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

Name of Entity: Bayer CropScience K.K.
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Approved Type 1 Use Regulation

Names of types of living modified organisms	Soybean tolerant to glufosinate herbicide, (*pat*, *Glycine max* (L.) Merr.)(A5547-127, OECD UI: ACS-GMØØ6-4).
Content of Type 1 Use of living modified organisms	Use for provision as food or animal feed purposes, cultivation, processing, storage, transportation and disposal, and other acts attendant with these.
Method of Type 1 Use of living modified organisms	—

Outline of 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.

a. Composition and origin of component elements.

The composition of the donor nucleic acid that was used for the development of the soybean tolerant to glufosinate herbicide (*pat, Glycine max* (L.) Merr.)(A5547-127, OECD UI: ACS-GMØØ6-4) (hereinafter referred to as “recombinant soybean A5547-127”) and origins of component elements are shown in Table 1.

Table1. Position, size, origin of each component element in the vector.

Component element	Size (bp)	Position in vector (bp)	Origin and function
—	188	1-188	A fragment of plasmid pUC19 (Yanisch-Perron *et al*., 1985).
RB	55	189-243	Right border derived from *Agrobacterium tumefaciens* Ti plasmid TiAch5 (Gielen *et al*.,1984).
—	217	244-460	A fragment of plasmid pUC19 (Yanisch-Perron *et al*., 1985).
P35S	543	461-1003	35S RNA promotor derived from Cauliflowers Mosaic Virus. It constitutively expresses *pat* gene in the plant (Odell *et al*., 1985).
pat	552	1012-1563	It encodes PAT proteins and confer tolerance to glufosinate herbicide, derived from *Streptomyces viridochromogenes*,. (Strauch *et al*., 1993). The codon usage of *pat* gene from *Streptomyces viridochromogenes* and

			transferred in recombinant soybean A5547-127 was modified to express in the plant (Strauch *et al*., 1993). However, the amino acid sequence of the enzyme which is produced by this modification remains unchanged.
T35S	203	1582-1784	35S RNA terminator derived from Cauliflowers mosaic virus. It terminates transcription and induces 3'polyadenylation of transcripts (Pietrzak *et al*., 1986).
ORI	550	2253-2803	A fragment of plasmid pUC19, which has replication origin (ColE1) on the position of 2257bp. This initiates the replication of the plasmid (Yanisch-Perron *et al*., 1985).
bla	861	3016-3876	*Escherichia coli*-derived ampicillin-resistance gene (*bla*) and this express the β-lactamase in the bacteria (Sutcliffe, 1978).
—	200	3877-4076	Sequence fragment of plasmid-pUC19 (Yanisch-Perron *et al*., 1985).

（Note: All the rights pertinent to the information in the table above and responsibility for the contents rest upon the applicant.）

b. Functions of component elements

① Function of individual component elements of donor nucleic acid, including target gene, expression regulatory region, localization signal, and selective marker.

Functions of individual component elements of donor nucleic acid which were used for development of recombinant soybean A5547-127 are shown n Table1.

② 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.

PAT Proteins

In the process of nitrogen metabolism, crops produce ammonia by the reduction of nitrate, the degradation of amino acid, photorespiration and so on. Glutamate synthases play a central role in detoxification of produced ammonia, though the glutamate synthase is inhibited if crops are sprayed with glufosinate herbicide, ammonia is accumulates, and the crops die.

On the other hand, in the plant in which the *pat* gene is transferred, phosphinothricin acetyl transferase (PAT proteins) is produced, and this enzyme acetylates glufosinate to transform it to N-acetylglufosinate, and then, inactivates the inhibition against glutamine synthase by glufosinate (OECD, 1999). This helps to prevent the inhibition of glutamine synthase by the glufosinate , ammonia is not accumulated in the plant , and the crop does not die even if it is sprayed with glufosinate.

Moreover, based on the amino acid sequence of PAT proteins, overall homology search was conducted with known allergens by using the AllelgenOnline data base of FARRP (Version 12), as a result, this protein did not show any homology with known allergens.

③ Contents of any change caused to the metabolic system of recipient organism.

