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

Applicant
Name: Dow Chemical Japan Limited
Michiro Kurita, Representative Director (Seal)
Address: 2-24 Higashi Shinagawa 2-chome, Shinagawa-ku, Tokyo

Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Soybean tolerant to aryloxyalkanoate herbicide and glufosinate herbicide (modified *aad-12*, *pat*, *Glycine max* (L.) Merr.) (DAS68416, OECD UI: DAS-68416-4)
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 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 and origins of donor nucleic acid used for production of soybean tolerant to aryloxyalkanoate herbicide and glufosinate herbicide (modified *aad-12*, *pat*, *Glycine max* (L.) Merr.) (DAS68416, OECD UI: DAS-68416-4) (hereinafter referred to as "modified soybean") are shown in Table **1** (p.2).

Table 1 Composition of donor nucleic acid, origins and function of component elements

Component element	**Origin and function**
T-DNA Border B	T-DNA boundary sequence derived from *Agrobacterium tumefaciens.*
RB7 MAR	Nuclear matrix attachment region derived from *Nicotiana tabacum* (Allen *et al.*, 1996). Stabilized expression of the gene.
Modified *aad-12* cassette	
AtUbi10	Polyubiquitin 10 (UBQ10) gene promoter derived from *Arabidopsis thaliana.* Contains a 5' terminal untranslated region and intron (Norris *et al.*, 1993). Expresses the gene for the entire plant body.
Modified *aad-12*	Aryloxyalkanoate dioxygenase gene derived from gram-negative bacillus *Delftia acidovorans* which is modified to a codon that is suitable for expressing in a plant body. Expresses the modified AAD-12 protein. Alanine is added to the second position of the amino acid sequence of the expressed modified AAD-12 protein for introduction of a cloning site (Wright *et al.*, 2007).
AtuORF23 3' UTR	A 3' terminal untranslated region consisting of the transcription termination point of ORF23 derived from the plasmid pTi15955 of *A. tumefaciens* and a polyadenylation site (Barker *et al.*, 1983). Terminates transcription of the gene.
pat cassette	
CsVMV	Promoter derived from *Cassava vein mosaic virus*. Contains a 5' terminal untranslated region (Verdaguer *et al.*, 1998). Expresses the gene for the entire plant body.
pat	Phosphinothricin acetyltransferase gene derived from *Streptomyces viridochromogenes* which is modified to a codon that is suitable for expressing in a plant body. Expresses the PAT protein. No modification is made to the amino acid sequence of the expressed PAT protein (Wohlleben *et al.*, 1988).
AtuORF1 3' UTR	A 3' terminal untranslated region consisting of the transcription termination point of ORF1 derived from the plasmid pTi15955 of *A. tumefaciens* (Barker *et al.*, 1983). Terminates transcription of the gene.

Component element	Origin and function
T-DNA Border A	T-DNA boundary sequence derived from *A. tumefaciens*.
T-DNA Border A	T-DNA boundary sequence derived from *A. tumefaciens*.
T-DNA Border A	T-DNA boundary sequence derived from *A. tumefaciens*.

(All the rights pertinent to the information in the table above and the responsibility for content rest upon Dow Chemical Japan Limited)

2) Functions of component elements

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

Functions of individual elements of the inserted genes are shown in Table **1** (p.2).

The donor nucleic acid contains the *RB7 MAR* sequence as a nuclear matrix attachment region. A nuclear matrix binding region is a region frequently observed in genomic DNA sequences, and is considered to play a role in adhering DNA to the nuclear matrix for the purpose of forming a loop structure of the DNA. It has been reported that a nuclear matrix binding region flanking to either side of an introduced gene enhanced the expression of the introduced gene or reduced gene silencing that suppresses expression of the gene (Allen *et al.*, 2000; Halweg *et al.*, 2005).

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

AryloxyAlkanoate Dioxygenase (hereinafter referred to as "modified AAD-12 protein) is an enzyme that catalyzes the reaction to introduce oxygen specifically to achiral and S- enantiomer of compounds with the aryloxyalkanoate structure. Moreover, this modified soybean exhibits herbicide tolerance by the modified AAD-12 protein catalyzing the reaction to introduce oxygen to aryloxyalkanoate herbicide and converting it to a compound with no herbicidal activity (Wright *et al.*, 2007). For instance, the modified AAD-12 protein catalyzes the reaction to introduce oxygen to herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) and coverts it to 2, 4-dichlorophenol (2, 4-DCP) and glyoxylic acid that have no herbicidal activity (Figure 1, p.4). the herbicides that become the substrate of the modified AAD-12 protein are shown in Attachment 1.

Investigation on the FARRP Allergen Database version 12 (2012) identified that the modified AAD-12 protein has no amino acid sequence that is structurally related to known allergens.

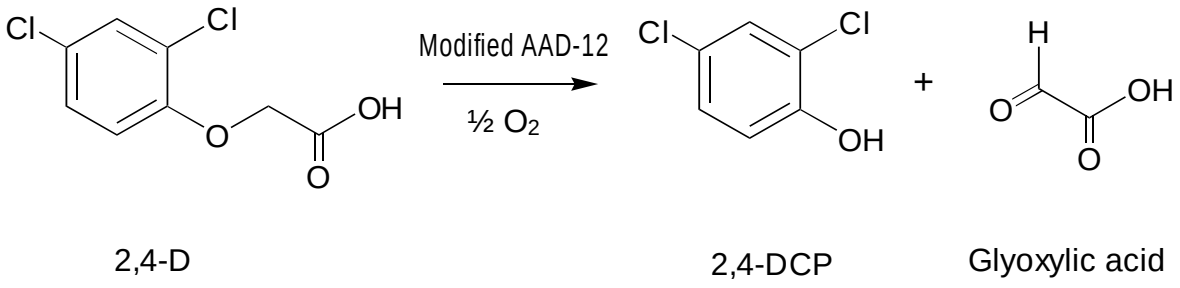

Figure 1 Action mechanism of modified AAD-12 protein

(All the rights pertinent to the information in the diagram above and the responsibility for content rest upon Dow Chemical Japan Limited.)

Phosphinothricin AcetylTransferase (hereinafter referred to as "PAT protein") swiftly coverts L-glufosinate to *N*-acetyl-L-glufosinate (2-acetamido-4-methylphosphinato-butyrate) which is a stable non-phytotoxic compound.

L-glufosinate is a structural analog of glutamate and a competitive inhibitor of glutamine

synthetase in microorganisms and plants, and has activity as a herbicide. Therefore, in plants that are not tolerant to herbicide glufosinate, a large amount of ammonia accumulates inside the cells due to inhibition of glutamine synthetase, which eventuates to plant cell death. Meanwhile, *N*-actyl-L-glufosinate does not inhibit glutamine synthetase and genetically modified plants that express PAT protein do not receive physiological effects of phytotoxin, and thus exhibits tolerance to herbicide glufosinate (OECD, 2002).

Investigation on the FARRP Allergen Database version 12 (2012) identified that PAT protein has no amino acid sequence that is structurally related to known allergens.

