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

Name: Monsanto Japan Limited
Applicant: Seiichiro Yamane, President
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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	*Lepidopteran* insect-protected soybean (Modified *cry1Ac*, *Glycine max* (L.) Merr.) (MON87701, OECD UI : MON-877Ø1-2)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, processing, storage, transportation, disposal, and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	The applicant performs the monitoring based on the monitoring plan specified separately.

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

(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 *Lepidopteran* insect-protected soybean (Modified *cry1Ac*, *Glycine max* (L.) Merr.) (MON87701, OECD UI : MON-877Ø1-2) (hereinafter referred to as "this recombinant soybean") are shown in Figure 1 (p. 3) and Table 1 (p. 4-6).

Seven amino acids of the Cry1Ac protein expressed by the *cry1Ac* gene introduced into this recombinant soybean are substituted, compared with the wild type (Genbank accession M11068). On the N-terminal region the four amino acids derived from the CTP1 protein were added to one another (Annex 5). Therefore, the *cry1Ac* gene introduced into this recombinant soybean and the expressed protein are referred to as the "modified *cry1Ac* gene" and the "modified Cry1Ac protein," respectively.

As for the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) which is expressed by the *cp4 epsps* gene introduced as a selective marker in the development process of this recombinant soybean, its nucleotide sequence was modified not to change the functional activity of the CP4 EPSPS: the 2nd residue from the N-terminal sequence is changed from serine to leucine, compared to the amino acid sequence from *Agrobacterium* sp. CP4 strain. Therefore, the *cp4 epsps* gene inserted in this recombinant soybean is referred to as the "modified *cp4 epsps* gene." However, this recombinant soybean is obtained by applying glyphosate herbicide to the R1 generation at a dose lower than normal and then selecting individuals damaged by the herbicide, that is, only individuals not containing the modified *cp4 epsps* gene due to genetic segregation are selected (Figure 3, p. 15).

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 donor nucleic acids used for the development of this recombinant soybean are shown in Table 1 (p. 4-6).

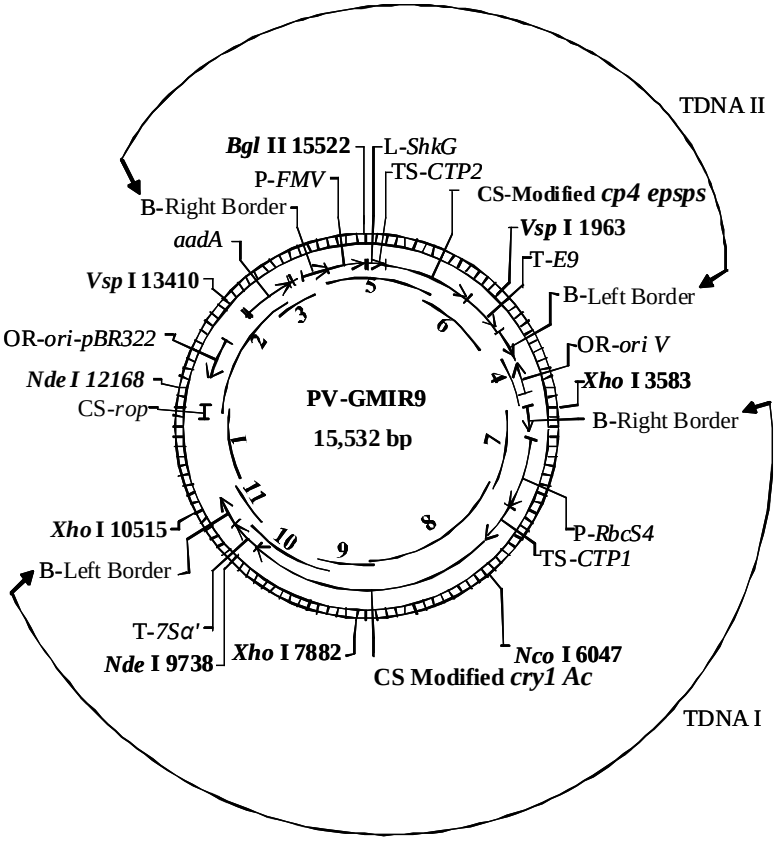


Figure 1. Plasmid map of PV-GMIR9[1]

In the process of the rearing of this recombinant soybean, individuals that contain the T-DNA I region but not the T-DNA II region shown above were selected.

[1]All the rights pertinent to the information in the figure above and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 1. Component elements of the donor nucleic acids, and their origins and functions [2]

Component elements	**Origin and function**
T-DNAII (It is not present in this recombinant soybean. It is continued from the 15,532 position in the plasmid.)	
Intervening Sequence	Sequence used in DNA cloning.
L[1]-*ShkG*	5'-terminal untranslated region of the *ShkG* gene of *Arabidopsis thaliana* (thale cress) coding for the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein (Klee et al., 1987; Herrmann, 1995). It is associated with the regulation of gene expression.
TS[2]-*CTP2*	Sequence coding for chloroplast transit peptides derived from the *ShkG* gene coding for the EPSPS protein of *A. thaliana* (Klee et al., 1987; Herrmann, 1995). It transports the target protein from the cytoplasm to the chloroplasts.
CS[3]-modified *cp4-epsps*	Coding sequence of the *aroA* gene coding for the 5-enolpyruvylshikimate-3-phosphate synthase derived from the *Agrobacterium* sp. CP4 strain (CP4 EPSP) (Padgette et al., 1996; Barry et al., 2001). In the amino acid sequence of the expressed protein, the serine at the second position from the N-terminal sequence is changed to leucine, compared with the amino acid sequence derived from the *Agrobacterium* sp. CP4 strain.
Intervening Sequence	Sequence used in DNA cloning.
T[4]-*E9*	3'-terminal untranslated region of the *RbcS2* gene coding for the ribulose-1,5-bisphosphate carboxylase small subunit of *Pisum sativum* (garden pea). It induces polyadenylation of mRNA(Coruzzi et al., 1984).
Intervening Sequence	Sequence used in DNA cloning.
B[5]-Left Border	DNA region derived from *Agrobacterium tumefaciens*. It contains the left border sequence used for the T-DNA transfer process (Barker et al., 1983).
Vector backbone region (absent in this recombinant soybean)	
Intervening Sequence	Sequence used in DNA cloning.
OR[6]-*ori V*	Origin of replication origin region derived from the broad-host-range plasmid RK2. It allows autonomous replication of vectors in *Agrobacterium* (Stalker et al., 1981).
Intervening Sequence	Sequence used in DNA cloning.