The PAT protein encoded by *pat* gene shows high affinity to L-type isomer of glufosinate, though it does not cause any acetyl group transfer to the other various amino acid, it has little affinity for glutamic acid which has specifically high structural similarity, and it causes virtually no transfer reaction in any living body (Thompson *et al*., 1987). In addition, even under the presence of excessive amount of various amino acid, the acetyl group transfer reaction to glufosinate by PAT proteins was never inhibited (Wehrmann *et al*., 1996). Accordingly, it is considered that the PAT proteins has high substrate specificity and it does not affect the metabolic system of the recipient organism.

Also, N-acetyl-L-glufosinate which is the metabolic products of glufosinate does not inhibit glutamate synthetase (OECD, 2002), and it is considered that it has no effect on the metabolic system of the recipient organism.

（2） Information concerning vectors

a. Name and origin

The plasmid used for preparing recombinant soybean A5547-127 was plasmid pB2/35SacK constructed based on plasmid- pUC19 and others (Figure 1, p.6).

b. Properties

① The number of base pairs and nucleotide sequence of vector

The number of base pairs of plasmid pB2/35SacK was 4,076 bp. The plasmid map is shown in Figure1 (p.6) and its entire sequences are shown in the Annex 1. (p.8).

② In case that there is any base sequence having any specific function, evaluate such function

plasmid pB2/35SAcK contains *bla*-gene, which confer ampicillin resistance as a selection marker. This gene was divided into two fragments when a digestion was carried out of plasmid-pB2/35SAcK by restriction enzyme prior to its transformation (Figure 2, p.7). Northern blotting analysis was performed using *bla*-gene as a probes with RNA extracted from the leaves, stems, roots and

seeds of recombinant soybean A5547-127 (T4 Generation, Figure 3, p.10). As a result, it is confirmed that any transcripts were not detected in any tissues (detection limit: 2pg) and this gene also was not expressed in recombinant soybean A5547-127 (Annex 2, p.15, Figure1.)

③ Presence or absence of the infectious characteristics of a vector, if present, the information concerning the host range.

The infectivity of plasmid pB2/35SAcK is not known.

