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

Applicant
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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Soybean tolerant to glyphosate herbicide (Modified *cp4 epsps*, *Glycine max* (L.) Merr.) (MON 89788, OECD UI: MON-89788-1)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

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

1. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of donor nucleic acid that was used for the development of soybean tolerant to glyphosate herbicide (Modified *cp4 epsps*, *Glycine max* (L.) Merr.) (OECD UI MON-89788-1) (hereinafter referred to as “this recombinant soybean”) and the origins and functions of component elements are shown in Figure 1 and Table 1.

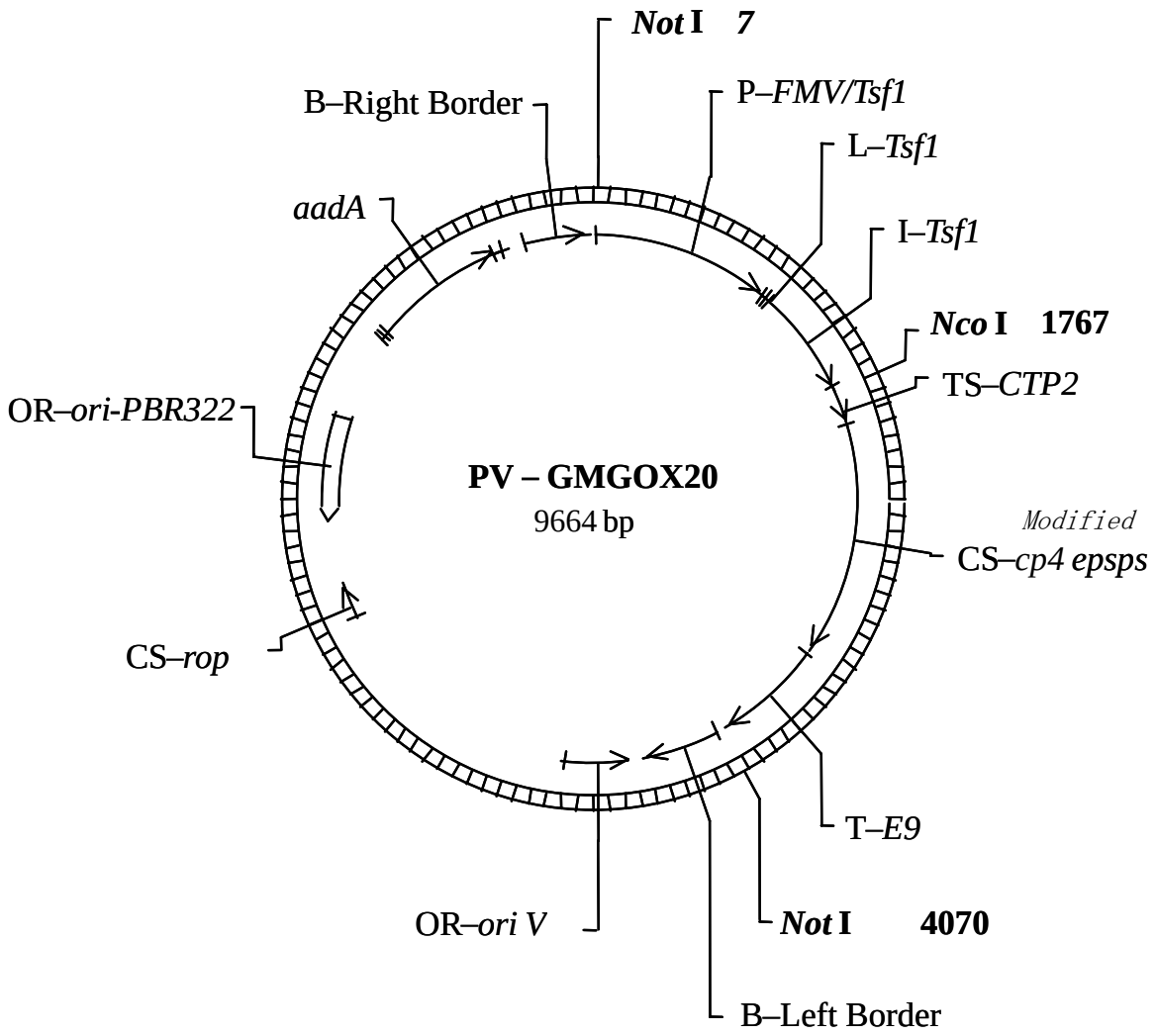

Figure 1: Plasmid map of PV-GMGOX20 [1]

The T-DNA region transferred in this recombinant soybean is from the B-Right Border to the B-Left Border in the above map in the clockwise direction.

