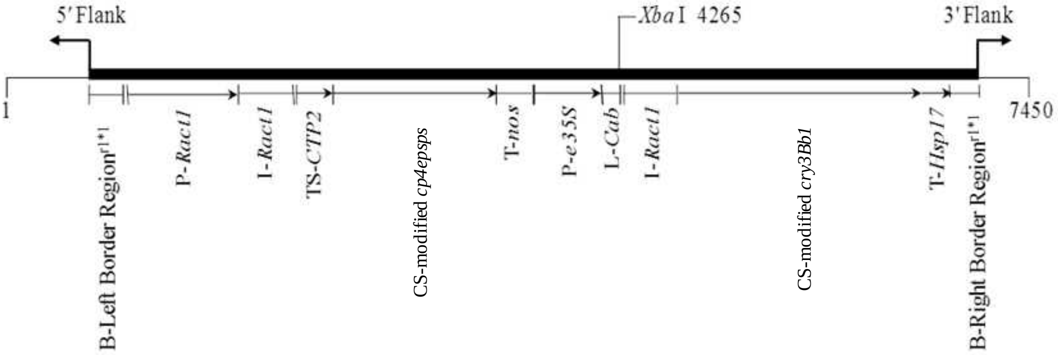


Figure 2. Structure diagram of the entire nucleic acid transferred to Cry1F line 1507
(All the rights pertinent to the information in this figure and the responsibility for the content remain with Dow Chemical Japan Ltd.)



[*1]: r1 means that the B-Left Border and Right Border Regions in the MON88017 are shorter than those before the transfection.

Figure 3. Structure diagram of the entire nucleic acid transferred to MON88017

The angled arrows in the structure diagram show the 5'- and 3'-terminal regions of the transferred genes and the subsequent adjacent endogenous sequences of maize. The positions of the component elements and sites cleaved by restriction enzymes are shown as estimated approximate positions.

(All the rights pertinent to the information in this figure and the responsibility for the content remain with Dow Chemical Japan Ltd.)

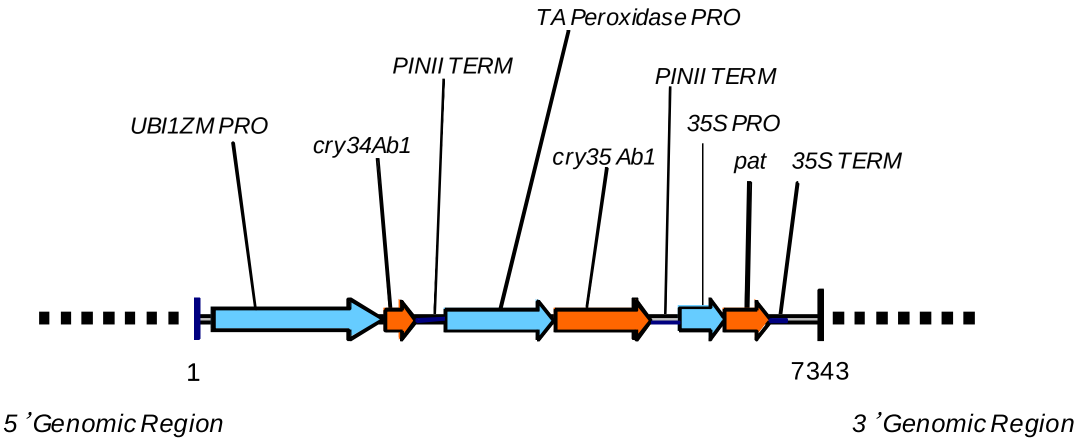


Figure 4. Structure diagram of the entire nucleic acid transferred to Event DAS-59122-7 (All the rights pertinent to the information in this figure and the responsibility for the content remain with Dow Chemical Japan Ltd.)