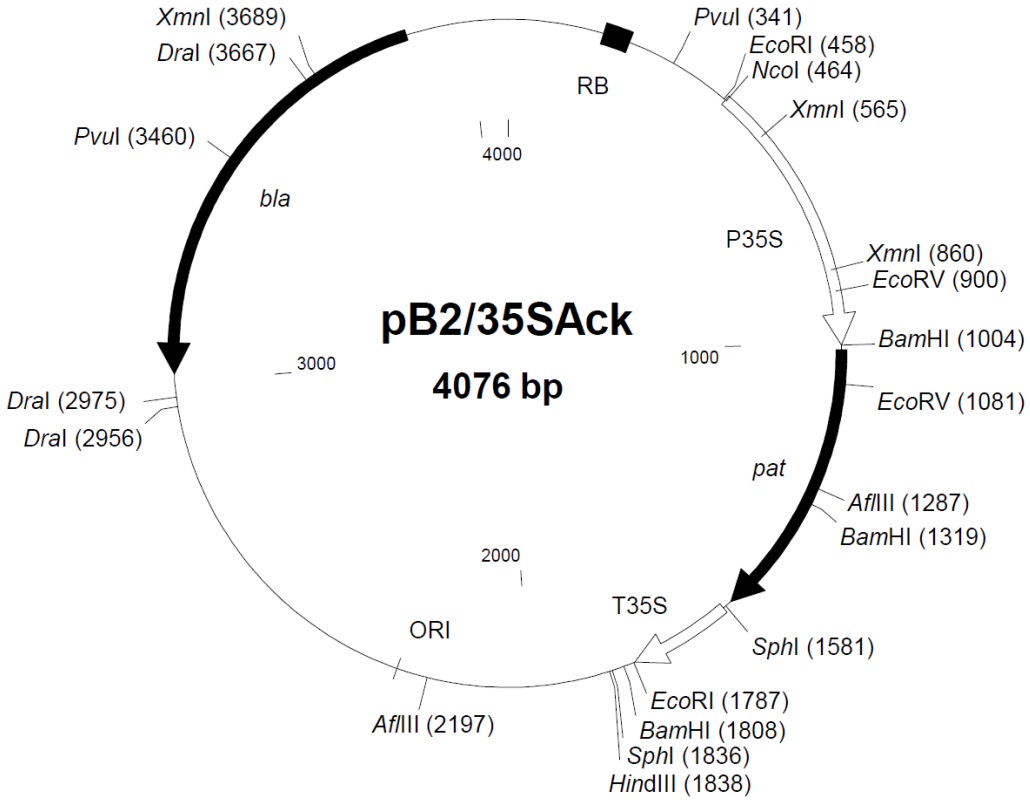


Figure1. Map of plasmid pB2/35SAcK and the digesting positions by restriction enzymes.

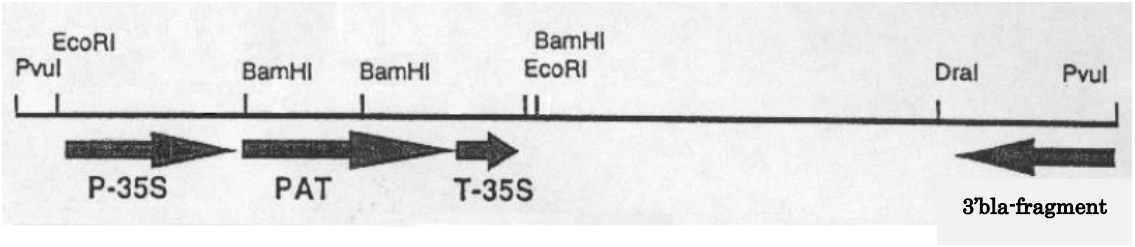
Note: All the rights pertinent to the information in the figure above and the responsibility for the contents rest upon the applicant.

（3）Method for preparing living modified organisms

a. Structure of the entire nucleic acid transferred in the recipient organism

The plasmid pB2/35SAcK was cut at the *Pvu*I-sites located on the upstream of 35S promotor and on the middle of *bla* gene, and divided into two fragments. Structure of the entire nucleic acid transferred in the recipient organism is shown in Figure2.

Fragment1



Fragment2

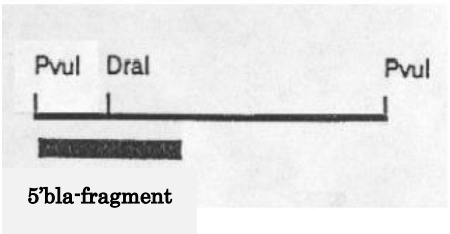


Figure 2 Structure of the transferred nucleic acid

（Note: All the rights pertinent to the information in the figure above and the responsibility for the contents rest upon the applicant.）

b. Method of transferring nucleic acid transfected to the recipient organism.

Two fragments (long and short) of plasmid pB2/35SAcK, which was cut at the two sites by the restriction enzyme *Pvu*I (Figure 2,p.7), were transferred into the shoot apical meristem of the recipient organism by the particle bombardment method.

c. Processes of the rearing of living modified organisms

① Mode of selecting the cells containing the transferred nucleic acid transfected

After transformation, the nucleic acid transferred cells were moved to a solid medium containing plant hormone and its shoot was induced, and then, the glufosinate tolerant plants was selected by using the medium containing glufosinate.

② Presence or absence of remaining *Agrobacterium* in case of using Agrobacterium method for transferring nucleic acid.

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③ Process of rearing and pedigree trees of the following lines; cells wot which the nucleic acid was transferred, the line to which confirmed the state of existence of replication products of transferred nucleic acid, the line subjected to isolated field tests; and the line used for collection of other necessary information for assessment of Adverse Effect of Biological Diversityfield

The selected transformants were cultivated in a greenhouse, the selection was done by glufosinate, and obtained the T0 generation of recombinant soybean A5547-127. Thereafter, the self-pollination was repeated to obtain the plants in individual generations used for each test. The pedigree tree was shown in Figure 3 (p.10).

The scope of this application is for the T3 genaration and subsequent generations. The approval status of recombinant soybean A5547-127 in Japan

was shown in Table 2.