[2]All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

L[1]: Leader (Leader Sequence); **TS[2]**: Targeting Sequence (Targeting Sequence); **CS[3]**: Coding Sequence (Coding Sequence); **T[4]**: Transcription Termination Sequence (Transcription Termination Sequence); **B[5]**: Border (Border Sequence); **OR[6]:** Origin of Replication (Replication initiation region); **P[7]**: Promoter (Promoter)

Table 1. Component elements of the donor nucleic acids, and their origins and functions (continued)

Component elements	Origin and function
T-DNA I	
B-Right Border	DNA region derived from *A. tumefaciens* containing the right border sequence, which is used for the T-DNA transfer process (Zambryski et al., 1982; Depicker et al., 1982).
Intervening Sequence	Sequence used in DNA cloning.
P^7-*RbcS4*	Promoter, leader, and 5'-terminal untranslated region of the *RbcS4* gene coding for the ribulose-1,5-bisphosphate carboxylase small subunit 1A of *A. thaliana* (Krebbers et al., 1988). It induces expression in the terrestrial part of the plant body.
TS-*CTP1*	Sequence coding for the transit peptide derived from the *RbcS4* gene of *A. thaliana* (Krebbers et al., 1988). It transfers the modified Cry1Ac protein to the chloroplasts.
CS-modified *cry1Ac*	Sequence coding for the modified Cry1Ac protein derived from *B. thuringiensis* (Fischhoff and Perlak, 1996). Seven amino acids of the modified Cry1Ac protein are different compared with the wild-type Cry1Ac protein generated from *B. thuringiensis* ssp. *kurstaki* HD-73 strain.
Intervening Sequence	Sequence used in DNA cloning.
T-*7S α'*	3'-terminal untranslated region of the *Sphas1* gene coding for the soybean 7Sα' seed storage protein of *G. max*. It terminates mRNA transcription and induces polyadenylation (Schuler et al., 1982).
Intervening Sequence	Sequence used in DNA cloning.
B-Left Border	DNA region derived from *A. tumefaciens* containing the left border sequence, which is used for the T-DNA transfer process (Barker et al., 1983).

Table 1. Component elements of the donor nucleic acids, and their origins and functions (continued)

Component elements	**Origin and function**
Vector backbone region (absent in this recombinant soybean)	
Intervening Sequence	Sequence used in DNA cloning.
CS-*rop*	Coding sequence of the repressor of primer protein derived from the ColE1 plasmid. It maintains the number of copies of plasmid in *E. coli* (Giza and Huang, 1989).
Intervening Sequence	Sequence used in DNA cloning.
OR-*ori-pBR322*	Origin of Replication separated from the pBR32. It allows autonomous replication of vectors in *E. coli* (Sutcliffe, 1979).
Intervening Sequence	Sequence used in DNA cloning.
CS-*aadA*	Bacterial promoter, coding sequence, and 3' untranslated region derived from the 3"(9)-*O*-nucleotidyltransferase, the aminoglycoside modified enzyme of transposon Tn 7 (Fling et al., 1985)(GenBank accession X03043). It confers resistance to spectinomycin and streptomycin.
Intervening Sequence	Sequence used in DNA cloning.
T-DNA II (absent in this recombinant soybean. Continued to the head of Table.)	
B-Right Border	DNA region derived from *A. tumefaciens* containing the right border sequence, which is used for T-DNA transfer (Zambryski et al., 1982; Depicker et al., 1982).
Intervening Sequence	Sequence used in DNA cloning.
P-*FMV*	Promoter of the FMV 35S RNA (Rogers, 2000). It induces transcription in plant cells.
Intervening Sequence	Sequence used in DNA cloning.

(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

【Modified *cry1Ac* gene】

This recombinant soybean develops resistance to certain *Lepidoptera* pests by expressing the modified Cry1Ac protein encoded by the modified *cry1Ac* gene derived from *Bacillus thuringiensis* subsp. *Kurstaki*.

It is known that the Bt proteins produced by a Gram-positive bacterium commonly present in soil, *B. thuringiensis*, is associated with a specific receptor 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; Van Rie et al., 1990).

The modified *cry1Ac* gene of this recombinant soybean was developed by connecting the first 1,398 base of the *cry1Ab* gene (1-466 positions of the amino acid sequence) (Perlak et al., 1990) and the 1,399-3,534 bases of the *cry1Ab* gene (467-1178 positions of the amino acid sequence) (Adang et al., 1985; Fischhoff and Perlak, 1996) (Figure 2, p. 10). The first 1,398 base of the *cry1Ab* gene, in which the silent mutation has been already introduced to increase its expression levels in plant bodies, that is, in this position, only six amino acids are different in the amino acid sequence from that of the wild type Cry1Ac protein (Adang et al., 1985; Genbank accession M11068). In the 1,399 to 3,534 bases of the *cry1Ab* gene, the silent mutation was newly introduced in the nucleotide sequence in order to increase the expression levels in plant bodies. In this position, only one amino acid is different in the amino acid sequence from that of the wild type Cry1Ac protein. This was the 766th amino acid and thought to be an amino acid mutation originally present in *B. thuringiensis* ssp. *kurstaki* HD-73 stain used for gene cloning (b in Figure 2, p. 9). Therefore, in the modified Cry1Ac protein expressed by the modified *cry1Ab* gene, seven amino acids are different from those of the wild-type Cry1Ac protein produced by *B. thuringiensis* ssp. *kurstaki* HD-73 strain. These substitutions are the same as for the modified Cry1Ac protein expressed in Cotton resistant to *Lepidoptera* pests (*cry1Ac*, *Gossypium hirsutum* L.)(531, OECD UI:

MON-ØØ531-6), which has already approved under the Type 1 Use Regulation (November 22, 2004). In the modified Cry1Ac protein expressed in this recombinant soybean, four amino acids derived from CTP1 were added to the N-terminal region as well as the above-mentioned substitution of seven amino acids (Annex 5).

The homology between the deduced amino acid sequence of the modified Cry1Ac protein expressed in this recombinant soybean and that of the wild type CrylAc protein produced by *B. thuringiensis* ssp. *kurstaki* HD-73 strain is 99.1%.