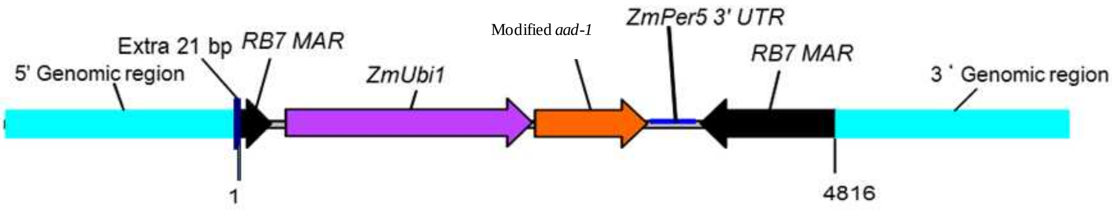


Figure 5. Structure diagram of the entire nucleic acid transferred to DAS40278 (All the rights pertinent to the information in this figure and the responsibility for the content remain with Dow Chemical Japan Ltd.)

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

The transferring nucleic acids to the recipient organism was performed using the following method.

MON89034: Agrobacterium method
Cry1F line 1507: Particle gun method
MON88017: Agrobacterium method
Event DAS-59122-7: Agrobacterium method
DAS40278: Whisker method[1]

3) Process of rearing of living modified organisms

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

The transformed cells were selected in the media added with the following:

MON89034: Paromomycin
Cry1F line 1507: Glufosinate
MON88017: Glyphosate
Event DAS-59122-7: Glufosinate
DAS40278: Haloxyfop

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

For MON89034 MON88017 and Event DAS-59122-7, *Agrobacterium* was removed by adding carbenicillin to the medium. It was confirmed that there are no residual *Agrobacterium* in MON89034 and MON88017 in the evaluation of the parent lines, in which MON89034 and MON88017 were transferred to the medium without carbenicillin and then there was no colonization of *Agrobacterium* in that medium. In addition, it was confirmed that there was no survival of *Agrobacterium* in Event DAS-59122-7 by microscopy of the plant body after Event DAS-59122-7 was

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[1] The embryonic suspension was obtained by liquid culture of the callus from immature embryos of Hi-II, the recipient maize. The linear DNA cut from the pDAS1740 with the restriction enzyme, *Fsp I,* and needle-like silicon carbide whisker fibers were added to the embryonic suspension and stirred, and then the silicon carbide whisker fibers created holes in the cells, which resultied in the transfer of the linear DNA to the recipient organism (Thompson *et al*., 1995).

transferred to the medium without carbenicillin. The nucleic acids were transferred to the recipient organisms of Cry1F line 1507 and DAS40278 using the particle gun and the whisker method, respectively. The agrobacterium method was not used.

(c) Process 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 Effects on Biological Diversity

For MON89034, R0 (regenerated plants) plants were bred with a conventional maize line LH172 to obtain LH172BC0F$_1$ plants. Based on the PCR method, LH172BC0F$_1$ plants were subsequently selected for the presence of the T-DNAI region and the absence of the T-DNAII region, and those containing the T-DNAII region were discarded.

Further selection was then performed for the selected individuals, based on the analysis of the transferred genes and the expression levels of the Cry1A.105 and modified Cry2Ab2 proteins. Subsequently, tests in the artificial climate chamber and the greenhouse were then performed and the actual resistance to pest insects and agronomic characters (morphological and growth characteristics, yield and productivity, pest insect sensitivity, etc.) were then examined in outdoor fields. MON89034 was selected upon comprehensive evaluation of these results.

For Cry1F line 1507, PCR analysis was done of leaf samples from the regenerated plant to confirm transferred gene presence or absence. ELISA analysis was used to confirm expression of modified Cry1F protein. Insect resistance was then determined by assay on larval European corn borer. T0 (regenerated plants) which demonstrated insect resistance were crossed with the same breeding lines obtain seeds. Finally, Cry1F line 1507 was selected in a comprehensive manner based on the resistance to European corn borer and agronomic characters examined in outdoor field trials.