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

[Modified AAD-12 protein]

The modified AAD-12 protein is an enzyme that catalyzes the reaction to introduce oxygen specifically to achiral and S-enantiomer of compounds with aryloxyalkanoate structure.

In the reaction where modified AAD-12 protein introduces oxygen to a substrate, under the presence of α-ketoglutarate, α-ketoglutarate is converted to succinate (Figure 2, p.5). In order to identify the possibility of compounds that exist inside plant bodies that are structurally or physiologically related to compounds with aryloxyalkanoate structure becoming the substrate of modified AAD-12 protein, enzyme-coupled assay of succinate was conducted for the reaction experiment of modified AAD-12 protein with α-ketoglutarate and formation of succinate acid has been confirmed. In the enzyme-coupled assay of succinate, for the reaction system using succinyl-CoA synthetase, pyruvate kinase and lactate dehydrogenase, the oxidation level of the reduced nicotinamide adenine dinucleotide was obtained by measuring the absorption at 340 nm and was converted to the formation of succinate (Luo *et al.*, 2006; Figure 3, p.6). As the compounds that exist inside plant bodies that are structurally or physiologically related to compounds with aryloxyalkanoate structure, some plant hormones (indole-3-acetic acid, abscisic acid, gibberellic acid (GA3) and aminocyclopropane-1-caoboxylic acid), some phenylpropanoid intermediates (*trans*-cinnamic acid, coumaric acid and sinapic acid), and 20 types of L-amino acids were used.

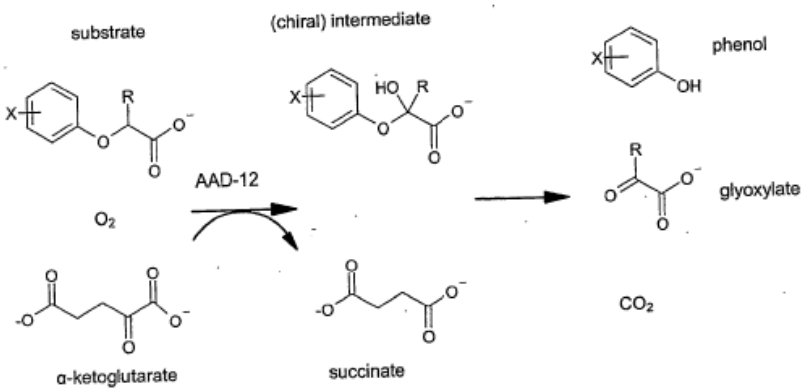

Figure 2 Enzyme reaction of modified AAD-12 protein under the presence of α-ketoglutarate

(All the rights pertinent to the information in the diagram above and the responsibility for content rest upon Dow Chemical Japan Limited.)

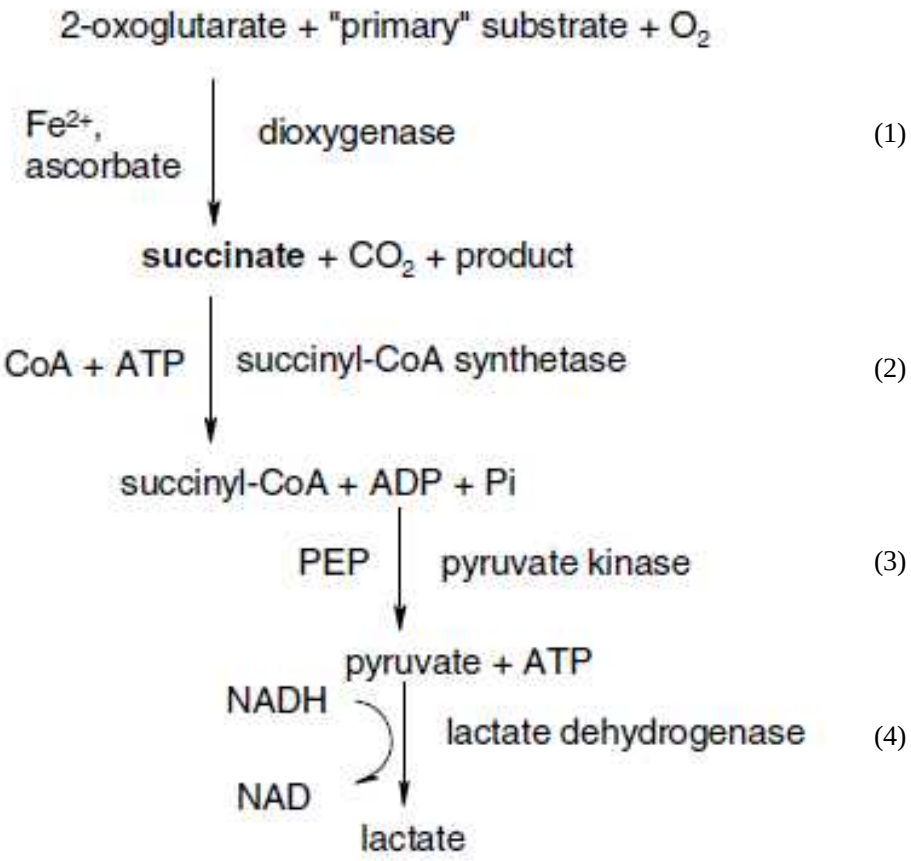

Figure 3 Reaction path of enzyme-coupled assay of succinate * A-ketoglutarate = 2-oxoglutarate

(1) In the reaction where dioxygenase introduces oxygen to the substrate, α-ketoglutarate (2-oxoglutarate) is converted to succinate.

(2) In the presence of coenzyme A (CoA) and adenosine-5'-triphosphate (ATP), succinate is converted to succinyl-CoA by succinyl-CoA synthetase and adenosine-5'-diphosphate (ADP) and phosphate (Pi) are produced simultaneously.

(3) Pyruvate and ATP are generated from phosphoenolpyruvate (PEP) and ADP by pyruvate kinase.

(4) In the presence of reduced nicotinamide adenine dinucleotide (NADH), pyruvate is converted to lactate by lactate dehydrogenase, and NAD is produced simultaneously. The amount of NAD produced in this reaction is considered equal to the amount of succinate.

Regarding the 20 types of L-amino acids, no production of succinate was identified for modified AAD-12 protein at a concentration of 1-μM, and it was considered that modified AAD-12 protein does not react with the 20 types of L-amino acids (Attachment 2, Table 1, p.18). Meanwhile, when 1-μM modified AAD-12 protein was acted on plant hormones and phenylpropanoid intermediates, production of a small amount of succinate was identified for coumaric acid, *trans*-cinnamic acid

and aminocyclopropane-1-caoboxylic acid (Attachment 2, Table 2, p.19). Further, when 5-μM and 10-μM modified AAD-12 protein was acted, production of succinate was identified for indole-3-acetic acid, gibberellic acid, abscisic acid, coumaric acid, *trans*-cinnamic acid and sinapic acid (Attachment 2, Table 3 and Table 4, p.20 and p.21). Like this, under various concentrations of modified AAD-12 protein, production of succinate was identified for some compounds. However, in enzyme-coupled assay, there is a possibility of uncoupling taking place that induces succinate products without undergoing oxidation of substrate (Hausinger, 2004). Therefore, in order to confirm oxidation of compounds that exhibited reaction in the enzyme-coupled assay, measurement of the primary oxides was carried out by the Fourier transform mass spectrometry (FT/MS). As a result, when 10-μM modified AAD-12 protein was acted, only the oxides of *trans*-cinnamic acid and indole-3-acetic acid were detected.