Table2. Application and approval status in Japan (as of October 2014)

Application submitted to:	Purpose	Approval status
Ministry of Agriculture, Forestry and Fisheries	Enviroment [1](import)	Certified in May 1999
Ministry of Agriculture, Forestry and Fisheries / Ministry of the Environment	Enviroment[2] (provisioin as food, provision as feed, processing, storage, transportation, disposal and acts incidental to them.)	Approved in November 2006.
Ministry of Agriculture, Forestry and Fisheries / Ministry of the Environment	Enviroment[2] (cultivation in isolated field, storage, tranportation , disposal and acts incidental to themfield)	Approved in December 2012.
Ministry of Labour, Health and Welfare	Food[3]	Approved in July 2002.
Ministry of Agriculture, Forestry and Fisheries	Feed[4]	Approved in March 2003.

[1]Based on the Guideline of the Use of Living Modified Organisms in the fields of Agriculture, Forestry and Fishery.

[2] Based on the Low concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms.

[3] Based on the Food Sanitation Act.

[4] Based on the Act on Safety Assurance and Quality Improvement of Feeds.

（Note: All the rights pertinent to the information in the table above and responsibility for the contents rest upon the applicant.）

Figure3. Pedigree tree of recombinant soybean A5547-127

(3) Status of existence of nucleic acid transfected in cells and the stability of traits caused by nucleic acid.

① Place where the replication product of transfected nucleic acid exists.

Based on a trial carried out in the United States in 1996, 31 resistant individuals were selected by the spraying glufosinate herbicide on the individuals of T2 generation (Figure 3) which was obtained by the self-pollination of T1 generation, a heterozygote with regard to *pat* genetic locus. Then, the seeds obtained by self-pollination of the glufosinate tolerant individuals (T3 generation: Figure3) were sown to the row by lines and the germinated seedlings were sprayed with glufosinate to see the segregation ration between glufosinate tolerant and glufosinate sensitive plants. As a result, a segregation ration of 1:2 was obtained between the 10 lines exhibiting tolerance to glufosinate in all seedlings (homozygote) and the 21 lines containing glufosinate tolerant seedlings and glufosinate sensitive seedlings (heterozygote).

In addition, as described in the next section (1. 2 (4) ②) , it was confirmed that one copy of inserted DNA was stably transferred over generations in recombinant soybean A5547-127 by the genomic southern blot analysis. Also, the soybean genome sequences before and after the insertion part as a query were compared with the soybean (*G. max*) genome stored in the NCBI database using BLASTn, and the insertion part was confirmed to exist on the soybean chromosome 18. (Annex6, p.115, Annex 16).

As a result of the above, it was deemed that the inserted gene transferred into recombinant soybean A5547-127 exits at the one point on the soybean genome.

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

Southern blotting and sequence analyses were done on the genomic DNA extracted from the leaves of recombinant soybean A5547-127 (T-4 generation: Figure3). As a result, it was confirmed that one copy of *pat* gene expression cassette was introduced into recombinant soybean A5547-127, and two *bla* gene fragments adjacent to the above *pat* gene expression cassette were inserted as following locations: one on 5' side lacking the sequence from 1 to 28 bp of 5'end in the upstream of *pat* gene cassette, and another one on 3' side in the downstream of it (Figure 4; Annex 3, p.17, Table 3, p.19-22, Figure 2-5; Annex 7, p.20, Figure 3).

In addition, to test the stability of the inserted gene, Southern blot analysis was done on the genome DNA extracted from the leaves of the generations of T3, T4, T5 and Tii (Figure 3 p.10) of recombinant soybean A5547-127 using *pat* gene expression cassette as the probe. As a result, the same bands were detected on each of the above generations, and it was confirmed that the inserted DNA was transferred stably over generations (Annex 8, p.11).

③In case that there are plural copies on the chromosome, evaluate whether they are adjacent or separated each other.

As shown in the above(4) ①, two divided fragments of *bla*-gene exist adjacent each other across one copy of *pat* gene expression cassette in recombinant soybean A5547-127 (Figure 4).