For MON88017, R0 (regenerated plants) plants were bred with a conventional maize line LH198 to obtain LH198BC0F1 plants. Individuals showing tolerance to glyphosate were selected and then the presence of the T-DNA region was confirmed by the PCR. For the selected individuals, further selection was performed based on the analysis of the transferred genes and the expression levels of the modified CP4 EPSPS and modified Cry3Bb1 proteins. Subsequently, the tests in the artificial climate chamber and the greenhouse were performed and then the insect resistance and agronomic characteristics (morphological and growth characteristics, yield and productivity, pest insect sensitivity, etc.) were examined in outdoor field trials. MON88017 was selected upon comprehensive evaluation of these results.

For Event DAS-59122-7, PCR analysis was done of leaf samples from the regenerated plant to confirm transferred gene presence or absence. ELISA analysis was used to confirm expression of Cry34Ab1 and Cry35Ab1. Insect resistance was then determined by assay on corn rootworms. T0 (regenerated plants) which demonstrated insect resistance were crossed with the same breeding lines as them to obtain seeds of the current generation of the recombinant (T0). Finally, Event DAS-59122-7 was selected in a comprehensive manner based on the resistance to corn rootworm and agronomic characters examined in outdoor field trials.

As for DAS40278, production of the modified AAD-1 protein was confirmed by spraying quizalofop, an aryloxyalkanoate herbicide to the regenerated plants (T0 generation). DAS40278 was selected based on comprehensive manner by evaluation of transgene analysis, confirmation of protein expression, the herbicide tolerance, and the agronomic characteristics in outdoor field trials conducted in US and Canada,.

The status of application and approval of MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, DAS40278, and this stacked maize line in Japan is described below (Table 6. p. 24).

Table 6. The status of application and approval of MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, DAS40278 and this stacked maize line in Japan

| | Food[1)] | Feed[2)] | Environment[3)] |
|---|---|---|---|
| MON89034 | November 2007<br>Confirmed safety | October, 2007<br>Confirmed safety | January, 2008<br>Approved for Type I Use Regulation |
| Cry1F line 1507 | July, 2002<br>Confirmed safety | March, 2003<br>Confirmed safety | March, 2005<br>Approved for Type I Use Regulation |
| MON88017 | October, 2005<br>Confirmed safety | March, 2006<br>Confirmed safety | April, 2006<br>Approved for Type I Use Regulation |
| Event DAS-59122-7 | October, 2005<br>Confirmed safety | March, 2006<br>Confirmed safety | April, 2006<br>Approved for Type I Use Regulation |
| DAS40278 | May, 2012<br>Confirmed safety | September, 2012<br>Confirmed safety | August, 2011<br>Approved for Type I Use Regulation Published public comments |
| This stacked maize line | November, 2012<br>Applied | November, 2012<br>Notified | May, 2012<br>Applied |

[1)] Food Sanitation Act

[2)] Act on Safety Assurance and Quality Improvement of Feeds

[3)] Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms

### 【Process of rearing of this stacked maize line】

This stacked maize line was developed from MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, and DAS40278 by crossing (Figure 6, p. 24).

Confidential information: not disclosed to unauthorized persons

Figure 6. Process of rearing of this stacked maize line

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

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

It was confirmed that the transferred genes in MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, and DAS40278 existed on the chromosome.

(b) The number of copies of replication products of transferred nucleic acid and stability of its inheritance across multiple generations

As a result of Southern blot analysis on the parent lines, it was confirmed that one copy of respective target genes existed at a site on the chromosomes for MON89034 (Rice *et al.*, 2006), Cry1F line 1507 (In-house report 1), MON88017 (Beazley *et al.*, 2002), Event DAS-59122-7 (In-house report 2), and DAS40278 (In-house report 3). In the evaluation of the parent lines, Southern blot analysis on multiple generations also showed that the transferred genes were stably inherited to subsequent generations.