Accordingly, the catalytic efficiency of modified AAD-12 protein was investigated by enzyme-coupled assay for *trans*-cinnamic acid and indole-3-acetic acid, and, as a control, for *S*-dichlorprop* which is a substance with aryloxyalkanoate structure and a chemical structure similar to 2,4-D. As a result, the catalytic efficiency, *Kcat/Km*, was 156.7 $M^{-1}s^{-1}$, 8.2 $M^{-1}s^{-1}$ and 30,175 $M^{-1}s^{-1}$ for *trans*-cinnamic acid, indole-3-acetic acid and *S*-dichlorprop, respectively (Attachment 2, Table 5, p.22). The values of *Kcat/Km* for *trans*-cinnamic acid and indole-3-acetic acid were 0.52% and 0.027% of that for *S*-dichlorprop, respectively, indicating substantially low catalytic efficiencies for *trans*-cinnamic acid and indole-3-acetic acid compared to that for *S*-dichlorprop. There was a report stating that the *Kcat/Km* value of modified AAD-12 protein for 2,4-D was 18,600 $M^{-1}s^{-1}$ (Wright *et al.*, 2010). Since the catalytic efficiency of modified AAD-12 protein is equivalent for 2,4-D and *S*-dichlorprop, the catalytic efficiency for *trans*-cinnamic acid and indole-3-acetic acid is considered substantially low compared to that for 2,4-D.

Additionally, it was reported that the *in vitro Kcat/Km* value of cinnamate-4-hydroxylase of *Arabidopsis thaliana* for *trans*-cinnamic acid was 3.4×10^6 $M^{-1}s^{-1}$ (Chen *et al.*, 2007). It also was reported that the *in vitro Kcat/Km* value of IAA amido synthetase of *Oryza sativa* for indole-3-acetic acid was 2.75×10^3 $M^{-1}s^{-1}$ (Chen *et al.*, 2009). Like these, the catalytic efficiency of cinnamate-4-hydroxylase and that of IAA amido synthetase are both high, and it is therefore considered that *trans*-cinnamic acid and indole-3-acetic acid are effectively and specifically used in the existing metabolic pathways in plant bodies. Meanwhile, the *Kcat/Km* value of modified AAD-12 protein for *trans*-cinnamic acid was 0.005% of that of cinnamate-4-hydroxylase for *trans*-cinnamic acid, and the *Kcat/Km* value of modified AAD-12 protein for indole-3-acetic acid was 0.3% of that of IAA amido synthetase for indole-3-acetic acid. Both of the *Kcat/Km* values of modified AAD-12 protein are substantially low.

Based on the abovementioned understanding, it is considered that while there is a possibility of modified AAD-12 protein oxidizing *trans*-cinnamic acid and indole-3-acetic acid, the catalytic efficiency is extremely low, and the possibility of identified oxidation reactions affecting the

---

* *S*- enantiomer of chiral herbicide dichlorprop. Only the *R*- enantiomer has herbicidal activity, and *S*-dichlorprop has no herbicidal activity.

metabolic pathways of plants is low.

Since no compound with aryloxyalkanoate structure is known to exist in plant bodies to date, it is considered to be unlikely that modified AAD-12 alters other metabolic systems of plant bodies.

Regarding the effect of 2,4-DCP as a decomposition product of herbicide 2,4-D on aquatic organisms, LC_{50} (median lethal concentration) identified in acute toxicity studies was 1.7 mg/L for freshwater fish and 1.4 mg/L for *Daphnia magna*, and EC_{50} (half maximal effective concentration) was 1.5 mg/L for Lemna. The NOEC (no observed effect concentration) identified in chronic toxicity studies was 0.14 mg/L for Lemna and 0.21 mg/L for *Daphnia magna*. Additionally, regarding effects on terrestrial organisms, LC_{50} was 125 mg/kg for earthworm and EC_{10} (10% effect concentration) was 0.7 mg/kg for *Folsomia candida* (OECD, 2006). Meanwhile, regarding effects of 2,4-D on aquatic organisms, LC_{50} identified in acute toxicity studies was 0.26 mg/L for freshwater fish and 2.2 mg/L for *Daphnia magna*, and EC_{50} was 0.2992 mg/L for *Lemna gibba*. The NOEC identified in chronic toxicity studies was 0.0476 mg/L for *Lemna gibba* and 0.20 mg/L for *Daphnia magna* (EPA, 2004).

Like these, the toxicity of 2, 4-DCP as a decomposition product of 2, 4-D is equivalent to or lower than that of 2, 4-D, and it is considered to be unlikely that the concentration of 2, 4-DCP will reach levels that cause adverse effects more than the sprayed 2, 4-D does, even if the maximum possible concentration of 2, 4-DCP is assumed for when 2, 4-D is sprayed.

The residual 2, 4-DCP concentration in grains was investigated after spraying the upper limit amount of the appropriate use range of 2,4-D onto this modified soybean. As a result, the maximum average residual amount was 0.047 mg/kg (Attachment 3, Table 2, p.26). LD_{50} of 2,4-DCP identified in acute toxicity studies on mice was 1,276-1,352 mg/kg body weight, and NOAEL (no observed adverse effect level) identified in chronic toxicity studies on rats was 440 mg/kg body weight/day for males and >250 mg/kg body weight/day for females (OECD, 2006), which is substantially larger than the residual amount of 2,4-DCP in this modified soybean. Therefore, it is considered to be unlikely that the imported seeds of this modified soybean cause adverse effects on wild animals or plants.

[PAT protein]

PAT protein is an enzyme that extremely specifically acetylates free amino groups of L-glufosinate that is the active ingredient of herbicide glufosinate, and never acetylates other amino acids or D-glufosinate (OECD, 1999). Additionally, acetylation of L-glufosinate by PAT protein is never affected, even under overabundance of L-amino acids (OECD, 1999). Therefore, it is considered to be unlikely that PAT protein alters other metabolic systems of plant bodies.

It has been confirmed that the toxicity (acute toxicity, subacute toxicity, chronic toxicity, carcinogenicity, and reproductive and developmental toxicity) of *N*-acetyl-L-glufosinate as a metabolic product of herbicide glufosinate on animals is lower than that of glufosinate (Food Safety Commission of Japan, 2010), and it is considered to be unlikely that the concentration of

N-acetyl-L-glufosinate becomes to the level that causes adverse effects more than the sprayed glufosinate does, even if the maximum possible concentration of *N*-acetyl-L-glufosinate is assumed for when glufosinate is sprayed. As a side note, *N*-acetyl-L-glufosinate is included in the compounds subject to the residue standards for soybeans.