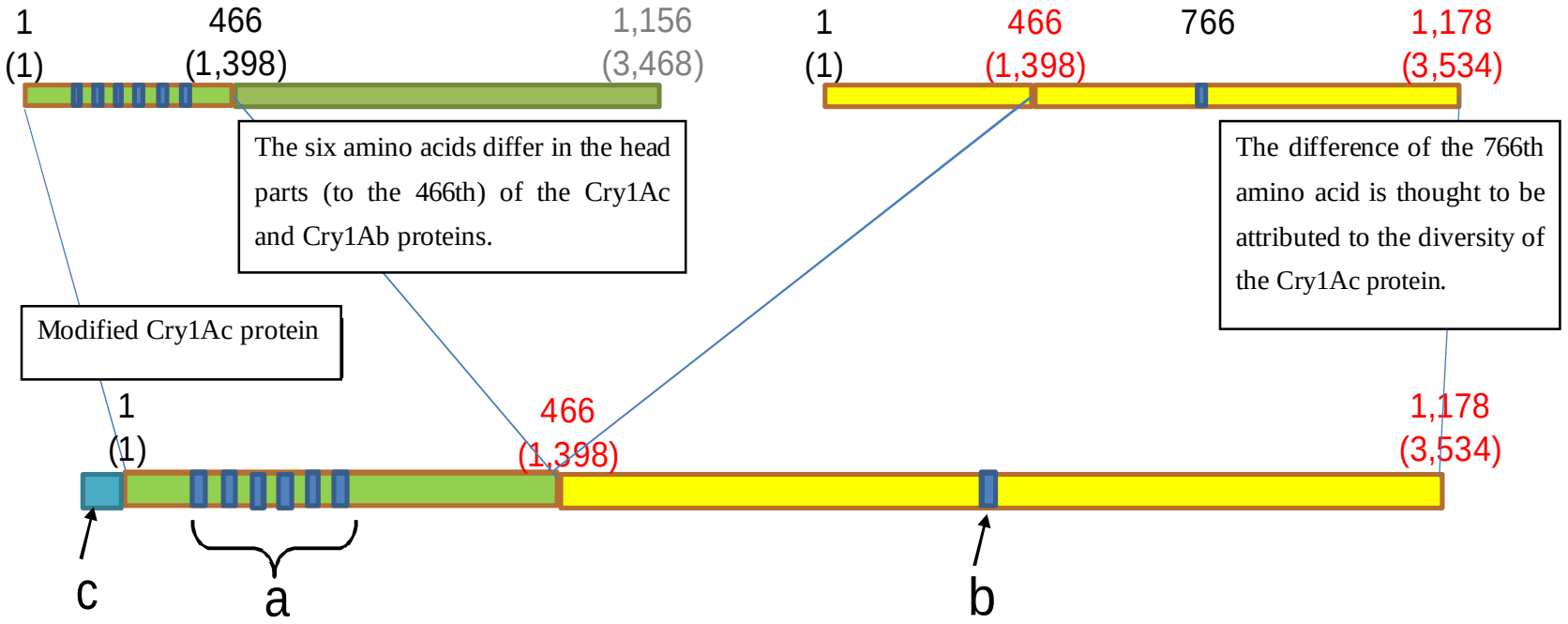
The Cry1A protein is known to have insecticidal activity against only *Lepidoptera* insects (Crickmore et al., 1998). Moreover, the protein classified as a Cry1Ac protein shows diversity within the degree of homology of 95% (Crickmore et al., 1998), and it is also known that there are several mutated forms of the Cry1Ac protein identified from *B. thuringiensi* (Von Tersch et al., 1991). As previously indicated, the homology between the modified Cry1Ac protein expressed in this recombinant soybean and the wild type Cry1Ac protein produced by *B. thuringiensis* ssp. *kurstaki* HD-73 strain was 99.1%, which is within the native degree of homology of 95% or more for the Cry1Ac protein, and therefore, the insecticidal spectrum of the modified Cry1Ac protein against *Lepidoptera* insects is thought to be the same as that of the Cry1Ac protein present in the natural world. It was identified from the literature review that the Cry1Ac protein does not have insecticidal activity against insect species other than *Lepidoptera* insects (Table 2, p 10). In addition, it is known that the sensitivity to the Cry1Ac protein differs with *Lepidoptera* insect species (Table 3, p.11).

This recombinant soybean has been cultivated in order to reduce or eliminate the use of pesticides currently used for *Lepidoptera* pest control mainly in some regions of South America classified as tropical and subtropic regions, which suffer serious damage by *Lepidoptera* pests. In fact, this recombinant soybean has been observed to exhibit insecticidal activity against major *Lepidoptera* pests in soybean cultivation in South America, such as velvetbean caterpillar (Biroud mame kemushi) (*Anticarsia gemmatalis*), soybean looper (*Pseudoplusia includes*), soybean axil borer (*Epinotia aporema*), and sunflower looper (*Rachiplusia nu*) (Annex 6-1,-2, -3 and -4).

It was examined whether or not the modified Cry1Ac protein shared similar amino acid sequences to those of known allergens by FASTA type algorithm, using allergen

database 10 (AD_2010[4]), the results showed no similarity of sequences with those of known allergens.

[4] AD_2010: It is the database developed based on the sequences obtained from Food Allergy Research and Resource Program Database (FARRP) (http://www.allergenonline.com), and contains 1,471 sequences.



a. The six differences are attributed to the differences in the amino acid sequences between the Cry1Ab protein, which is where the head part of the modified Cry1Ac protein is derived from, and the wild type Cry1Ac protein.
b. The difference in the 766th amino acid is attributed to the diversity of the Cry1Ac protein of *B. thuringiensis*.
c. Four amino acids derived from CTP1 are added to the N-terminal region of the modified Cry1Ac protein.

Figure 2. Method for constructing the modified Cry1Ac protein [5]

[5]All the rights pertinent to the information in the figure above and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 2. Insecticidal activity of the Cry1Ac protein by order[1,6]

Order	Insecticidal activity	Number of species examined	Number of sensitive species
Lepidoptera	+	47	46
Diptera	-	1	0
Coleoptera	-	8	0
Neuroptera	-	1	0
Hymenoptera	-	6	0
Hemiptera	-	6	0
Isoptera	-	1	0
Blattaria	-	1	0
Collembola	-	2	0
Acari	-	2	0
Haplotaxida	-	1	0

[1.] Prepared based on a literature review of 75 papers (for these papers, see Annex 7).