The results of the analysis of the nucleotide sequences of the transferred genes in MON89034 showed that the 5’-terminal region of P-*e35S*, which controls expression of the *cry1A.105* gene, and the neighboring right border region were substituted by the left border region within the T-DNA II region and the 5’-terminal region of P-*35S* controlling the expression of the *nptII* gene due to homologous recombination. However, the homologous recombination did not occur in the protein coding regions and normal Cry1A.105 protein expression was confirmed in individual tissues as to the Cry1A.105 protein coding region which was the nearest open reading frame. Therefore, it was concluded that a new open reading frame was not generated by this homologous recombination.

The results of the analysis of the nucleotide sequences of nucleic acid transferred to Cry1F line 1507 showed that a part of the modified *cry1F* gene sequence, a part of the *pat* gene sequence, and a part of the *ORF25PolyA Terminator* sequence were contained in the 5’-terminal region, the 5’- and 3’-terminal regions, and the 3’-terminal region of the nucleic acid transferred, respectively. However, Northern blot analysis confirmed that transcription of these gene fragments into mRNA did not occur and that the gene fragments were not functional (In-house report 4).

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

This item is not applicable because there is only one copy each of MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, and DAS40278.

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

The stability of expression of the parent lines was identified by the evaluation of the parent lines as follows.

MON89034: Confirming the expression of protein by western blot analysis (Hartmann *et al.*, 2006).

Cry1F line 1507: Confirming the expression of protein by ELISA (In-house report 5), bioassay using Lepidoptera pests and the glufosinate spraying test (Isolated field test report 1)

MON88017: Confirming the expression of the protein by the glyphosate spraying test and ELISA.

Event DAS-59122-7: Confirming the expression of the protein by ELISA (In-house report 6), bioassay using Coleoptera pests (Bing, *et al.*, 2003) and the glufosinate spraying test (Isolated field test report 2).

DAS40278: Confirming the expression of protein by ELISA

(e) 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 sequences of the nucleic acids transferred to MON89034, MON88017, Cry1F line 1507, Event DAS-59122-7, and DAS40278 do not contain any sequences that allow gene transmission. Therefore, it is unlikely that these genes would be transmitted through virus infection and/or any other routes to wild animals and wild plants.

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

In order to detect and identify MON89034, the DNA sequences of the transferred genes and the nearby regions of the plant genomes are used as primers. As a result, MON89034 is specifically detectable (Rice *et al.*, 2006).

For the detection and identification of Cry1F line 1507, the real-time quantitative PCR using the DNA sequences of the transferred genes and the nearby regions of the plant genomes as primers has been developed (JRC, 2005a).

In order to detect and identify MON88017, the DNA sequences of the transferred genes and the nearby regions of the plant genomes are used as primers. As a result, MON88017 is specifically detectable (Beazley *et al.*, 2002).

For the detection and identification of Event DAS-59122-7, the real-time quantitative PCR using the DNA sequences of the transferred genes and the nearby regions of the plant genomes as primers has been developed (JRC, 2005b).

In order to detect and identify DAS40278, the PCR using the DNA sequences of the transferred genes and the nearby regions of the plant genomes as primers has been developed (Dow AgroSciences LLC, 2009).

In order to detect and identify this stacked 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

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

This stacked maize line contains the following traits derived from individual parent lines.

MON89034: Resistance to the Lepidoptera pests due to Cry1A.105 protein and modified Cry2Ab2 protein derived from the transferred genes

Cry1F line 1507: Resistance to Lepidoptera pests due to modified Cry1F protein and tolerance to glufosinate due to PAT protein. The proteins are derived from the transferred genes

MON88017: Tolerance to glyphosate due to modified CP4 EPSPS and resistance to Coleoptera pests due to modified Cry3Bb1 proteins. The proteins are derived from the transferred genes

Event DAS-59122-7: Resistance to Coleoptera pests due to Cry34Ab1 and Cry35Ab1 proteins and tolerance to glufosinate herbicide due to the PAT protein. The proteins are derived from the transferred genes

DAS40278: Tolerance to aryloxyalkanoate herbicides due to modified AAD-1 protein derived from the transferred genes

The possibility of the functional interaction of these proteins was examined in terms of proteins with resistance to pest insects and herbicides.