(2) Information concerning vector

1) Name and origin

The vector pDAB2407 which became the base of introduced pDAB4468 was derived from *A. tumefaciens* and *Escherichia coli*.

2) Properties

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

The total number of base pairs in the expression vector is 12,154 bp. The nucleotide sequence of pDAB4468 is shown in Attachment 4.

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

The expression vector pDAB4468 possesses the *spec*R gene that confers tolerance to spectinomycin. While the *spec*R gene was used as a selective marker on establishment of the expression vector pDAB4468, it is located outside the T-DNA region and thus is not introduced into this modified soybean.

Southern blot analysis of this modified soybean confirmed no existence of the *spec*R gene (Attachment 5, Table 2, p.5).

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

The T-DNA region of the vector which became the base of the expression vector pDAB4468 is rewritten to the donor nucleic acid shown in Table **1** (p.2) and does not contain a sequence that allows for infection of *Agrobacterium tumefaciens*; infectivity has not been identified yet.

(3) Method of preparing living modified organisms

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

The map of the expression vector pDAB4468 is shown in Figure 4 (p.10). The production process of the expression vector pDAB4468 is shown in Attachment 6.

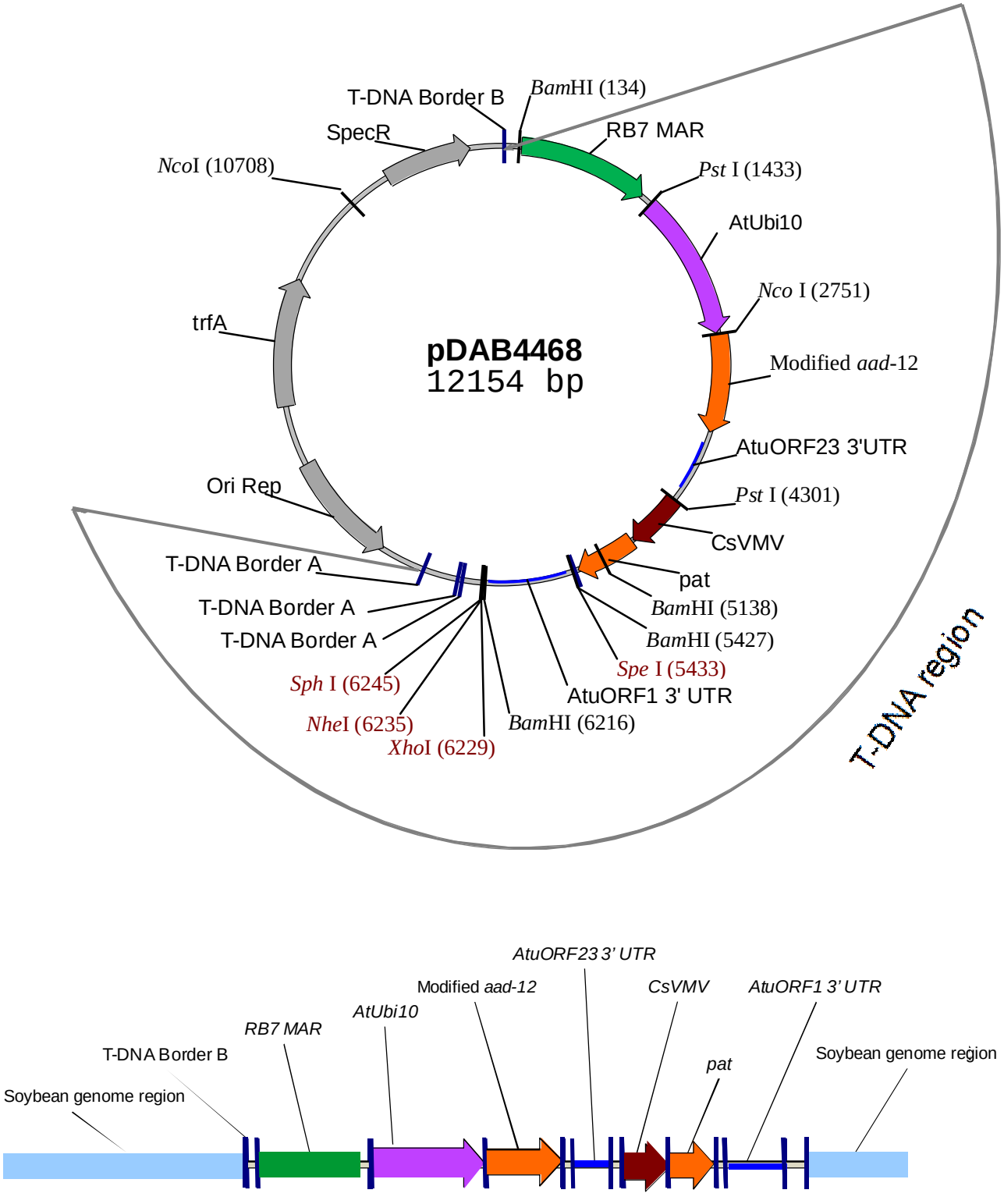

Figure 4 Map of expression vector pDAB4468 (top) and schematic of insertion of T-DNA region (bottom)

* The numbers in parenthesis in the top figure refer to the restriction enzyme cleavage site on the plasmid with T-DNA Border B as the starting point.

(All the rights pertinent to the information in the diagram above and the responsibility for content rest upon Dow Chemical Japan Limited.)

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

Introduction of nucleic acid into the recipient organism was carried out by *Agrobacterium* method.

3) Processes of rearing living modified organisms

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

Selection was made by culturing adventitious buds and shoots formed from cultured *Agrobacterium*-infected tissues in a medium containing herbicide glufosinate.

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

Agrobacterium was sterilized by adding antibiotics to the adventitious bud-inducing medium and adventitious bud-elongating medium. Subsequently, the formed shoots were cultured in a regeneration medium containing no antibiotics, and absence of remaining *Agrobacterium* was confirmed.

(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

Individual bodies with tolerance were selected by applying glufosinate to the re-differentiated plant bodies. For the selected plant bodies, introduced genes were analyzed by the PCR method and Southern blot analysis. This modified soybean was selected by comprehensively assessing the analysis of introduced genes in progenies, confirmation of protein expression, herbicide tolerance and agronomic characters identified at outdoor fields in the US and Canada. The range of application is progenies from T3 generation onward.

Details are shown in Figure 5 (p.12).

The status of application and approval of this modified soybean in Japan is summarized below (as of June 2013).

August 2009 Approved for Type 1 Use Regulation (isolated field test) by the Ministry of Agriculture, Forestry and Fisheries (MAFF) and the Ministry of the Environment (MOE) based on the “Law Concerning the Conservation and Sustainable Use of Biological Diversity Through Regulations on the Use of Living Modified Organisms” (Period of use: August 28, 2009 to March 31, 2011).