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

Table 1: Origins and functions of the component elements of PV-GMGOX20 used for the development of this recombinant soybean [2]

Component elements	Origin and function
T-DNA region	
B[1]-right border	A DNA region derived from *Agrobacterium tumefaciens*, including the right border sequence; used as the initiation point of T-DNA transfer (Reference 23).
P[2]-*FMV*/*Tsf1*	Chimaera promoter with the enhancer sequence of Figwort Mosaic Virus (FMV) 35S promoter (Reference 25) bound to *Arabidopsis thaliana Tsf1* promoter (Reference 24). Involved in the constant expression of the target gene in the entire tissue of plant body.
L[3]-*Tsf1*	*Tsf1* gene leader sequence encoding the translation elongation factor EF-1 alpha of *Arabidopsis thaliana* (exon 1) (Reference 24). The ribosome binding site for translation.
I[4]-*Tsf1*	*Tsf1* gene intron sequence encoding the translation elongation factor EF-1 alpha of *Arabidopsis thaliana* (Reference 24); Enhances the expression of the transferred gene.
TS[5]-*CTP*2	A sequence encoding the chloroplast transit peptide derived from *shkG* gene of *Arabidopsis thaliana* EPSPS (Reference 26). Transports the modified CP4 EPSPS protein to the plastid which synthesizes the aromatic amino acid.
CS[6]-modified *cp4 epsps*	A coding sequence of *aroA* (*epsps*) gene that encodes 5-enol-pyruvylshikimate-3-phosphate synthase derived from *Agrobacterium* CP4 strain (CP4 EPSPS) (Reference 27; Reference 28). A modification is given to the nucleotide sequence to enhance its expression in plants without changing the function of the CP4 EPSPS protein. Only a single modification is transferred to the amino sequence: the second amino acid from the N-terminal is modified to leucine, instead of serine.
T[7]-*E9*	3' untranslated region sequence of pea (*Pisum sativum*) ribulose-1, 5-biphosphate carboxylase small subunit (*RbcS2*) E9 gene (Reference 29). Terminates transcription of mRNA and induces polyadenylation.
B-left border	A DNA region derived from *A. tumefaciens*, including the left border sequence; used as the termination point of T-DNA transfer (Reference 30).

Table 1 **(Continued):** Origins and functions of the component elements of PV-GMGOX20 used for the development of this recombinant soybean

Component elements	**Origin and function**
Component elements outside T-DNA (does not exist in this recombinant soybean)	
OR[8]-*ori* V	The replication origin region of *Agrobacterium*, derived from broad recipient organism plasmid RK2. Permits autonomous replication of vectors in *A. tumefaciens* (Reference 31).
CS-*rop*	Coding sequence of repressor of primer proteins. Maintains the number of copies of plasmid in *Escherichia coli* (Reference 32).
OR-*ori-PBR322*	The replication origin region isolated from pBR322. Permits autonomous replication of vectors in *E.coli* (Reference 33).
aadA	Bacteria promoter and coding sequence for the 3'(9)-O-nucleotidyltransferase, the aminoglycoside modified enzyme, derived from transposon Tn7 (Reference 34). Confers resistance to spectinomycin and streptomycin.

[1] B－Border
[2] P－Promoter
[3] L－Leader
[4] I－Intron
[5] TS－Targeting Sequence
[6] CS－Coding Sequence
[7] T－3' nontranslated transcriptional termination sequence and polyadenylation signal sequence
[8] OR－Origin of Replication

2) Functions of component elements

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

Functions of component elements of the donor nucleic acid that was used for the development of this recombinant soybean are shown in Table 1 on pages 4 through 10. The modified *cp4 epsps* gene, the target gene for this recombinant soybean, is detailed below.