Functional interaction among proteins with resistance to pest insects

The Cry1A.105, modified Cry2Ab2 and modified Cry1F proteins exhibit insecticidal activity against Lepidoptera pests, and the modified Cry3Bb1 and Cry34Ab1/Cry35Ab1 proteins against Coleoptera pests. Because the structures of the regions involved in specificity of insecticidal activity of these proteins are not changed in his stack maize line, it is not thought that insecticidal activity will not be affected. In addition, it is possible that the insecticidal activity of this stack maize line may increase additively, but it is difficult to think that a synergistic or antagonistic action would result from an interaction among the proteins in the stack.

Functional interaction among of proteins with tolerance to herbicides

The PAT, modified CP4 EPSPS, and modified AAD-1 proteins have high substrate specificity, and therefore, are not thought to change the metabolic system of recipient organism. Each protein substrate is different and each involves an independent metabolic pathway. Therefore, it is difficult to think that these proteins will interact to produce an unexpected metabolite.

Functional interaction between proteins with resistance to pest insects and proteins with tolerance to herbicides

Since the proteins with resistance to pest insects and proteins with tolerance to herbicides have different functions, it is difficult to think that both types of proteins interact each other.

Based on the above, it is unlikely that the expressed proteins derived from respective parent lines interact with one another in this stacked maize line.

Therefore, differences in physiological and ecological properties between this stacked maize line and a maize line, the species of the taxonomy to which the recipient organism belongs were evaluated, based on the results of the individual examination of the parent lines, MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, and DAS40278.

(b) 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

The Biological Diversity Risk Assessment Report of respective parent lines was completed and confirmed that the following physiological or ecological properties were not different between the respective parent lines and their controls, non-recombinant maize. As for the information on the physiological or ecological properties, see the website of the Japan Biosafety Clearing House[2].

a Morphological and growth characteristics  
b Cold-resistance and heat-resistance at the early stage of growth  
c Wintering ability and summer survival of the mature plant  
d Fertility and size of the pollen  
e Production, shedding habit, dormancy, and germination rate of the seed  
f Crossability  
g Productivity of harmful substances

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[2] [MON89034, Cry1F line 1507, MON88017, Event Das-59122-7]

1. In the website, https://ch.biodic.go.jp/bch/OpenSearch.do, input “Maize” in the box of “Name of Organism” and then select “Search/View.”
2. Select “Name of Organism-Maize” of the appropriate line.
3. Select “Attached documents.”
4. Select “Document 1.”

[DAS40278]

1. In the website, http://www.bch.biodic.go.jp/bch_3_1.html, select “Agriculture, Forestry and Fisheries-2011.”
2. Select “Outline of the application form-PDF” of the 47th appropriate line.

## II. Review by persons with specialized knowledge and experience concerning Adverse Effects 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 Effects on Biological Diversity**

Maize resistant to Lepidoptera and Coleoptera pests, and tolerant to glufosinate, glyphosate and aryloxyalkanoate herbicides (including the progeny lines which are isolated from the maize lines, MON89034, *B.t.* Cry1F maize line 1507, MON88017, *B.t.* Cry34/35Ab1 Event DAS-59122-7, and DAS40278, that contain a combination of their respective transferred genes (except those already granted an approval regarding Type I Use Regulation)) (hereinafter referred to as "this stacked line") was developed with the following lines by crossing.