May 2012 Approved anew for Type 1 Use Regulation (isolated field test) by MAFF and MOE based on the “Law Concerning the Conservation and Sustainable Use of

Biological Diversity Through Regulations on the Use of Living Modified Organisms" for the purpose of conducting additional tests at isolated fields (Period of use: May 29, 2012 to March 31, 2014).

May 2013 Applied to the Ministry of Health, Labour and Welfare for safety confirmation as use for food based on the "Food Sanitation Act".

May 2013 Applied to MAFF for safety confirmation as use for feed based on the "Act on Safety Assurance and Quality Improvement of Feeds".

Confidential and non-disclosed

Figure 5 Process of rearing of modified 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 exists

Transferred nucleic acid obeys Mendel's law once incorporated into the plant chromosome. Segregation of traits introduced into modified soybean in the F2 generation (Figure 5, p.12) was analyzed (2009, Indiana, USA). One F1 generation individual body that was obtained by crossing the T4 generation line and non-modified soybean was self-pollinated, and the presence or absence of modified AAD-12 protein expression in the F2 group was investigated using the lateral flow strip method*. As a result, it was confirmed that transferred nucleic acids exist on the soybean chromosome since the observed values were consistent with Mendel's law of segregation for nuclear genes (Table **2**, p.12).

Table 2 Segregation analysis of F2 generation of modified soybean

Generation	**Total number of individual bodies**	**Estimated ratio**	**Estimated value**[1)]		**Observed value**[1)]		χ^2	**p-value**[2)]
			AAD-12 +	**AAD-12 –**	**AAD-12 +**	**AAD-12 –**		
F2	146	3:1	110.25	36.75	101	45	2.64	0.10

[1)] AAD-12 +: Number of individual bodies in which modified AAD-12 protein was detected
AAD-12 –: Number of individual bodies in which modified AAD-12 protein was not detected

[2)] $p < 0.05$

---

* Method to visually identify accumulation of a marker that is an immune complex consisting of the antigen in the specimen, a labelled antibody and capture antibody while the specimen moves on a membrane by the capillary phenomenon. In the present test, presence or absence of expression of modified AAD-12 by modified soybean was confirmed by capturing the protein with the antibody on a membrane and visual inspection of the band.

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

In order to confirm the number of copies of transferred nucleic acids, Southern blot analysis was carried out from T3 generation to T5 generation. As a result, it was confirmed that one copy each of *RB7 MAR*, modified *aad-12* cassette and *pat* cassette were introduced to the modified soybean, and are inherited stably over multiple generations (Attachment 5, Table 2, p.4).

In order to confirm insertion of individual component elements to the T-DNA region, cloning and sequencing were performed for the entire inserted gene for the modified soybean including the host genome border region. The base sequences were determined for the total of 10,212 bp including the transferred nucleic acid region with 6,400 bp, sequence flanking the 5' terminal with 2,730 bp and the sequence flanking the 3' terminal with 1,082 bp (Attachment 7). As a result, it was clarified that all of T-DNA Border A and part of T-DNA Border B were not transferred, while all other component elements were transferred in complete form (Figure 4, bottom, p.10).

(3) Positional relationship in the case of multiple copies existing in chromosome

No multiple copies exist in the chromosome.

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

For the T4 generation and T6 generation of modified soybean, expression level of modified AAD-12 protein and PAT protein in leaves was investigated using the ELISA method (2008-2009, Indiana, USA). As a result, it was confirmed that expression of modified AAD-12 protein and PAT protein was stably conferred to both generations (Table 3, p.13 and Table 4, p.14).

Table 3 Expression level of modified AAD-12 protein in leaves of T4 and T6 generations of modified soybean[1)]

(ng/mg dry weight)

Generation	Average	Standard deviation	Number of samples
T4	51.42	25.22	16
T6	74.99	8.53	28
Non-modified soybean	< LOD [2)]	–	28

[1)] Leaves in 5th leaf stage were provided for testing.

[2)] Below limit of detection (0.5 ng/mg).

Table 4 Expression level of PAT protein in leaves of T4 and T6 generations of modified soybean[1)]

(ng/mg dry weight)

Generation	Average	Standard deviation	Number of samples
T4	9.17	2.99	16
T6	7.13	2.80	28
Non-modified soybean	< LOD [2)]	–	28

[1)] Leaves in 5th leaf stage were provided for testing.

[2)] Below limit of detection (0.06 ng/mg).

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

Modified soybean does not include transmittable sequences and the nucleic acids introduced to modified soybean will not be transferred to wild animals or wild plants.

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

A PCR method that uses the base sequence specific to modified soybean as the primer has been developed as a method to detect and identify this modified soybean. Regarding the contamination ratio of modified soybean to non-modified soybean, the limit of detection using the current PCR is 0.04% as the DNA content ratio. Regarding reproducibility, assurance of inter-laboratory transferability was confirmed at US Dow AgroSciences and US Eurofins Genescan (Attachment 8, Table 12, p.28, Study ID 101716).

(6) Difference between modified organism and recipient organism or species to which recipient organism belongs

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

For modified soybean, modified *aad-12* gene and *pat* gene are introduced, and they confer tolerance to aryloxyalkanoate herbicide and herbicide glufosinate by expressing modified AAD-12 protein and PAT protein, respectively. By growing modified soybean with tolerance to aryloxyalkanoate herbicide, farmers will have a wider range of herbicide products to choose from and will be able to control weeds that gain resistance to other herbicides. Additionally, the tolerance to herbicide glufosinate was used as the marker for selection.

During isolated field tests conducted in 2012 at the Ogori Development Center, Dow Chemical Japan Limited, herbicide 2,4-D and glufosinate tolerance test was conducted for modified soybean (T10 generation) and non-modified soybean as a control (Maverick). Seventeen days after seeding (around the second leaf stage of true leaf), the modified soybean and non-modified soybean (12 individual bodies each) were sprayed with 2,4-D at 1,120 g a.e./ha* (normal application) or

glufosinate to at 374 g a.i./ha* (normal application). In 2 weeks after application, all non-modified soybeans withered, while all modified soybean plants exhibited adequate herbicide tolerance with no chemical damage observable (“Isolated Field Test Results Report”, Figure 1, p.2).

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

In 2012, isolated field tests were conducted at the Ogori Development Center, Dow Chemical Japan Limited, and the differences between modified soybean (T10 generation) and non-modified soybean as a control (Maverick) were investigated.

(a) Morphological and growth characteristics

As morphological and growth characteristics, comparison between modified soybean and non-modified soybean was carried out referencing the Screening Standards by Agricultural, Forestry and Fishery Plant Species for agricultural soybean species (Ministry of Agriculture, Forestry and Fisheries, 2012), regarding germination rate, uniformity of germination, beginning period of flowering, ending period of flowering, mature period, lobular form, amount of trichome, growth type, main stem length, position of lowest pod shooting node, number of nodes on the main stem, number of branches, aboveground fresh weight during harvest period, number of fertile pods, total grain weight per plant, mature grain weight per plant, weight of a hundred grains, and shape of grain.