[6]All the rights pertinent to the information in this table and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 3. Insecticidal spectrum of the Cry1Ac protein[7]

Scientific name	Common name	LC_{50} (μg/ml diet)	95% Confidence Interval	Reference
Target insects of this recombinant soybean				
Anticarsia gemmatalis	Velvetbean caterpillar (Biroud mame kemushi)	0.039	0.012 - 0.094	Travalini et al. (2003)
Pseudoplusia includes	Soybean looper	0.21-0.48	0.16 - 0.65	Luttrell et al. (1999)
Epinotia aporema	Soybean axil borer	0.45	0.32 - 0.58	Bledig et al. (2001)
Rachiplusia nu	Sunflower looper	0.27	-	Bledig et al. (2001)
Other *Lepidoptera* insects				
Manduca sexta (L.)	Tobacco hornworm (Tabako suzumega)	0.036	0.028 - 0.048	MacIntosh et al. (1990)
Trichoplusia ni (Hübner)	Cabbage looper (Irakusa ginnuwaba)	0.09	0.018 - 0.18	MacIntosh et al. (1990)
		0.31	0.23-0.61	Moar et al. (1990)
Heliothis virescens (Fabricius)	Tobacco budworm	1	0.55 - 2.35	MacIntosh et al. (1990)
Helicoverpa zea (Boddie)	Corn earworm	10	6.4 - 24.8	MacIntosh et al. (1990)
Agrotis ipsilon (Hufnagel)	Black cutworm (Tamayanaga)	18	10.36 - 36.1	MacIntosh et al. (1990)
		>200	-	Gilliland et al. (2002)
		202.5	-	Lu and Yu (2008)
Ostrinia nubilalis (Hübner)	European corn borer (Yoroppa awanomeiga)	37	17.8 - 115.9	MacIntosh et al. (1990)
Spodoptera exigua (Hübner)	Beet army worm (Shiroichimonjoto)	44	41.9 - 46.4	MacIntosh et al. (1990)

[7]All the rights pertinent to the information in this table and the responsibility for the contents rest upon Monsanto Japan Limited.

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

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(2) Information concerning vectors

1) Name and origin

The vector PV-GMIR9 used for the development of this recombinant soybean was constructed from several plasmid vectors including plasmid pBR322 derived from *E. coli*.

2) Properties

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

The total number of base pairs in the PV-GMIR9 used for the development of this recombinant soybean is 15,532 bp.

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

As a marker gene for selecting the constructed vector in *E. coli*, the *aadA* gene derived from the transposon Tn7 conferring resistance to spectinomycin and streptomycin is present outside the T-DNA region.

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

The infectivity of this vector is not known.

(3) Method of preparing living modified organisms

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

The component elements of the plasmid vector transferred to the recipient organism are listed in Table 1 (p. 4-6). The positions of the component elements of the donor nucleic acid and sites cleaved by restriction enzymes in the vector are shown in Figure 1 (p. 3).

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

The Agrobacterium method was used to transfer the plasmid vector PV-GMIR9 into the apical meristems of plumules of the non-recombinant soybean variety A5547.

3) Process of rearing of living modified organisms

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

Apical meristems isolated from plumules of the conventional soybean variety A5547 were co-cultivated with *A. tumefaciens* ABI strain containing the plasmid vector PV-GMIR9, which were subsequently incubated on the tissue culture medium containing glyphosate to select transformed cells.

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

Carbenicillin, Cefotaxime, and ticarcillin-clavulanic acid were added to the tissue culture medium to remove any residual *Agrobacterium* used for transformation. Then, at the R5 generation of this recombinant soybean, PCR analysis was conducted for the backbone region of the plasmid vector PV-GMIR9 used for transformation. As a result, the plasmid vector PV-GMIR9 backbone region was not detected from this recombinant soybean (Annex 8), and thus, it was considered that there was no residual *Agrobacterium* used for transformation in this recombinant soybean.

(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

The transformed regenerated plants (R0) were self-pollinated, and the subsequent R1 plants were screened for the presence of the modified *cp4 epsps* gene by applying glyphosate herbicide at a dose lower than normal. Plants damaged by glyphosate were selected as individuals not containing the T-DNA II region (region containing the modified *cp4 epsps* gene expression cassette). Then using the selected R1 plants which did not contain the T-DNA II region, further selection was carried out based on the TaqMan PCR to select individuals containing the T-DNA I region (region containing the modified *cry1Ac* gene expression cassette) in a homozygous state. The progenies of the selected plants were subjected to the analysis of the inserted genes and morphological assessments, and based on the results, MON87701 line was selected as the final commercial line.

The process of rearing of this recombinant soybean is illustrated in Figure 3 (p. 15). The scope of this application covers the R5 generation and all progeny hybrid lines derived from the R5 generation.

【Confidential: not disclosed to unauthorized persons】

Figure 3. Process of rearing of this recombinant soybean

【Confidential: not disclosed to unauthorized persons】

(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

In order to examine whether the transferred gene of this recombinant soybean exists on the chromosome, this recombinant soybean having the transferred gene in a homozygous state (R5 generation) was crossed with a soybean variety not containing the modified *cry1Ac* gene (MSOY8329) to produce F1 plants. The F1 plants were subsequently self-pollinated to produce the F2 generation. Of the plants of the F2 generation, one individual having the modified *cry1Ac* gene in a heterozygous state was self-pollinated to produce the F3 generation. The genotypes of the transferred genes of these F2 and F3 generations were examined using the TaqMan PCR and tested for the segregation ratio. As a result, the segregation ratio of the transferred gene was matched to the presumed segregation ratio according to Mendel's law, 1:2:1 (Table 4, p 16; Table 2 in Annex 9). Therefore, it was concluded that the transferred gene resides on the chromosome of this recombinant soybean.

Table 4. Segregation ratio of the transferred gene in the F2 and F3 generations of this recombinant soybean[8]

					1:2:1 Segregation				
Generation	Number of plants tested	Observed value +/+	Observed value +/−	Observed value −/−	Expected value +/+	Expected value +/−	Expected value −/−	χ^2	p value
F2	297	79	148	70	74.25	148.50	74.25	0.5	0.76
F3	263	73	121	69	65.75	131.50	65.75	1.8	0.41

[1] The F3 generation was produced by self-pollination of a heterozygote out of the individuals of the F2 generation.

[8]All the rights pertinent to the information in this table and the responsibility for the contents rest upon Monsanto Japan Limited.

(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 for existence of the transferred gene, it has been confirmed that a single copy of the T-DNA I region was transferred at a single site in the genome of this recombinant soybean (Figures 4-6 in Annex 10, p. 38-40) and was stably inherited in offspring across multiple generations (R4, R5, R6, R8, and R9 generations) (Figure 14 in Annex 10, p. 52). In addition, it has been confirmed that the backbone region and the T-DNA II region were not transferred to this recombinant soybean (Figures 7-9 in Annex 10, p. 41-43).

(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 (Figures 4-6 in Annex 10, p. 38-40).