Figure 4. Schematic diagram of Inserted DNA in recombinant soybean A5547-127

（Note: All the rights pertinent to the information in the figure above and responsibility for the contents rest upon the applicant.）

④ Inter-individual or inter-generational expression stability under a natural environment with respect to the characteristics that were accompanied by the expression of the transferred nucleic acid

ELISA analysis was conducted using the PAT protein in the roots, stems and leaves of five individuals of each of recombinant soybean A5547-127 and the non-recombinant soybean which were cultivated in a greenhouse in the United States in 2002. PAT proteins were detected in all tissues of all individuals of recombinant soybean A5547-127 (Table 3).

Table3. Expression contents of PAT proteins in each tissues of recombinant soybean A5547-127

Line	tissue	Average PAT protein content (μg/g fresh weight) ± SD	Crude protein / fresh weight (%)	PAT protein / crude protein (%)
recombinant soybean A5547-127	Root	3.73 ± 0.98	2.15	0.017
	Stem	11.5 ± 1.8	3.62	0.032
	Leaf	19.0 ± 5.0	6.70	0.028
Non-recombinant soybean	Root	＜LOD	2.39	-
	Stem	＜LOD	4.30	-
	Leaf	＜LOD	7.13	-

The plants were grown in United States in 2002(n=5). Limit of detection (LOD) of PAT protein by fresh weight of each tissue was 2.72ng/g (root), 3.72ng/g (stem) 9.76ng/g (leaf).

Note: All the rights pertinent to the information in the table and the responsibility for the contents rest upon the applicant.

In the isolated field of Akeno site, Bayer CropScience K.K. (hereinafter referred to as “ isolated field”) in 2013, recombinant soybean A5547-127 (T iv -generation, Figure 3, p.10), the non-recombinant soybean, and harvested seeds from these (T v -generation, Figure 3, p.10) were cultivated and sprayed with glufosinate herbicide. As a result, all of the individuals of recombinant soybean A5547-127 showed resistant to glufosinate (Table 4, Annex 9, p.17).

Table4. Herbicide resistance by expression of introduced gene

System	T iv Seed			T v Seed（harvested seed）		
	Number of sprayed individuals	Number of resistant individuals	Rate of resistant individuals (%)	Number of sprayed individuals	Number of resistant individuals	Rate of resistant individuals (%)
recombinant soybean A5547-127	20	20	100	100	100	100
non-recombinant soybean	20	0	0	100	0	0

Plants were grown until the primary leaf stage, glufosinate was sprayed, and resistance to herbicide was confirmed two weeks after spray (dosage: 55.5g a.i. (content of active ingredient equivalent to /450L/10a)

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Based on the results discussed above, it was confirmed that PAT protein in recombinant is stably expressed among individuals and over generations.

⑤Presence 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.

Recombinant soybean A5547-127 contains no DNA sequence which possesses transmittable factor and therefore, there is no possibility taht introduced nucleic acids are transferred into wild animals and plants under a natural environment.

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

The real-time PCR method using TaqMan® probes is available for specific detection method of this recombinant soybean A5547-127 (Annex 10).

The detectable limit of this method is 0.08% as the genomic DNA content ratio (Annex 10).

Regarding the reliability (reproducibility), assurance of inter-laboratory transferability was confirmed by 12 external institutions (Annex 11).

（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 nuclear acid.

Recombinant soybean A5547-127 has resistant to glufosinate herbicide by the expression of PAT protein by introduced *pat* gene.

③ With respect to the physiological or ecological characteristics listed below, presence or absence of difference between recombinant crop and taxonomic species to which the recipient organism belongs, and the degree of difference, if any.