[Modified *cp4 epsps* gene]

The wild-type *cp4 epsps* gene was isolated from *Agrobacterium* CP4 strain and encodes for the 5-enol-pyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein. The CP4 EPSPS protein has high tolerance to glyphosate herbicide. The modified *cp4 epsps* gene has the nucleotide sequences as provided in Annex 1 and the deduced amino acid sequence as shown in Figure 2 on page 7. The modified *cp4 epsps* gene has been produced by modifying the nucleotide sequence of the wild-type *cp4 epsps* gene to enhance the expression level in plants without changing the functional activity of the wild-type CP4 EPSPS protein. Regarding the amino acid sequence, only serine, the second from the N-terminal, is modified to leucine. As a promoter for the modified *cp4 epsps* gene expression cassette, chimera promoter is used in order to enhance constant expression of the target gene in the entire tissue that consists of enhancer sequence of Figwort Mosaic Virus (FMV) 35S promoter (Reference 25) bound to *Tsf1* promoter derived from *Arabidopsis thaliana* (Reference 24). In addition, in order to permit the modified CP4 EPSPS protein to function in the chloroplast, the site for biosynthesis of aromatic amino acids, the nucleotide sequence (CTP2) which encodes the chloroplast transit peptide derived from the *shkG* gene of *Arabidopsis thaliana* EPSPS is incorporated upstream of the modified *cp4 epsps* gene (Reference 26) (Figure 1).

Glyphosate herbicide is the active ingredient of Roundup, a nonselective herbicide, and inhibits the activity of 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) (E.C.2.5.1.19), one of the enzymes in the shikimate synthesis pathway for aromatic amino acid biosynthesis, by specifically binding to the enzyme (Reference 35, Reference 36). As a result, plants treated with glyphosate cannot synthesize enough amounts of the aromatic amino acids essential for protein synthesis due to the inhibition of EPSPS, resulting in the death of the plant. The activity of the CP4 EPSPS protein produced by the modified *cp4 epsps* gene is not inhibited even under the presence of glyphosate, thus the recombinant plants that express this protein have normal functions of shikimate synthesis pathway and can grow.

1…..MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL
51…..LEGEDVINTG KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA
101…..TGCRLTMGLV GVYDFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD
151…..RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI TTVIEPIMTR
201…..DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID VPGDPSSTAF
251…..PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED
301…..VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL
351…..RVKESDRLSA VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT
401…..HLDHRIAMSF LVMGLVSENP VTVDDATMIA TSFPEFMDLM AGLGAKIELS
451…..DTKAA

Figure 2: Amino acid sequence of the modified CP4 EPSPS protein, deduced from the modified *cp4 epsps* gene used for the development of this recombinant soybean[2]

(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 which is known to possess any allergenicity

In order to investigate whether the modified CP4 EPSPS protein shares functionally important amino acid sequences with know allergens, the modified CP4 EPSPS protein was compared with allergens in the Allergen Database 5 (AD5[3]) based on the FASTA type algorithm. As a result, the CP4 EPSPS protein did not have any structurally similar sequence homology to those of known allergens.

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

Due to the reasons described below, it is considered unlikely that the expression of the modified CP4 EPSPS protein could change the metabolic system of the recipient organism.

EPSPS is one of the enzymes that catalyze the shikimate pathway for biosynthesizing the aromatic amino acids specific for plants and microbes, and exists in chloroplasts or plastids in plants (Reference 37). The shikimate pathway is an important metabolic pathway that is considered to be involved in one fifth of carbon fixation in plants (Reference 38; Reference 36). This pathway is regulated by 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase, which is involved in the first step of the pathway, but it has been clarified to be extremely unlikely that the stages from DAHP through the production of 5-enol-pyruvylshikimate-3-phosphate (EPSP), which is catalyzed by EPSPS, to the synthesis of chorismic acid are inhibited or suppressed by metabolic intermediates or end products of this pathway (Reference 39; Reference 40). This suggests that EPSPS is not the rate-determining enzyme in this pathway, and as such it is not considered that enhanced EPSPS activity will increase the concentration of

[2] All the rights pertinent to the information in the diagram above and the responsibility for the content rest upon Monsanto Japan Limited.
[3] An integrated database containing “llergen, gliadin and glutenin” searched from GenBank, EMBL, PIR, RCSB PDB-NRL3D, SwissProt and other database

aromatic amino acids, the end products of this pathway. In practice, it is reported that plant cells that produce 40 times as much EPSPS as compared to normal do not synthesize excessive aromatic amino acids (Reference 41). Moreover, it has been demonstrated that there are no compositional differences in aromatic acid content between MON 89788 and the non-recombinant control soybean, according to the determination of amino acid composition. Similarly, the amino acid content in other glyphosate-tolerant crops, conducted as part of the food/feed safety evaluation of glyphosate-tolerant crops (soybean, oilseed rape, cotton, maize, alfalfa and sugar beet) which have been developed by Monsanto Company, have no relevant composition differences to date.