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

Based on Western blot analysis, it has been confirmed that the modified Cry1Ac protein is stably expressed across multiple generations (R4, R5, R6, R8, and R9 generations) of this recombinant soybean (Figure 2 in Annex 11, p. 15).

Moreover, this recombinant soybean was cultivated in three replicated plots in five field sites in the U.S. (Alabama, Arkansas, Georgia, Illinois, and North Carolina), and the expression levels of the modified Cry1Ac protein in the over-season leaf (OSL), roots, terrestrial part, and seeds were analyzed by the ELISA (Annex 12). The leaves were sampled four times at different growth stages (OSL-1: 3-4 leaf stages, OSL-2: 6-8 leaf stages, OSL-3: 10-12-leaf stages, and OSL-4:14-16 leaf stages). The expression levels of the modified Cry1Ac protein in pollen (including anthers) of this recombinant soybean, which was cultivated in one replicated plot in a field in the US (Illinois), was also determined.

As a result, the mean expression levels of the modified Cry1Ac protein were the highest in leaves (30-53 µg/g fwt), next, in terrestrial part (8.1 µg/g fwt), seeds (4.2

μg/g fwt), and pollen (2.3 μg/g fwt), in that order. The expression levels of the modified Cry1Ac protein in roots were undetectable (LOD=0.347 μg/g fwt) (Table 5, p. 18 and Table 1 and 2 in Annex 12, p. 18-19).

In the rearing process, the selection was performed, while confirming the expression levels of the modified Cry1Ac protein in each generation.

Table 5. Expression levels of the modified Cry1Ac protein in the tissues of the MON87701 line (2007, US)[9]

Tissue type [9]	Cry1Ac µg/g fwt Mean (SD)[1,3]	Range [4] (µg/g fwt)	Cry1Ac µg/g dwt Mean (SD)[2]	Range (µg/g dwt)	LOQ/LOD (µg/g fwt)
OSL-1	30(8.5)	12-40	220(70)	110-350	2.5/0.74
OSL-2	38(16)	18-80	260(100)	130-500	2.5/0.74
OSL-3	34(17)	14-77	240(110)	94-480	2.5/0.74
OSL-4	53(36)	15-110	340(290)	78-960	2.5/0.74
Root	< LOD	< LOD	NA[5]	NA[5]	0.4/0.347
Terrestrial part	8.1(7.2)	2.5-26	29(28)	8.2-95	2.0/0.55
Harvested seeds	4.2(0.73)	3.1-5.0	4.7(0.79)	3.4-5.7	1.0/0.47
Pollen/anther [6]	2.3(0.58)	1.8-3.1	NA[7]	NA[7]	ND[8]

1. As for protein expression levels, the protein weight (µg) per tissue weight (g) was expressed per fresh weight (fwt).
2. Protein expression levels were expressed as µg/g per dry weight (dwt). The dry weight values were calculated by dividing the fresh weight (fwt) by the dry weight conversion factors obtained from moisture analysis data.
3. The mean and standard deviation (SD) were calculated for each tissue type (OSL-1: n=13, terrestrial part: n=14, pollen/anther: n=4, others: n=15)
4. Minimum and maximum values were determined for each tissue type.
5. When the value per the fresh weight was undetectable, it was not converted to that per the dry weight.
6. Because of the small amount of samples, the accuracy was not confirmed for the evaluation of pollen/anther, however, the optimized ELISA was used.
7. Because of the small amount of samples of pollen/anther, no conversion to values per dry weight was performed.
8. Because of the small amount of samples of pollen/anther, the limit of detection (LOD) and the limit of quantitation (LOQ) were not determined.

[9] All the rights pertinent to the information in this table and the responsibility for the contents rest upon Monsanto Japan Limited.

9 OSL1-4 means OSL1: 3-4 leaf stages, OSL2: 6-8 leaf stages, OSL3: 10-12 leaf stages, and OSL4: 14-16 leaf stages. In each growth stage, leaf samples were collected. As for terrestrial part, the plant body in the R6 stage (full seed) was collected.

(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 transferred nucleic acid does not have the function allowing transmission. Therefore, it is considered unlikely that it could be transmitted through virus infection and/or other routes to any other wild animals and wild plants.

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

This recombinant soybean can be specifically detected and identified by the End-Point TaqMan PCR using a primer set specifically binding to this recombinant soybean (Annex 13). The recommended DNA concentration for this assay is 5-10 ng per PCR reaction, so the assay can be performed using a single seed.

The reproducibility and reliability of this method was verified using 91 seeds from this recombinant soybean and 44 seeds from the non-recombinant soybean (Annex 13).

(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

The modified *cry1Ac* gene transferred into this recombinant soybean expresses the modified Cry1Ac protein to impart resistence to *Lepidoptera* pests (Annex 6-1, 2, 3, and 4).

(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[10]

From 2009 to 2010 isolated field tests were carried out in Kawachi Research Farm, Monsanto Japan Limited, using this recombinant soybean. The tests were

[10] All the rights pertinent to the information in a. through g. in this section and the responsibility for the contents rest upon Monsanto Japan Limited.

conducted using the R9 generation of this recombinant soybean (Figure 3, p. 15). As the non-recombinant control soybean, A5547, the host plant of this recombinant soybean for gene transfer was used. The wintering ability and the fertility and size of the pollen at the early stage of growth were tested in the Monsanto Company (US).

a. Morphological and growth characteristics

The differences in morphological and growth characteristics were investigated for 20 items, based on the designated items for classification of seeds and seedling characteristics for registration of seeds and seedlings (initiation of germination, date of germination, uniformity of germination, number of germinated plants, germination rate, shape of leaflet, trichome quantity, time of flower initiation, time of flower completion, growth type, maturation period, main stem length, number of main stem nodes, number of branches, the lowest main stem node position with pod, plant type, weight of plant at harvest time, and appearance of harvested seed (seed hull color, uniformity of seeds, and seed shape)). As for the items on which statistical analyses were performed (number of germinated plants, main stem length, number of main stem nodes, number of branches, the lowest main stem node position with pod, and weight of plants at harvest time), statistically significant differences were observed in the number of germinated plants between this recombinant soybean and the non-recombinant control soybean (Table 2 in Annex 14, p. 9). The number of germinated plants of this recombinant soybean, 913, was smaller than that of the non-recombinant control soybean, 973.