An isolated field trial of recombinant soybean A5547-127was conducted at an isolated field in 2013. The generation used for this test was the T iv generation of recombinant soybean A5547-127 (Figure 3, p.10). As a comparison, a non-recombinant soybean, variety A5547, was used, which has the genetic background of recombinant soybean A5547-127 (hereinafter referred to as “non-recombinant soybean”).

a Morphological and growth characteristics

Regarding the morphological and growth characteristics, a comparison between recombinant soybean A5547-127 and non-recombinant soybean was done for the following 20 items based on the “Test Guidelines of plant variety of Agricultural, Forestry and Aquatic plants: Soybean” (MAFF, 2012); the time of germination, complete germination date, the plant growth type, the color of trichome, the volume of trichome, the flower color, the shape of leaflet, the time of beginning of flowering, anthesis , the fertility and the size of pollen, the time of maturity, the length of main stem, the number of nodes of main stem, the number

of side shoot, the weight of above-ground part of one plant, the color of matured pods, the shape of seed, the color of seed coat and the hilum color. Statistical analyses were performed of the fertility and size of pollen, the length of main stem, the number of nodes of main stem, the number of side shoot, the weight of above-ground part of one plant and the shape of seeds. Observations were compared of the time of germination, complete germination date, the time of beginning of flowering, anthesis, the time of maturity, the plant growth type, the color of trichome, the volume of trichome, the flower color, the shape of leaflet, the color of matured pods, the color of seed coat and the hilum color. As a result, the statistically significant difference was observed in the average number of nodes of main stem, which were 20.1 in recombinant soybean A5547-127 and18.1 in the non-recombinant soybean. Also, concerning other morphological and growth characteristics, statistically significant difference or observed difference in between recombinant soybean A5547-127 and non-recombinant soybean was not found (Annex 9, p5-10).

b Cold-tolerance at the early stage of growth.

The cold tolerance of this recombinant soybean A5547-127 and non-recombinant soybean as a wilting at early stage of growth was investigated at the condition of 5℃ and 10 hours of daylight length. As a result, no statistically significant difference was observed between recombinant soybean A5547-127 and non-recombinant soybean during entire trial period (Annex 9, Figure 5, p11).

c Overwintering ability of the matured plant

In the isolated field, cultivation of recombinant soybean A5547-127 and non-recombinant soybean after their harvest time of December had continued, which was sowed in June 2013. All plants were confirmed to be died due to low temperature and frost in February 2014 (Annex 9, p.12).

d Fertility and size of pollens.

The pollens were collected from recombinant soybean A5547-127 and non-recombinant soybean cultivated in the isolated field, and the fertility and size of pollen were compared after dyeing by acetocarmine solution. As a result, no statistically significant differences of the fertility and size of pollen were observed in between recombinant soybean A5547-127 and non-recombinant soybean

(Annex 9, Table 4, p.9 and Figure 7, p.10).

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

Concerning the characteristics referring of the production, based on “Test Guidelines of plant variety of Agricultural, Forestry and Aquatic plants: Soybean” (MAFF, 2012), 6 items were investigated such as total seeds weight per one plant (coarse seeds weight), matured grain weight per plant (fine seed weight), number of matured grain per plant, number of grains per pod, number of matured pod per plant, and 100 grain weight. As a result of statistical treatment on these items, the average value of the number of grain per seed pod was 2.03 on non-recombinant soybean compared to 2.17 on recombinant soybean A5547-127, and statistically significant difference was observed. Regarding other measured items, there was no statistically significant difference between recombinant soybean A5547-127 and non-recombinant soybean (Annex 9, Table 4, p.9).

With regard to the shedding habit, a comparison of the number of burst pod as the difficulty of pod burst was done in between recombinant soybean A5547-127 and non-recombinant soybean which were grown in the isolated field and harvested at their maturity stage. As a result, both of recombinant soybean A5547-127 and non-recombinant soybean were difficult to burst the pods, and no difference in pod burst was observed (Annex 9, Figure 4, p.9).