Besides, EPSPS is the enzyme that catalyzes a reversible reaction to produce EPSP and inorganic phosphate (Pi) from phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Reference 42), and is known to specifically react with these substrates (Reference 43). The only substance that is known to react with EPSPS other than these is shikimate, an analogue of S3P. However, the reactivity with shikimate is only one two millionth of the reactivity with S3P, and it is unlikely that shikimate acts as the substrate of EPSPS in the living body.

Consequently, it is considered to be extremely unlikely that the production of the modified CP4 EPSPS protein, which is functionally parallel to plant EPSPS protein, has an effect in any way on the metabolic pathways of plants.

(2) Information concerning vector

1) Name and origin

The vector used for the production of this recombinant soybean is assembled from plasmids including pBR322 derived from *E. coli* (Reference 44).

2) Properties

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

The total number of base pairs of the plasmid vector PV-GMGOX20 used to develop this recombinant soybean is 9,664bp. The entire nucleotide sequences of this plasmid vector are provided in Annex 1.

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

This vector contains the *aadA* gene derived from *E. coli* transposon Tn7 as a selectable marker gene, which expresses the 3'(9)-O-nucleotidyltransferase that confers resistance to spectinomycin and streptomycin (Reference 34).

c) Presence or absence of infectivity 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 in the recipient organism

The plasmid vector PV-GMGOX20 constructed for the production of this recombinant soybean was prepared and used for the transfer of the nucleic acid. The plasmid vector PV-GMGOX20 is composed of the T-DNA region including the modified *cp4 epsps* gene expression cassette ([P-*FMV*/*Tsf1*]-[L-*Tsf1*]-[I-*Tsf1*]-[TS-*CTP2*]-[CS-modified *cp4 epsps*]- [T-*E9*]), and the region outside the T-DNA to construct, select, maintain and grow the plasmid in *E. coli* (Figure 1, Table 1). The outside of the T-DNA region of the plasmid vector PV-GMGOX20 contains the *aadA* gene which permits the expression of 3'(9)-O-nucleotidyltransferase which confers resistance to spectinomycin and streptomycin as a selectable marker gene in *E. coli*, though the outside of the T-DNA region has not been transferred in the recipient organism since the transfer of the nucleic acid is based on the *Agrobacterium* method.

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

The plasmid vector PV-GMGOX20 was transferred to meristematic tissue extracted from the seed of the non-recombinant soybean variety A3244 by the *Agrobacterium* method.

3) Processes of rearing of living modified organisms

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

The *A. tumefaciens* ABI strain containing the meristematic tissue of conventional soybean variety A3244 and the plasmid vector PV-GMGOX20 was co-cultivated and then, cell selection was performed on the tissue culture medium to which glyphosate, carbenicillin and Claforan were added. In this process, Untransformed cells were removed by the glyphosate and *Agrobacterium* was removed by carbenicillin and Claforan.

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

Agrobacterium was removed from the cultured medium by addition of carbenicillin and Claforan.

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

Regenerated individuals (R_0) obtained by regeneration on the medium from dividing cells were self-propagated. In the R_1 generation, evaluation was made for the expression of the modified CP4 EPSPS protein, the tolerance to glyphosate and the homozygosity of the transferred genes to select individuals and then their progeny were subjected to analysis for the transferred genes and examination for

the morphological characteristics. As a result, the MON89788 line was finally selected as a commercialization line. The process of rearing of this recombinant soybean is shown in Figure 3. In this Biological Diversity Risk Assessment Report, this recombinant soybean line, MON89788, refers to the re-differentiated generation obtained by transferring of gene (=R0 generation) and all its subsequent generations.

The following shows the approvals received from organizations in Japan.

May, 2006: The Ministry of Agriculture, Forestry and Fisheries and the Ministry of the Environment granted the approval of Type I Use Regulations (Cultivation in isolated field, storage, transportation. disposal and acts incidental to them) in accordance with the "Law concerning the conservation and sustainable use of biological diversity through regulations on the use of living modified organisms."

February, 2007: An application was filed to the Ministry of Health, Labour and Welfare for approval of the safety of use of the cultivar as food based on the "Safety Evaluation Criteria for Food and Additives derived from Recombinant-DNA Techniques".