As for the items on which statistical analyses were not performed (initiation of germination, date of germination, uniformity of germination, germination rate, shape of leaflet, trichome quantity, time of flower initiation, time of flower completion, elongation type, maturation period, plant type, appearance of harvested seed (seed hull color, uniformity of seeds, and seed shape)), no differences were observed in all items other than uniformity of germination between this recombinant soybean and the non-recombinant control soybean. Since the germination rates of this recombinant soybean and the non-recombinant soybean did not reach 80%, the uniformity of germination could not be observed (Table 2 in Annex 14, p. 9).

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

Cold tolerance at the early stage of growth was evaluated in a climate chamber in the Monsanto Company (US) in 2007. In the cold tolerance at the early stage of growth, this

recombinant soybean, the non-recombinant control soybean A5547, and six conventional commercial cultivars were grown in a greenhouse, and 19 days after sowing, the seedlings were transferred to and grown in a climate chamber at 15°C (day)/8°C (night) for 20 days to examine and compare plant vigor, main stem length, growth stage, fresh weight, and dry weight. As a result, for all items subjected to statistical analyses, no statistically significant differences were observed between this recombinant soybean and the non-recombinant control soybean (Table 4 in Annex 15, p. 21).

c. Wintering ability and summer survival of the mature plant

This recombinant soybean and the non-recombinant control soybean raised in an isolated field were left to grow after the maturation period to observe the growth conditions in winter in Japan. As a result of observation made on January 5, 2010, in the plot for investigation of wintering ability, this recombinant soybean and the non-recombinant control soybean were both found dead (Figure 6 in Annex 14, p. 12).

d. Fertility and size of the pollen

Pollens were sampled from this recombinant soybean and the non-recombinant control soybean grown in an isolated field, and the samples were stained with iodine potassium iodide solution to observe their fertility and size. As a result, no significant difference was observed in pollen fertility between this recombinant soybean and the non-recombinant control soybean. Furthermore, no difference was observed in shape or size of pollen (Figure 7 in Annex 14, p. 13).

In addition, in 2007, pollens were sampled from this recombinant soybean and the non-recombinant control soybean grown in a field in Illinois, U.S., to examine their fertility and size. As a result, no statistically significant difference was observed in pollen fertility or size (Table 2 in Annex 16, p.16; Figure 1, p. 17).

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

Items related to seed production (number of ripe pods, approximate grain weight per plant, precise grain weight per plant, and 100-seed weight) were examined in this recombinant soybean and the non-recombinant control soybean grown under the same conditions in the isolated field, and the obtained data was subjected to statistical analyses. As a result, no statistically significant differences

were observed between this recombinant soybean and the non-recombinant control soybean (Table 3 in Annex 14, p. 16).

Regarding the shattering habit, this recombinant soybean and the non-recombinant control soybean grown in an isolated field were harvested during the maturation period, and the harvested plants were left to air-dry in a vinyl house before examining the degree of pod shattering. As a result, this recombinant soybean and the non-recombinant control soybean were both found to be shatter-resistant and showed no difference in the pod shattering habit (Table 3 in Annex 14, p. 16).

Regarding dormancy and germination rate, seeds were collected immediately after harvesting this recombinant soybean and the non-recombinant control soybean grown in an isolated field, and the seeds were incubated on a Petri dish at 25ºC to examine the number of germinated plants over time. As a result, the germination rates of this recombinant soybean and the non-recombinant control soybean were both high (91.7% and 94.4%, respectively) and showed no statistically significant differences in the number of final germinated plants (Table 3 in Annex 14, p. 16).

f. Crossability

This recombinant soybean was used as the pollen parent to examine the crossability between this recombinant soybean and the non-recombinant control soybean. The frequency of occurrence of hybrids in the harvested seeds of the non-recombinant control soybean was identified to examine the crossability. Identification of the hybrid was based on whether or not the protein was expressed in this recombinant soybean used as the pollen parent.

In the plot for investigation of morphological and growth characteristics, seeds were harvested from non-recombinant control soybean plants that were cultivated in rows (except three plants at each end) neighboring this recombinant soybean. The non-recombinant control soybean plants were neighbored by this recombinant soybean in the plot on the southeast or northwest border at a distance of 1.65 m (Figure 2 in Annex 14, p. 5). The plot was not covered with an insect net during the flowering period. Four hundred and eighty seeds were randomly selected from the harvested seeds and sown in pots in a greenhouse. As soon as they reached 2- to 3-leaf stages, whether or not the protein was expressed in leaves was determined per one seed, using the lateral-flow method.

The expressed proteins were detected in none of the 480 seeds sown in this study (Annex 14). It was concluded that no crossing was observed in this study.

g. Productivity of harmful substances

To confirm whether or not this recombinant soybean produces any substances affecting soil microbes and other plants, a soil microflora test, a plow-in test and a succeeding crop test were conducted. As a result, no statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean regarding the number of soil microbes and the number of germinated roots and dry weight of radish (Tables 5-7 in Annex 14, p. 20).

II. Results of 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 Effects on Biological Diversity (called Experts) for possible Adverse Effects 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

This recombinant soybean was developed by transferring the T-DNA region of the plasmid PV-GMIR9, constructed based on the plasmid pBR322, etc., derived from *Escherichia. coli*, by the *Agrobacterium* method.

Based on the segregation ratio of the transferred gene and the Southern blot analysis, it has been confirmed that a single copy of the T-DNA region, which contains the modified *cry1Ac* gene encoding the modified Cry1Ac protein derived from *Bacillus thuringiensis*, resides on the chromosome of this recombinant soybean and is stably inherited across multiple generations. In addition, it has been confirmed by Western blot analysis and ELISA that the target gene is stably expressed across multiple generations.

The content of the Type 1 Use of Living Modified Organism of this recombinant soybean was evaluated, within the scope of the provision as food, provision as feed, processing, storage, transportation, disposal, and acts incidental to them, for risk of Adverse Effects on Biological Diversity in terms of the following 1) to 3).

1) Competitiveness

Soybean, the taxonomical species to which the recipient organism belongs, has been cultivated for a long time in Japan, but there is no report that it grows voluntarily in the natural environment in Japan.

Studies on various characteristics related to competitiveness of this recombinant soybean were carried out in a climate chamber in the U.S. in 2007 and in isolated fields in Japan in 2009-2010. As a result, no differences were observed between this recombinant soybean and the non-recombinant control soybean.

It is known that weeds competing with other wild plants have one or more of characteristics including dormancy, a shattering habit or seed dispersal mechanism. However, based on the above-mentioned results of the examination and others, it was

thought that those characteristics did not change between this recombinant soybean and the non-recombinant control soybean. Therefore, it is difficult to conclude that there is competitiveness causes only by the resistance to *Lepidoptera* pests, which are conferred on this recombinant soybean, under the natural environment in Japan.