At the isolated field, both modified soybean and non-modified soybean started germinating 4 days after seeding. Regarding germination rate, there was a significant difference identified between modified soybean (99.0%) and non-modified soybean (80.2%) (“Isolated Field Test Results Report”, Table 1, Germination Rate-1, p.3). When an indoor germination test was conducted using the seeds of modified soybean, and non-modified soybean for the seeding in the field, no significant difference was identified regarding the germination rate between modified soybean (98.3%) and non-modified soybean (95.0%) (“Isolated Field Test Results Report”, Table 1, Germination Rate-2, p.3). Subsequent growth was identical in the field, and no difference was observed between modified soybean and non-modified soybean in terms of beginning period of flowering, ending period of flowering, or mature period (“Isolated Field Test Results Report”, Table 2, p.4).

Regarding items other than germination rate, no statistically-significant differences were observed (“Isolated Field Test Results Report”, Tables 3-6, p.4-6).

---

* Active ingredient content of herbicide is expressed with active ingredient (a.i.) or acid equivalent (a.e.). Active ingredient (a.i.) content refers to the component weight, and acid equivalent (a.e.) refers to the amount of free acids. Herbicide preparations contain the active ingredient in the form of salt or as is. When the active ingredient exists in the form of salt, the active component is an acid, and the base part varies depending on preparation. Since various preparations exist that contain 2,4-D with different base parts, acid equivalent was used in order to accurately apprehend the amount of active component.

(b) Cold tolerance at the early stage of growth

The cold tolerance of modified soybean and non-modified soybean at an early stage of growth was investigated. The modified soybean and non-modified soybean that grew to the first leaf developing period (6 individuals each) were grown in an incubator set to 4°C and 16-hour day lengths, and their growing conditions were observed. As a result, both modified soybean and non-modified soybean exhibited symptoms of leaf whitening, plant body shrinkage and severe growth inhibition after 31 days, and there was no difference observed in the level of the symptoms ("Isolated Field Test Results Report", Figure 3, p.7).

(c) Wintering ability of the mature plant

The wintering ability of the mature plant of modified soybean and non-modified soybean was investigated. Mature plants grown in the field (16 plants) were left untouched without harvesting until the following year, and the conditions of the plant bodies under the natural winter conditions were observed. As a result of observation on individuals served for testing in January 2013, both modified soybean and non-modified soybean were found to be withered ("Isolated Field Test Results Report", Figure 4, p.7).

(d) Fertility and pollen size

No difference was observed in pollen shape ("Isolated Field Test Results Report", Figure 5, p.8). The fullness and pollen size of modified soybean and non-modified soybean that were stained with iodine solution were investigated. As a result, no statistically-significant difference was observed between modified soybean and non-modified soybean ("Isolated Field Test Results Report", Table 7, p.9).

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

Regarding the production of the seed, comparison between modified soybean and non-modified soybean was made on the number of fertile pods, total grain weight per plant, mature grain weight per plant, and the weight of a hundred grains. As a result, no statistically-significant difference was observed for all items, and it was judged that there was no difference between modified soybean and non-modified soybean in the production of the seed ("Isolated Field Test Results Report", Table 5, p.5).

Regarding pod dehiscence, the level of dehiscence during the mature period was observed for modified soybean and non-modified soybean. As a result, the level of dehiscence of both modified soybean and non-modified soybean were difficult, and there was no observable difference ("Isolated Field Test Results Report", Table 8, p.9).

The dormancy of modified soybean and non-modified soybean was assessed by germinating the harvested seeds in a plate immediately after harvesting without conducting dormancy breaking and investigating the germination rate. As a result, both modified soybean and non-modified soybean

exhibited a high germination rate, and it was judged that the dormancy was extremely shallow. No statistically-significant difference was observed (“Isolated Field Test Results Report”, Table 7, p.9).

(f) Crossability

Modified soybean and non-modified soybean were planted alternately to the crossability test plot (“Isolated Field Test Results Report”, Attachment Figure 2, p.15) with an interval of 25 cm, and 3,200 seeds obtained from non-modified soybeans were again sowed inside the isolated field. Among the 3,200 seeds that were sowed, 3,129 seeds germinated (germination rate 97.8%). The survivability was then investigated by treating them with 1,120g ae/ha of herbicide 2,4-D at the second leaf stage of true leaf and counting the survived individuals. As a result, survival of 5 individuals out of the 3,129 individuals was confirmed, with a survival rate of 0.16% (“Isolated Field Test Results Report”, Table 9, p.10).

(g) Productivity of harmful substances

In order to compare the productivity of harmful substances for modified soybean and non-modified soybean, a succeeding crop test, plow-in test and soil microflora test were conducted.

< Succeeding crop test>

Root area soil of modified soybean and non-modified soybean in the harvest period was collected from 8 locations per plot, mixed (8 plant/plot, 4 repeated plots) and filled into a 25-cell seedling growing tray. Single radish seed was sowed to each cell. The germination rate was investigated 7 days after seeding, and the plant height and dry weight were investigated 21 days after seeding.

As a result, regarding the germination rate, plant height and dry weight of assay plant radish, no statistically-significant differences were observed between the test plots of s modified soybean and non-modified soybean (“Isolated Field Test Results Report”, Table 10, p.11).

<Plow-in test>

Aboveground part of plant bodies of modified soybean and non-modified soybean in the harvest period were reaped (4 plant/plot, 4 repeated plots), dried, crushed, and mixed well with horticultural soil, where 4 plants were regarded as 1 sample (the weight ratio of dried powder: approx. 0.6%). The mixed soil was placed into a 25-cell seedling growing tray, and a single radish seed was sowed to each cell. The germination rate was investigated 7 days after seeding, and the plant height and the dry weight were investigated 22 days after seeding.

As a result, regarding the germination rate, plant height and dry weight of assay plant radish, no statistically-significant differences were observed between the test plots of modified soybean and non-modified soybean (“Isolated Field Test Results Report”, Table 11, p.11).

<Soil microflora test>

Soil of modified soybean and non-modified soybean after harvesting was collected from 3 locations per plot (3 sample/plot, 4 repeated plots). Bacterial count, actinomycetal count, and filamentous fungal count were measured using the dilution plate technique. As a result, no statistically-significant differences were observed between the test plots of modified soybean and non-modified soybean (“Isolated Field Test Results Report”, Table 12, p.12).

II. Results of the review by persons with specialized knowledge and experience concerning Adverse Effects on Biological Diversity

A review was conducted by persons with specialized knowledge and experience concerning Adverse Effects on Biological Diversity (called Experts) for possible Adverse Effects on Biological Diversity caused by 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) Results of the assessment of Adverse Effects on Biological Diversity

Modified soybean was produced by introducing the T-DNA region of pDAB4468 established from the plasmid pDAB2407 derived from *Agrobacterium tumefaciens* and *Escherichia coli* by *Agrobacterium* method.

For modified soybean, it has been confirmed that one copy of T-DNA region containing modified *aad-12* gene that codes modified AAD-12 protein derived from *Delftia acidovorans*, *pat* gene that codes PAT protein derived from *Streptomyces viridochromogenes* and the nuclear matrix atatchment region *RB7 MAR* derived from *Nicotiana tabacum* (stabilizes expression of introduced gene) is introduced and is inherited stably over multiple generations by the segregation system of genes and Southern blot analysis.