February, 2007: An application was filed to the Ministry of Agriculture, Forestry and Fisheries for approval of the safety of use of the cultivar as feed based on the "Safety Evaluation Criteria for Feed and Additives Produced by Recombinant-DNA Techniques".

Figure 3　Pedigree of this recombinant soybean

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

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

The transferred genes in this recombinant soybean are inherited in the subsequent generations following Mendel's law of heritability and then, the transferred nucleic acid resides on the chromosome (Annex 2).

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

As a result of Southern blotting analysis for existence of the transferred gene, it was confirmed that one copy of the T-DNA region was transferred at a site in the genome of this recombinant soybean (Figure 4 of Annex 3). In addition, it was also confirmed that there were no other backbone regions inserted into this recombinant soybean except the T-DNA region (Figure 5 on p32 of Annex 3) and that all the component elements of the modified *cp4 epsps* gene expression cassette in the T-DNA region were transferred (Figures 6 to 9 of Annex 3). Moreover, it was revealed based on the result of Southern blotting analysis for multiple generations that the transferred genes are stably inherited in offspring (Figure 10 of Annex 3).

The map of transferred genes to this recombinant soybean is shown in Figure 4.

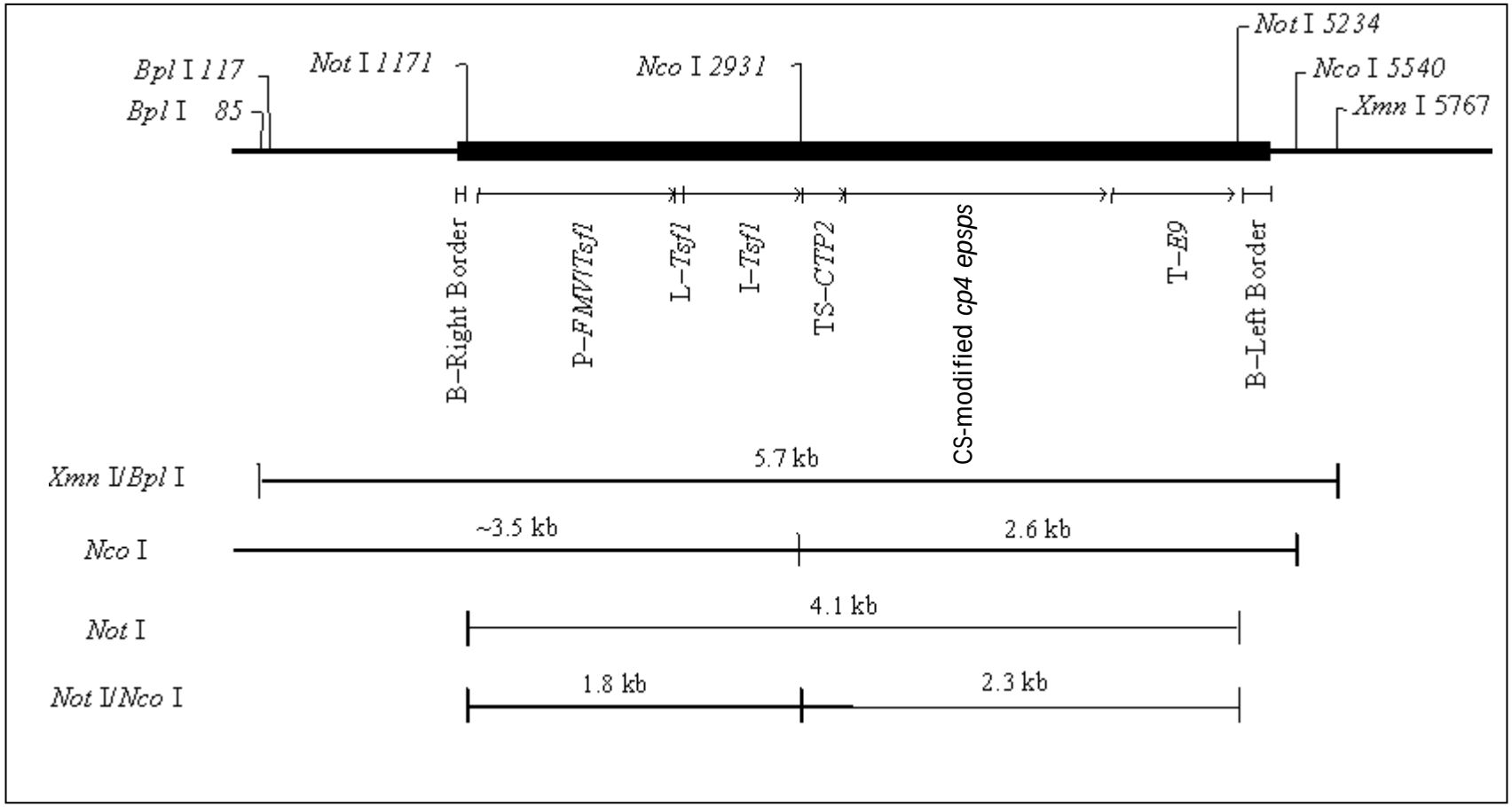

Figure 4: Map of transferred genes to this recombinant soybean[4]

[4] All the rights pertinent to the information in the diagram above and the responsibility for the content remain with Monsanto Japan Limited.

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

This item is not applicable because there is only one copy (Figure 4 of Annex 3).

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

Stability of the expression of the modified CP4 EPSPS protein in this recombinant soybean was examined based on Western blot analysis. The Western blot analysis was conducted using the polyclonal antibody specific to the modified CP4 EPSPS protein for the protein extracted from the leaves and seeds in four generations of inbred progeny of this recombinant soybean ($R4^a$, $R5^b$, $R6^c$, $R6^d$, $R6^e$, $R7^f$, and $R7^g$). As a result, in all the generations analyzed, the band referring to the molecular weight of the modified CP4 EPSPS protein was detected; therefore, it was confirmed that the target traits have been stably expressed in multiple generations (Figure 2 of Annex 4).

Based on the above results, it was found that the modified *cp4 epsps* gene transferred in this recombinant soybean is stably inherited in multiple generations and that the modified CP4 EPSPS protein is expressed in the subsequent generations.

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

Regarding the plasmid vector PV-GMGOX20, the region of recipient organism, which allows autonomous replication, is limited to gram-negative bacteria such as *E. coli*. Therefore, there is no possibility that the plasmid might be transmitted to any wild animals and wild plants under natural environment.