Based on the above, within the scope that the content of the Type 1 Use of Living Modified Organism of this recombinant soybean includes the provision as food, provision as feed, processing, storage, transportation, disposal, and acts incidental to them, it was judged that the conclusion made by the applicant that the wild animals and wild plants likely to be affected cannot be specified and that this recombinant soybean poses no significant risk of Adverse Effects on Biological Diversity attributable to competitiveness is reasonable.

2) Productivity of harmful substances

Regarding the plant species of soybean to which the recipient organism belongs, there is no report that it produces any substance harmful to wild animals and wild plants.

This recombinant soybean expresses the modified Cry1Ac protein, and it has been confirmed that the modified Cry1Ac protein expressed in this recombinant soybean does not have similar amino acid sequence to that of known allergens. In addition, the modified Cry1Ac protein that does not have enzyme activity was considered unlikely to affect the metabolic pathway of the recipient organism and produce any harmful substances.

In addition, as a result of soil microflora tests, plow-in tests and succeeding crop tests carried out in the isolated field in Japan to examine the production of harmful substances by this recombinant soybean (the substances secreted from the roots, which can affect other plants and microorganisms in soil and substances existing in the plant body, which can affect other plants after dying), no difference was observed between this recombinant soybean and the non-recombinant control soybean.

As the wild animals likely to be affected by this recombinant soybean, 26 *Lepidoptera* insect species listed as endangered species or near threatened species inhabited Japan and 18 *Lepidoptera* insect species consuming *Glycine soja* were identified.

In the following cases, the effects on the identified *Lepidoptera* insect species were studied:

(a) when directly consuming this recombinant soybean,

(b) when consuming the pollen released from this recombinant soybean, and

(c) when consuming the hybrid of *Glycine soja* and this recombinant soybean with resistance to *Lepidoptera* pests and its progeny.

Then, based on the following results, it was concluded that the identified *Lepidoptera* insects were very unlikely to be affected by the modified Cry1Ac protein at the population level.

(a) a place where the imported this recombinant soybean falls during transportation and subsequently grows is predicted to be a place neighboring the road for transportation. However, it is unlikely that the population of the identified *Lepidoptera* insects locally inhabits such a place and is dependent only on soybean;

(b) since the production of pollen of soybeans is extremely low and the pollen is unlikely to disperse due to its stickiness, it is unlikely that larvae of the identified *Lepidoptera* insects consume the pollen of this recombinant soybean. In addition, the identified *Lepidoptera* insects do not locally inhabit some places along the road where soybeans fall, so it is extremely unlikely that the population of the insects is affected; and

(c) it is unlikely that the identified *Lepidoptera* insects are dependent only on *Glycine soja*, and as described below 3) Crossability, it is also very unlikely that this recombinant soybean imported to Japan falls during transportation, subsequently grows, and are crossed with *Glycine soja* to produce the hybrid. Therefore, it is very unlikely that the population of *Lepidoptera* insects is affected by consuming the hybrid with resistance to *Lepidoptera* pests and its progeny.

Based on the above, within the scope that the content of the Type 1 Use of Living Modified Organism of this recombinant soybean includes the provision as food, provision as feed, processing, storage, transportation, disposal, and acts incidental to them, it was judged that the conclusion made by the applicant that his recombinant soybean poses no significant risk of Adverse Effects on Biological Diversity attributable to Productivity of harmful substances is reasonable.

3) Crossability

Since *Glycine soja*, which is known as a wild relative of soybean, has the same chromosome number (2n=40) as soybean and can be crossed with soybean, it was specified and assessed as a potentially affected wild plant as described below.

There is no obstacle to the growth of the hybrid obtained from artificial crossing between soybean and *G. soja*. Thus, in the case where this recombinant soybean and *G. soja* crossed with each other in the natural environment in Japan, there is a possibility that the hybrid would grow and that the gene transferred into this recombinant soybean

could diffuse among the population of *G. soja* through crossing of the hybrid to *G. soja*. Moreover, since *G. soja* grows voluntarily and widely throughout Japan on riversides, banks, around fields, in orchards, etc., in the case where this recombinant soybean grows near *G. soja*, they could cross with each other.

However,

(a) there is a report regarding hybridization between soybean and *G. soja* and gene penetrance to the progenies that, in a follow-up study conducted on *G. soja* population located near soybean fields throughout Japan for several years, the occurrence of crossing between *G. soja* and soybean was checked using genetic markers, etc., but none of the obtained results demonstrated continuous existence of hybrid progenies;

(b) it is known that the flowering times of soybean and *G. soja* rarely overlap with each other, and there is also a report that, even when a soybean variety whose flowering time overlaps that of *G. soja* and *G. soja* were alternately grown at distances of 50 cm, the crossing rate was 0.73%; and

(c) there is a report that, in a crossing study conducted by sowing seeds of recombinant soybean (herbicide glyphosate-tolerant) and *G. soja* at different timings and growing the two species with the *G. soja* vines wrapped around the soybean plants, among the seeds harvested from *G. soja*, one seed was found to be a hybrid with soybean in a plot (11,860 seeds) in which the blooming times of the two species were the closest to each other.

In addition, in an isolated field test conducted in Japan in 2009, the occurrence of natural crossing of this recombinant soybean and the non-recombinant soybean was examined, but no crossing was observed. Moreover, when various characteristics related to reproduction were compared between this recombinant soybean and the non-recombinant control soybean, no differences in the morphology and fertility of pollen and no statistically significant differences in seed productivity were observed. Based on these findings, it is likely that the crossing rate of this recombinant soybean and *G. soja* was extremely low, as in the case of conventional soybean.

Since the hybrid between soybean and *G. soja* and its progeny carry soybean genes at a constant rate, their adaptability to the natural environment is lower than that of *G. soja* and then they disappear rapidly. On the other hands, when the modified *cry1Ac* gene is transferred to *G. soja*, it is likely that due to the conferred resistance to *Lepidoptera* pests its adaptability increases, however, the degree of the effect is considered to decrease based on the the following:

(a) observation of *Lepidoptera* insects living on *G. soja*,

(b) literature review on sensitivity of those insects to the Cry1Ac protein,

(c) observation of feeding damage to *G. soja* by various insects and defoliation study

on *G. soja*, and other examinations.