(a) Competitiveness

The species of soybean to which the recipient organism belongs has been grown in Japan over a long period of time, yet there has been no report made stating that soybean became a weed under the natural environment.

In 2012, investigations on various traits pertaining to competitiveness of modified soybean were conducted in an isolated field in Japan. As a result, while a statistically-significant difference was identified for the germination rate among morphological and growth traits, this difference was considered not to increase competitiveness of modified soybean. For items other than the germination rate, no difference was identified between modified soybean and non-modified soybean as a control.

For modified soybean, modified *aad-12* gene and *pat* gene are introduced, and they confer tolerance to aryloxyalkanoate herbicide and herbicide glufosinate by expressing modified AAD-12 protein and PAT protein, respectively. It is unlikely that such herbicide tolerance enhances competitiveness under natural conditions where application of such herbicides is unlikely to take place.

Based on the abovementioned understanding, it was judged that the conclusion by the applicant, stating that no wild animal or plant that has a possibility to be affected was identified and modified soybean would pose no risk in causing Adverse Effects on Biological Diversity

attributable to its competitiveness is reasonable.

(b) Productivity of harmful substances

There has been no report stating that the species of soybean, to which the recipient organism belongs, produces substances that are harmful to wild animals or plants.

While modified soybean produces modified AAD-12 protein that confers tolerance to aryloxyalkanoate herbicide and PAT protein that confers tolerance to herbicide glufosinate, there has been no report on the relevant proteins as harmful substances, and it has been confirmed that they contain no amino acid sequence related to known allergens.

In order to compare the productivity of harmful substances for modified soybean and non-modified soybean, a succeeding crop test, plow-in test and soil microflora test were conducted. As a result, in all of the tests, no statistically-significant differences were observed between the test plots of modified soybean and non-modified soybean. It has been confirmed that the toxicity (acute toxicity, subacute toxicity, chronic toxicity, carcinogenicity, and reproductive and developmental toxicity) of *N*-acetyl-L-glufosinate as a metabolic product of herbicide glufosinate on animals is lower than that of glufosinate, and it is considered to be unlikely that the concentration of *N*-acetyl-L-glufosinate will reach levels that cause more adverse effects than sprayed glufosinate.

Based on the abovementioned understanding, it was judged that the conclusion by the applicant, stating that no wild animal or plant that has a possibility to be affected was identified and modified soybean would pose no risk in causing Adverse Effects on Biological Diversity attributable to its productivity of harmful substances is reasonable.

(c) Crossability

As a wild relative of soybean, *Glycine soja* grows wild in Japan. The chromosome number is 2n = 40 for soybean and *Glycine soja*, and they are crossable. To that end, *Glycine soja* was identified as a wild plant that has a possibility to be affected, and investigations were carried out as below.

No specific defects are observable in the growth of crossbreeds that were artificially crossed between soybean and *Glycine soja*. Therefore, when crossing occurs between modified soybean and *Glycine soja* in the natural Japanese environment, it is likely that the crossbreed will grow normally and the gene introduced to modified soybean will spread among *Glycine soja* through crossing of this crossbreed and *Glycine soja*. In Japan, *Glycine soja* is distributed throughout the country and grows wild on dry river beds, river banks, and around farms and orchards. Therefore, when modified soybean is grown near such places, it is likely that crossing with *Glycine soja* takes place.

However, there are reports stating,

(1) Soybean and *Glycine soja* are self-fertilizing plants, and it is known that in general the flowering season of *Glycine soja* is later than that of soybean and their flowering seasons rarely overlap. Therefore, it is considered to be unlikely that crossing of soybean and

Glycine soja occurs. In an actual example, the average crossing rate of Tanba Black (soybean specific to Japan that has a relatively late flowing season) and *Glycine soja* was 0.73%;

(2) Under conditions where *Glycine soja* was twining around modified soybean and the flowering season overlapped, only one individual body was crossbred out of 11,860 individual bodies germinated from seeds collected from *Glycine soja*; and,

(3) In an experiment where two types of modified soybean with late flowering seasons (AG6702RR and AG5905RR) were used (i.e. flowering peak closer to *Glycine soja*) and *Glycine soja* was twining around modified soybeans, the number of crossbreeds out of 25,741 individual bodies was 25 individual bodies (0.097%) for AG6702RR and 10 individual bodies (0.039%) for AG5905RR. When *Glycine soja* was grown 2, 4, 6, 8, and 10 m away from modified soybean (AG6702RR), the number of crossbreeds was 1 (out of 7,521 individual bodies), 1 (7,485), 1 (14,952), 0 (14,964), and 0 (21,749), respectively.

Additionally, regarding the fullness of pollen, no difference was identified between modified soybean and non-modified soybean. Similarly, no differences were identified in the shape or size of the pollen.

As above, the flowering seasons of soybean and *Glycine soja* are unlikely to overlap, and the crossing rate is low. Additionally, no differences were identified between modified soybean and non-modified soybean in pollen properties. Therefore, it is considered that the crossing rate of modified soybean and *Glycine soja* under natural conditions is extremely low. Furthermore, it can be assumed that crossbreed progenies of soybean and *Glycine soja* do not survive for a long period of time at locations where *Glycine soja* grows wild. Therefore, it was considered that the possibility of gene propagation of modified soybean through *Glycine soja* occurring is extremely low.

Based on the abovementioned understanding, it was judged that the conclusion by the applicant stating that modified soybean would pose no risk in causing Adverse Effects on Biological Diversity attributable to its crossability is reasonable.

(2) Conclusion based on the Biological Diversity Risk Assessment Report

Based on the abovementioned understanding, it was judged that the conclusion of the Biological Diversity Risk Assessment Report, stating that the use of modified soybean in accordance with Type 1 Use Regulation would pose no risk in causing Adverse Effects on Biological Diversity in Japan, is reasonable.

References

1. Allen, George C.; Hall Jr., Gerald; Michalowski, Susan; Newman, Winnell; Spiker, Steven; Weissinger, Arthur K.; Thompson, William F. High-level transgene expression in plant cells: Effects of a strong scaffold attachment region from tobacco. The Plant Cell. 1996, 8(5), p. 899-913.

2. Allen, George C.; Spiker, Steven; Thompson, William F. Use of matrix attachment regions (MARs) to minimize transgene silencing. Plant Molecular Biology. 2000, 43(2-3), p.361-376.

3. Barker, R.F.; Idler, K.B.; Thompson, D.V.; Kemp, J.D. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. Plant Molecular Biology. 1983, 2(6), p.335-350.

4. Chen, Hao; Jiang, Hanxiao; Morgan, John A. Non-natural cinnamic acid derivatives as substrates of cinnamate 4-hydroxylase. Phytochemistry. 2007, 68(3), p.306–311.