For production of this recombinant soybean, the *Agrobacterium* method was used, though it has been confirmed that there is no residual *Agrobacterium*. As a result, there is no risk that any DNA fragment can be transmitted to 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 by using the DNA sequence of the transferred genes and the nearby regions of the plant genome as primers (Annex 5).

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

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

The modified *cp4 epsps* gene transferred into this recombinant soybean encodes the modified CP4 EPSPS protein which possesses high tolerance to glyphosate herbicide. By the expression of this protein in the plant tissues, this recombinant soybean can grow without any effect of glyphosate herbicide (Figure 4 of Annex 6).

2) Differences between the recombinant plant and the taxonomic species to which the recipient organism belongs[5]

Isolated field tests were carried out in Kawachi Research Farm (KRF), Monsanto Japan Limited, in 2006 using this recombinant soybean. The soybeans tested included the R7 generation of this recombinant soybean (Figure 3). As the non-recombinant control soybean, A3244, the mother plant of this recombinant soybean for gene transfer, was used.

(a) Morphological and growth characteristics

The differences in morphological and growth characteristics between this recombinant soybean and the non-recombinant control soybean were investigated in reference to a total of 23 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, trichome color, time of flower initiation, time of flower completion, color of flower, elongation type, maturation period, main stem length, number of main stem nodes, number of branches, the lowest main stem node position of podding, the lowest main stem node height of podding, plant shape, weight of plant at harvest time, and shape of harvested seed (seed hull color, uniformity of seeds and seed shape)]. As a result, none of the items showed any difference between this recombinant soybean and the non-recombinant control soybean (Table 2, Figures 5 to 8 of Annex 6).

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

This recombinant soybean and the non-recombinant control soybean were grown in a closed greenhouse set at a minimum temperature of 20℃, and the seedlings at around the second-leaf stage were grown in a climate chamber set at 5℃ (12-hour day length) for 35 days to observe the growth conditions. As a result, this recombinant soybean and the non-recombinant control soybean began withering and dying 35 days after transfer to the climate chamber, and no difference was observed between the both plants in the withering and dying (Figures 9 and 10 of Annex 6).

(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 even after the maturation period to observe the growth conditions in winter season in Japan. As a result of observations made on November 7 on the growth conditions in winter season, this recombinant soybean and the non-recombinant control soybean were both found dead, and no difference was observed between the two types of plant at this stage (Figure 11 of Annex 6).