Regarding the dormancy and the germination rate, the germination rate of the seeds of recombinant soybean A5547-127 and non-recombinant soybean grown in the isolated field were investigated. These seeds were air dried for one month just after harvest, sowed in pots, and the germination rates of recombinant soybean A5547-127 and non-reocombinant soybean were almost 100% after two weeks of sowing. As a result, no statistically significant difference in between recombinant soybean A5547-127 and non-recombinant soybean was observed, so dormancy was not observed (Annex 9, Table9, p16).

f Crossability

The seeds of non-recombinant soybean harvested at isolated field were used to test the crossability. At the morphological and growth characteristics tested plots of non-recombinant soybean, selected the plants adjacent to recombinant

soybean A5547-127 by the distance of 1.2 to 1.4m, randomly selected seeds from these plants were sown in a greenhouse, and sprayed glufosinate (dosage 55.5g a.i./450L/10a equivalent) on the seeding at the developmental stage from cotyledon to the first leaves, the number of survival plants after spraying glufosinate was counted. As a result, the forty four individuals out of 4,093 plants were survived after two weeks of glufosinate herbicide sprayed, so glufosinate was sprayed again. Eighteen individuals survived one week after the re-spray. Then, DNA was extracted from these 18 individuals, PCR was performed using the event specific primers to detect recombinant soybean A5547-127 (Annex 10). As a result, these 18 individuals were amplified with event specific primers of recombinant soybean A5547-127. Therefore, these 18 individuals were considered as the hybrid between non-recombinant soybean and recombinant soybean A5547-127, and the crossability was 0.44 %(Annex 9, p18).

g Productivity of harmful substance

In order to compare the productivity of harmful substances for recombinant soybean A5547-127 and non-recombinant soybean, succeeding crop test, plow-in test and soil microflora test were conducted at isolated field test.

Succeeding crop test

The soil of the cultivated area in the isolated field of recombinant soybean A5547-127 and non-recombinant soybean were collected after cultivating them for about six months until their harvest. The radish as assay plant were grown using each obtained soil to measure germination rate, plant height, fresh weight, and dry weight. As a result, no statistically significant differences were measured in all items in between recombinant soybean A5547-127 and non-recombinant soybean (Annex 9, Table 6, P.13).

Plow-in test

Aboveground parts of plants of recombinant soybean A5547-127 and non-recombinant soybean which were cultivated in the isolated field for about six months until their harvest time were harvested, dried, and crushed into powder. 1 % of these samples were mixed with the soil, radish as assay plant was grown, and germination rate, plant height, fresh weight and dry weight were compared. As a result, regarding the germination rate, plant height and dry weight, no statistically significant differences were observed in between the test plots of

recombinant soybean A5547-127 and non-recombinant soybean (Annex 9, Figure7, p14). A statistically significant difference in fresh weight was observed, and the fresh weight per 10 individuals of recombinant soybean A5547-127 was 0.56 g more than that of non-recombinant soybean (Annex 9, Table 7, p.14).

Soil microflora test

Soil of recombinant soybean A5547-127 and non-recombinant soybean after about 6 months of cultivation in the isolated field was collected, and filamentous fungus, actinomycete, and bacterium were counted using dilution plate technique. As a result, no statistically significant differences were observed in any above tests in between the test plots of recombinant soybean A5547-127 and non-recombinant soybean (Annex9, Table7, p15).

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

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

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

This recombinant soybean A5547-127 was produced by the introduction of restriction enzyme digested plasmid pB2/35SAcK constructed by plasmid pUC19 and so on originated from *Escherichia coli* by the particle bomberdment method.

The insertion of one copy of *pat* gene encoding PAT protein derived from *Streptomyces viridochromogenes* was confirmed by segregation ratio of *pat* gene, southern blot analysis, and sequence analysis. Also, the stable inheritance of *pat* gene over multiple generations was confirmed by southern blot analysis. In addition, stable protein expression over multiple generations was confirmed by ELISA analysis.

(1) Competitiveness

The soybean, the biological species of 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.

Isolated field trial was done in Japan from 2013 to 2014, and on the various characteristics related to the competitiveness of recombinant soybean A5547-127 was investigated. As a result, no statistically significant differences in between recombinant soybean A5547-127 and non-recombinant soybean were observed except the number of nodes of main stem and the number of seeds per pod, but these were all within the rage of the average of regular soybeans, so the possibility that recombinant soybean A5547-127 increases the competitiveness is low.

Recombinant soybean A5547-127 has a resistance to glufosinate by the expression of PAT protein. However, it is hard to consider that the glufosinate exerts a selective pressure under the natural environment because herbicides are not used under natural environment.