(a) Maize resistant to Lepidoptera pests, to which the *cry1A.105* gene coding the Cry1A.105 protein and the modified *cry2Ab2* gene coding the modified Cry2Ab2 protein were transferred (MON89034),

(b) Maize resistant to Lepidoptera pests, and tolerant to glufosinate herbicide, to which the modified *cry1F* gene coding the modified Cry1F protein and the *pat* gene coding the PAT protein (phosphinothricin acetyltransferase) were transferred (*B.t.* Cry1F maize line 1507),

(c) Maize tolerant to glyphosate herbicide and resistant to Coleoptera pests, to which the modified *cp4 epsps* gene coding for the modified CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the modified *cry3Bb1* gene coding for modified Cry3Bb1 protein were transferred (MON88017),

(d) Maize resistant to Coleoptera pests and tolerant to glufosinate, to which the *cry34Ab1*, *cry35Ab1*, *pat* genes coding for the Cry34Ab1, Cry35Ab1, and PAT (phosphinothricin acetyltransferase) proteins were transferred, respectively (*B.t.* Cry34/35Ab1 Event DAS-59122-7)

(e) Maize tolerant to aryloxyalkanoate herbicide, to which the modified *aad-1* gene coding the modified AAD-1 protein (aryloxyalkanoate dioxygenase) was transferred (DAS40278).

It was not thought that the respective Bt proteins (Cry1A.105, modified Cry2Ab2, modified Cry1F, modified Cry3Bb1, and Cry34Ab1/Cry35Ab1 proteins) derived from the genes transferred to this stacked line interact with one another to change the specificity of the insecticidal activity in these proteins, because the specificity regions of the individual proteins were not changed. In addition, the substrates and actions of the PAT, modified CP4 EPSPS, and modified AAD-1 proteins are different, their involved metabolic pathways are independent, and there has been no report that Bt proteins exhibit enzyme activity. Therefore, it was concluded that these proteins did not interact to change the metabolic system of the recipient organism to produce an unexpected metabolite in this stacked line.

Based on the above, it was unlikely that these proteins derived from respective parent lines functionally interact with one another in the plant body of this stacked maize line, and therefore it was concluded that there were no trait changes to be evaluated, except having the traits which the parent line had.

The examination of the respective evaluation items has already been completed*. Based on the results of the examination, the conclusion described in the Biological Diversity Risk Assessment Report that the use of the respective parent lines in accordance with the Type I Use Regulation causes no Adverse Effect on Biological Diversity in Japan has been judged to be reasonable.

**a. Competitiveness**

**b. Productivity of harmful substances**

**c. Crossability**

* The results of the evaluation of the respective parent lines are available as described below.

[MON89034, Cry1F line 1507, MON88017, Event DAS-59122-7, DAS40278]

1. In the website, https://ch.biodic.go.jp/bch/OpenSearch.do, input “Maize” in the box of “Name of Organism” and then select “Search/View.”
2. Select “Name of Organism-Maize” of the appropriate line.
3. Select “Attached documents.”
4. Select “Document 2.”

(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 maize line, in accordance with the Type 1 Use Regulation, causes Adverse Effects on Biological Diversity in Japan. It was judged that the conclusion above made by the applicant is reasonable.

## References

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In-house report 3: DAS40278, Number of the transferred genes and their stability between generations and in one generation (In-house report)

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In-house report 5: Confirmation that the Cry1F and PAT proteins are stably produced in the progeny lines (In-house report)

In-house report 6: Confirmation that the Cry34Ab1 and Cry35Ab1, and PAT proteins are stably produced in the progeny lines (In-house report)

Isolated field test report 1: Evaluation of the safety of Bt Cry1F Maize line 1507 resistant to pest insects and tolerant to glufosinate in the isolated field environment (In-house report)

Isolated field test report 2: Environmental impact evaluation of Maize resistant to *Coleoptera* pests, and tolerant to glufosinate herbicide (*B.t.* Cry34/35Ab1 Event

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