(d) Fertility and size of the pollen

Pollen was sampled from this recombinant soybean and the non-recombinant control

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

soybean, and the samples were stained with iodine potassium iodide solution to observe their fertility and sizes for investigation. As a result, this recombinant soybean and the non-recombinant control soybean both exhibited high fertility values, showing no significant difference. Furthermore, no difference was observed either in shape and size of pollen (Figures 12 and 13 of Annex 6).

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

The differences were investigated between this recombinant soybean and the non-recombinant control soybean cultivated under the same condition regarding the items for seed production (the number of ripe pods, approximate grain weight per plant (g), precise grain weight per plant (g), and 100-kernel weight (g)). As a result, a statistically significant difference was observed in 100-kernel weight between this recombinant soybean and the non-recombinant control soybean, though no statistically significant difference was observed in other items (Table 3 of Annex 6). The 100-kernel weight, in which a statistically significant difference was observed, was 17.12 g in this recombinant soybean and 18.54 g in the non-recombinant control soybean.

Regarding the shedding habit, this recombinant soybean and the non-recombinant control soybean were harvested during the maturation period, and the plant body was left to air-dry in a vinyl house for 10 days to examine whether or not the pod became broken. As a result, this recombinant soybean and the non-recombinant control soybean were both found to have difficulty shedding the pods, and showed no difference (Table 3 of Annex 6).

Regarding the germination rate, seeds collected immediately after harvesting were placed in a Petri dish at 25℃ to examine the germination rate over time. As a result, no significant difference was observed in the germination rate between this recombinant soybean and the non-recombinant control soybean, and no statistically significant difference was observed in the final number of germinated plants (Tables 3 and 4 of Annex 6).

(f) Crossability

In the plot for investigation of morphological and growth characteristics, the non-recombinant control soybean was defined as the seed parent, while this recombinant soybean was defined as pollen parent, and the frequency of occurrence of hybrids in the harvested seeds of the non-recombinant control soybean was identified to examine the crossability of this recombinant soybean. Identification of the hybrid was based on the indicator whether or not the tolerance to glyphosate herbicide is given just as this recombinant soybean, the pollen parent, possesses.

In the non-recombinant control soybean cultivated in the plot for investigation of morphological and growth characteristics, seeds were harvested in bulk from 8 individuals per replication. The individuals from which seeds were harvested were cultivated in the inner two rows in the plot, a minimum of 1.15 m distant from this recombinant soybean in the neighboring plot. Five hundred (500) seeds per replication were selected at random and sown, and then were grown in a closed greenhouse until the primary leaf developed. On the 10th day after sowing,

glyphosate herbicide (Product name: Roundup High Road, a 1 to 100 solution) was sprayed. On the 8th day after herbicide spraying the number of individuals which were found surviving due to the tolerance to glyphosate (the number of hybrids) was counted. For any individuals which were found growing so poorly at the time of herbicide spraying as its primary leaf failed to develop, it was studied based on the lateral flow detection method whether or not the modified CP4 EPSPS protein expresses since the effects of herbicide might fail to appear and the identification of herbicide tolerance was difficult in some cases.

A total of 1,500 seeds of the non-recombinant soybean plot were sown and 1,487 germinated (Table 5 of Annex 6). Among them, 11 individuals were found to grow poorly. As a result of the glyphosate herbicide spraying test and lateral flow test, no hybrid was identified in the harvested seeds of the non-recombinant control soybean, and the crossability of this recombinant soybean and the non-recombinant control soybean was 0%.

(g) Productivity of harmful substances

To confirm whether or not this recombinant soybean produces any substances affecting soil microbes and other plants, soil microflora tests, plow-in tests and succeeding crop tests were conducted. As a result, in all the items examined, no statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean (Tables 6 to 8 of Annex 6).

Root nodule bacteria live in symbiosis with the roots of soybean. Then, in order to identify if this recombinant soybean produces any substances that can affect the root nodule bacteria, possible effects on the root nodule bacteria were examined. From 12 individuals each of this recombinant soybean and the non-recombinant control soybean (4 individuals per lot, 3 replications), root nodules adhering to the roots were collected and the number and weight of the root nodules having a diameter of 2 mm or more were measured. As a result, no statistically significant difference was observed in the number of root nodule bacteria and the weight of root nodule bacteria between this recombinant soybean and the non-recombinant control soybean (Table 9 of Annex 6).