Based on the above understanding, it was judged that the conclusion by the applicant that the wild animals and plants likely to be affected cannot be specified, and the use of this recombinant soybean A5547-127 poses no significant risk of Adverse Effect on Biological Diversity attributable to competitiveness is reasonable.

(2) Productivity of harmful substances.

The soybean, the biological species of the recipient organism belongs, is not reported to produce any harmful substances.

Recombinant soybean A5547-127 produces PAT protein conferring resistance to glufosinate herbicide, but this protein has not been reported as a harmful substance, and it was confirmed that it had no homology with known allergens. PAT protein has high substrate specificity, and it is not considered to produce new harmful substances by affecting on the metabolic pathways of the recipient organism. Although N-acetyl-L-glufosinate is produced by the effect of PAT protein during spraying of glufosinate, it is confirmed that its toxicity to animals is lower than the toxicity of glufosinate itself.

In order to compare the productivity of harmful substances between recombinant soybean A5547-127 and non-recombinant soybean, succeeding crop test, plow-in test and soil microflora test were conducted. As a result, no statistically significant differences in between recombinant soybean A5547-127 and non-recombinant soybean were observed.

Based on the above understanding, it was judged that the conclusion by the applicant that the wild animals and plants likely to be affected cannot be specified, and that the use of this recombinant soybean A5547-127 poses no significant risk of Adverse Effect on Biological Diversity attributable to productivity of harmful substances is reasonable.

(3) Crossability

Since *Glycine soja*, a closely relative wild species that can cross with soybean (*G. max*), grows naturally in Japan, and *G. soja* was specified as the wild plant

likely to be affected.

In the case where this recombinant soybean A5547-127 and *G. soja* are crossed with each other in the Japanese natural environment, there is a possibility that the cross between hybrid and *G. soja* are repeated, and the introduced gene may diffuse within *G. soja* population. Also, *G. soja* grows naturally and widely throughout Japan, so in case where this recombinant soybean grows near *G. soja*, it cannot be denied that there are chances where both plants cross with each other. However,

① Both *Glycine max* and *G. soja* are self-fertilizing plants, and it is rare that their flowering time is at the same in Japan.

② It is generally known that the flowering time of soybean and *G. soja* is unlikely to match with each other, and even in the case when the both plants were cultivated alternately to artificially match the flowering time, the rate of crossability was reported as 0.73%.

③ As a result of crossability test in which the flowering time of another herbicide tolerant soybean and *G. soja* was matched each other and the both plants were cultivated adjacent each other, and maximum crossability rate was reported as 0.14%.

④ For several years, genetic analyses were performed toward the population of *G. soja* inhabit around the soybean field in every regions of Japan. As a result, it was confirmed that the progenies resulted on the cross did not continuously survive.

In addition, regarding on the isolated field trial conducted in Japan from 2013 to 2014 to see the characteristics of reproduction of recombinant soybean A5547-127 and non-recombinant soybean, no significant differences were observed in fertility and size of pollen. Therefore, it was considered that the rate of crossability between recombinant soybean A5547-127 and *G. soja* was very low, which is the same as that of between *G. soja* and conventional soybeans.

Furthermore, even if any cross is done between recombinant soybean A5547-127 and *G. soja*, it is unlikely that such herbicide tolerance enhances the competitiveness under the natural environment where application of such glufosinate herbicide is unlikely to take place. Therefore, it was hard to consider that the progeies occupies in *G. soja* population would be happened.

As described above, the possibility that the cross between recombinant

soybean A5547-127 and *G. soja* is done, and the possibility that the transferred gene diffuses among the population of *G. soja* without remaining at a low level is also considered to be very low stochastically. Consequently, it was judged that the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to crossability is reasonable.

2. Conclusion based on the Biological Diversity Risk Assessment Report

Based on the above understanding, It was judged that the conclusion of the Biological Diversity Risk Assessment Report, stating that the use of this recombinant soybean in accordance with the Type 1 Use regulation would pose no risk in causing Adverse Effects on Biological Diversity in Japan, is reasonable.

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List of Annexes

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Annex2 : Evaluation of cryptic gene expression of the *bla* gene in Liberty Link soybean event A5547-127.

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