G. soja suffers feeding damage and other damages from many organisms with no sensitivity to the Cry1Ac protein. In fact, the study on the population of *G. soja* growing wild in Ibaraki and Saga prefectures in 2011 indicated that the degree of feeding damage by *Lepidoptera* insects was up to approximately 5% of leaf area. Therefore, considering together with the results of the defoliation study in the above (c), the degree of feeding damage by the identified *Lepidoptera* insects was not enough to affect growth rate and seed production of *G. soja*. In addition, the population dynamics of *G. soja* was limited by mainly environmental factors and competition with grass and perennial weeds and feeding damage by *Lepidoptera* insects was not become a big limiting factor.

Considering the above, it was concluded that competitiveness of the hybrid between this recombinant soybean and *G. soja* was low, as with that of the hybrid between conventional soybean and *G. soja*.

When the use of this recombinant soybean was limited to import and processing and others, and if it fell during transportation and subsequently grew, the possible occurrence of crossing between this recombinant soybean and *G. soja* was examined based on the investigation on import experience and modes of transportation and others. The results showed the following:

(a) The use of this recombinant soybean is classified roughly into oil expression, provision as feed, and provision as food except oil expression (tofu, natto, miso etc.), and the possibility of falling of soybean for provision as feed was thought to be higher than that for the other uses, based on the following reasons:

i. since soybeans for oil expression are directly brought into a plant adjacent to the port, the possibility of their falling related to inland transportation is low;

ii. soybeans for provision as feed are transported overland by sealing means such as a flexible container, and most of those for the other uses are transported overland in bulk; and

iii. as for soybeans for provision as food except oil expression, recombinant soybeans themselves are unlikely to be used and they are transported in a highly sealed state, such as a paper bag.

However, the amount of fallen soybeans is thought to be small and it is thought that the amount of the fallen soybean seeds imported into Japan is extremely limited, considering the following points:

a. they are loaded in a deep-box-type dump truck with loading depth;

b. the load is covered with double sheets; and

c. the measures to prevent seed falling, such as prohibiting overloading, are taken and the plants are also limited.

(b) The possibility of growing of the soybean seed falling during transportation is low, considering growing characteristics (weediness) and growing environment, including herbicidal activities on highways, which are thought to be transportation routes.

(c) It is thought to be unlikely that the individuals grown from the fallen seeds grow next to *G. soja* and are crossed with it, in terms of the habitat of *G. soja*, the flowering seasons of soybean and *G. soja*, and the crossing rate.

Based on the above, soybean seeds imported for the provision as food or feed are extremely unlikely to fall during transportation, grow, and are crossed with *G. soja*.

The number of hybrid seeds of *G. soja* crossed with soybeans grown to flowing from seeds, which fell during transportation from the port to respective feed plants, was estimated, on the assumption that the occurrence of crossing between *G. soja* of this recombinant soybean growing from the seeds falling during transportation is the highest, based on annual imports, use amount by use, status of the use, mode of transportation of soybeans in Japan, and the number of the soybean plants confirmed to grow determined by the field studies of modified plants by the Ministry of Agriculture, Forestry and Fisheries of Japan (2009 and 2010).

Next, as an example, using the route estimated to have the biggest "number of soybeans, which may fall during transportation and grow next to *G. soja*," further estimation was performed, considering along with the two conditions characterizing the current situation ((a) the number of soybean individuals, which fell during transportation from the port to feed plants, decreases every 5 km of transportation distance, (b) judgment whether or not soybeans falling during transportation grow next to *G. soja* based on the investigation of land utility from using a GIS (geographic information system)). The results showed that the number of possible soybean individuals, which fell during transportation from the port to feed plants, and grow next to *G. soja*, was up to 11.99 individuals and that the number of the hybrid seeds, which is likely to be produced by crossing between the said soybean individuals and *G. soja*, was up to 0.75 seeds.

Therefore, the number of hybrid seeds of *G. soja* crossed with soybeans, whose seeds were imported into Japan, fell during transportation from the port to respective feed plants, grow next to *G. soja*, and are crossed with *G. soja*, was thought to be very small.

The population of plants habiting a stable natural ecosystem is generally maintained at a certain level. The number of the seeds growing to fruit the next year, out of the hybrid seeds between this recombinant soybean and *G. soja*, was estimated to be up to 0.0015 (0.75×0.0020) seeds, based on the proportion of the seeds, which grow to fruit the next year, of the fruited seeds was approximately 0.13 to 0.20% (488 to 741 seeds per an individual), given the seed production per an individual of *G. soja*, was the seed production necessary to maintain the population of *G. soja*. Therefore, the progeny of the hybrid seeds was extremely unlikely to grow.

Based on the above, within the scope that the content of the Type 1 Use of Living Modified Organism of this recombinant soybean includes the provision as food, provision as feed, processing, storage, transportation, disposal, and acts incidental to them, it was judged that the conclusion made by the applicant that this recombinant

soybean poses no significant risk of Adverse Effects on Biological Diversity attributable to Productivity of harmful substances is reasonable.

In addition, in order to understand the changes of the situations used as premises for those evaluations and the growth situation of this recombinant soybean, a monitoring by the applicant based on the monitoring plan is included in the Type 1 Use Regulation for the Type 1 Use of this recombinant soybean and the monitoring will be performed.

(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 recombinant soybean, 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.

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List of Annexes for *Lepidopteran* insect-protected soybean (Modified *cry1Ac*, *Glycine max* (L.) Merr.) (MON87701, OECD UI : MON-877Ø1-2)

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Annex 14 Biological Diversity Risk Assessment Report of *Lepidopteran* insect-protected soybean (Modified *cry1Ac, Glycine max* (L.) Merr.) (MON87701, OECD UI : MON-877Ø1-2) in Isolated Field (Confidential)

Annex 15 An Assessment of the Effect of Cold Stress on Insect-Protected Soybean MON87701 under Growth Chamber Conditions (MSL0021174) (Confidential)

Annex 16 Viability and Morphology Evaluation of Pollen from Insect-Protected Soybean MON87701 Produced in a U.S. Field Trial During 2007 (MSL0021055) (Confidential)

Annex 17 Habitats and terrestrial part for *Lepidoptera* insects included in the Ministry of the Environment Red List (Confidential)

Annex 18 Insecticidal Activity of Purified -Endotoxins from *Bacillus thuringiensis* Against *Colias lesbia* (Fab.) (*Lepidoptera*: Pieridae)

and *Spilosoma virginica* (Fab.) (*Lepidoptera*: Arctiidae) (MSL18020) (Confidential)

Annex 19 Independent and combined activity of Cry1Ac and in Cry2Ab2 *in vitro* and plant tissue studies (MSL16204) (Confidential)

Annex 20 Discussion of possibility that soybean individuals, which fall during transportation, germinate, and grow, grow next to *Glycine soja* (Confidential)