II. Review by persons with specialized knowledge and experience concerning Adverse Effect on Biological Diversity

A review was made by persons with specialized knowledge and experience concerning Adverse Effect on Biological Diversity (called Experts) for possible Adverse Effect on Biological Diversity caused by the use in accordance with the Type 1 Use Regulation for Living Modified Organism based on the "Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms." Results of the review are listed below.

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

(1) Competitiveness

The soybean plant (*Glycine. max* (L.) Merr.) 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 Japan.

This recombinant soybean is given traits to be tolerant to glyphosate herbicide due to the transferred modified *cp4 epsps* gene. However, it is not believed that the glyphosate herbicide functions as a selective pressure under the natural environment in Japan; therefore, it is hard to consider that these characteristics enhance the competitiveness of this recombinant soybean.

As a result of examination in the isolated fields in Japan regarding the characteristics relating to competitiveness, a significant difference was observed from the non-recombinant control soybean only in the 100-kernel weight. However, it is considered unlikely that this difference could cause this recombinant soybean to become competitive.

Based on the above understanding, it was judged that the conclusion by the applicant that the wild animals and wild plants likely to be affected cannot be specified and that the use of this recombinant soybean poses no significant risk of Adverse Effect 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 a harmful substance to wild animals and wild plants.

This recombinant soybean is given the ability to produce the modified CP4 EPSPS protein, though there is no report that this protein would be a harmful substance, and it has not been shown to offer amino acid sequence homology with any known allergens. In addition, this protein has high substrate specificity and thus, it is considered unlikely to affect the metabolic system of the recipient organism.

In addition, the ability of this recombinant soybean to produce harmful substances (the substances secreted from the roots to affect other plants, the substances secreted from the roots to affect microorganisms in soil, and the substances contained in the plant bodies to

affect other plants after dying out) was investigated in isolated fields of Japan, and no significant difference between this recombinant soybean and the non-recombinant control soybean was observed.

Based on the above understanding, it was judged that the conclusion by the applicant that the wild animals and wild plants likely to be affected, if cannot be specified and that the use of this recombinant soybean poses no significant risk of Adverse Effect on Biological Diversity attributable to productivity of harmful substances is reasonable.

(3) Crossability

1) Identification of wildlife likely to be affected

Since it is known that if the *Glycine soja* Sieb. et Zucc. that grows voluntarily in Japan is crossed with soybean (*Glycine max* (L.) Merr.), it produces fertile seeds, the *Glycine soja* was specified as a wild plant that potentially could be affected.

2) Evaluation of concrete details of adverse effect

Existing documents do not show any obstacle to the growth and reproduction of the hybrid obtained from soybean and *G. soja*. So, in the case where this recombinant soybean and *G. soja* are crossed with each other in the Japanese natural environment, there is possibility that the hybrid would grow and that the gene transferred into this recombinant soybean through the back crossing from the hybrid to *G. soja* could diffuses among the population of *G. soja* without remaining at a low level.

3) Evaluation of likelihood of adverse effect

Glycine soja grows voluntarily and widely throughout Japan in sunny fields, on the roadsides and the like. So, in the case where this recombinant soybean is cultivated in Japan, it cannot be denied that there are chances where both grow closely to each other.

(a) Both *Glycine max* and *Glycine soja* are typical autogamous plants engaged in cleistogamy, and the flowering time of *Glycine soja* is generally later than that of *Glycine max* by about one month;

(b) According to existing documents, even when *Glycine max* pedigree whose flowering time overlaps that of *Glycine soja* was grown adjacent to *Glycine soja*, the crossing rate was less than 1%.

(c) According to the results of isolated field tests in Japan, it can be considered that the crossability of this recombinant soybean is equivalent to that of conventional *Glycine max* and that the crossability of this recombinant soybean with *Glycine soja* is also similar to that of conventional *Glycine max*; and

(d) It is considered unlikely that the glyphosate tolerance given by the expression of the modified *cp4 epsps* gene functions dominant over selection pressures under the natural environment:
Even in the case where this recombinant soybean grows near *Glycine soja* as a

rare case, it is considered extremely low that they could cross with each other and that the transferred gene could diffuse among the group of *Glycine soja* under the Japanese natural environment without remaining at a low level.

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 Type 1 Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion above made by the applicant is reasonable.

Bibliography