5. Chen, Qingfeng; Zhang, Baichen; Hicks, Leslie M.; Wang, Shiping; Jez, Joseph M. A liquid chromatography–tandem mass spectrometry-based assay for indole-3-acetic acid–amido synthetase. Analytical Biochemistry. 2009, 390(2), p.149-154.

6. Chiang, Y.C.; Kiang, Y.T. Geometric position of genotypes, honeybee foraging patterns and outcrossing in soybean. Bot. Bull. Academia Sinica. 1987, 28(1), p.1-11.

7. EPA. Environmental Fate and Effects Division’s Risk Assessment for the Reregistration Eligibility Document for 2,4-Dichlorophenoxyacetic Acid (2,4-D). 2004, http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2004-0167-0003, (see 2013-2-12).

8. FAO. FAOSTAT. (updated: 2013-1-16), http://faostat.fao.org/default.aspx?lang=en, (see 2013-2-12).

9. Fujita, R.; Ohara, M.; Okazakl, K.; Shimamoto, Y. The extent of natural cross-pollination in wild soybean (*Glycine soja*). The Journal of Heredity. 1997, 88(2), p.124-128.

10. Halweg, Christopher; Thompson, William F.; Spiker, Steven. The Rb7 matrix attachment region increases the likelihood and magnitude of transgene expression in tobacco cells: A flow cytometric study. The Plant Cell. 2005, 17(2), p.418-429.

11. Hausinger, Robert P. Fe (II) /α-ketoglutarate-dependent hydroxylases and related enzymes.

Critical Reviews in Biochemistry and Molecular Biology. 2004, 39(1), p.21-68.

12. Kuroda, Yosuke; Kaga, Akito; Tomooka, Norihiko; Vaughan, Duncan A. Gene flow and genetic structure of wild soybean (*Glycine soja*) in Japan. Crop Science. 2008, 48(3), p.1071-1079.

13. Kuroda, Y.; Kaga, A.; Tomooka, N.; Vaughan, D. The origin and fate of morphological intermediates between wild and cultivated soybeans in their natural habitats in Japan. Molecular Ecology. 2010, 19(11), p.2346–2360.

14. Luo, Lusong; Pappalardi, Melissa B.; Tummino, Peter J.; Copeland, Robert A.; Fraser, Marie E.; Grzyska, Piotr K.; Hausinger, Robert P. An assay for Fe(II)/2-oxoglutarate-dependent dioxygenases by enzyme- coupled detection of succinate formation. Analytical Biochemistry. 2006, 353(1), p. 69–74.

15. Mizuguti, Aki; Yoshimura, Yasuyuki; Matsuo, Kazuhito. Flowering phenologies and natural hybridization of genetically modified and wild soybeans under field conditions. Weed Biology and Management. 2009, 9(1), p.93–96.

16. Mizuguti, Aki; Ohigashi, Kentaro; Yoshimura, Yasuyuki; Kaga, Akito; Kuroda, Yosuke; Matsuo, Kazuhito. Hybridization between GM soybean (*Glycine max* (L.) Merr.) and wild soybean (*Glycine soja* Sieb. et Zucc.) under field conditions in Japan. Environ. Biosafety Res. 2010, 9(1), p.13–23.

17. Nakayama, Yuichiro; Yamaguchi, Hirofumi. Natural hybridization in wild soybean (*Glycine max* ssp. *soja*) by pollen flow from cultivated soybean (*Glycine max* ssp. *max*) in a designed population. Weed Biology and Management. 2002, 2(1), p.25–30.

18. Norris, Susan R.; Meyer, Sandra E.; Callis, Judy. The intron of *Arabidopsis thaliana* polyubiquitin genes is conserved in location and is a quantitative determinant of chimeric gene expression. Plant Molecular Biology. 1993, 21(5), p.895-906.

19. OECD. Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. Series on Harmonization of Regulatory Oversight in Biotechnology No.11. 1999.

20. OECD. Consensus document on the biology of *Glycine max* (L.) Merr.(soybean). Series on Harmonization of Regulatory Oversight in Biotechnology No.15. 2000.

21. OECD. Module II: Phosphinothricin. Series on Harmonization of Regulatory Oversight in Biotechnology No.25. 2002.

22. OECD. '2,4-Dichlorophenol'. OECD Existing Chemicals Database. 2006, http://webnet.oecd.org/HPV/UI/handler.axd?id=3d43ed35-5ef5-4323-bddd-4aa527db0765, (see 2013-2-12)

23. Sleper, D.A.; Nickell, C.D.; Noel, G.R.; Cary, T.R.; Thomas, D.J.; Clark, K.M.; Rao Arelli, A.P. Registration of ‘Maverick’ soybean. Crop Science. 1998, 38(2), p.549-550.

24. Verdaguer, Bertrand; de Kochko, Alexandre; Fux, Charles I.; Beachy, Roger N.; Fauquet, Claude. Functional organization of the cassava vein mosaic virus (CsVMV) promoter. Plant Molecular Biology. 1998, 37(6), p.1055–1067.

25. Wohlleben, W.; Arnold, W.; Broer, I.; Hillemann, D.; Strauch, E.; Pühler, A. Nucleotide sequence of the phosphinothricin *N*-acetyltransferase gene from *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana tabacum*. Gene. 1988, 70(1), p.25-37.

26. Wright, Terry R.; Lira, Justin M.; Walsh, Terence, Authony; Merlo, Donald J.; Jayakumar, Pon Samuel; Lin, Gaofeng. Novel herbicide resistance genes. 2007, WO 2007/053482 A2.

27. Wright, Terry R.; Shan, Guomin; Walsh, Terence A.; Lira, Justin M.; Cui, Cory; Song, Ping; Zhuang, Meibao; Arnold, Nicole L.; Lin, Gaofeng; Yau, Kerrm; Russell, Sean M.; Cicchillo, Robert M.; Peterson, Mark A.; Simpson, David M.; Zhou, Ning; Ponsamuel, Jayakumar; Zhang, Zhanyuan. Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proc. Natl. Acad. Sci. USA. 2010, 107(47), p.20240-20245.

28. Yoshimura, Yasuyuki; Matsuo, Kazuhito; Yasuda, Koji. Gene flow from GM glyphosate-tolerant to conventional soybeans under field conditions in Japan. Environ. Biosafety Res. 2006, 5(3), p.169–173.

29. Kaga, Akito; Tomooka, Norihiko; Phuntsho, Ugen; Kuroda, Yosuke; Kobayashi, Nobuya; Isemura, Takehisa; Gilda, Miranda-Jonson; Vaughan, Duncan A. Exploration and Collection for Hybrid Derivatives Between Wild and Cultivated Soybean: Preliminary Survey in Akita and Hiroshima Prefectures, Japan. Annual Report on Exploration and Introduction of Plant Genetic Resources. Vol. 21, National Institute of Agrobiological Sciences, 2005, p.59-71.

30. Kuroda, Yosuke; Kaga, Akito; Apa, Anna; Vaughan, Duncan A.; Tomooka, Norihiko; Yano, Hiroshi; Matsuoka, Nobuyuki. Exploration, Collection and Monitoring of Wild Soybean and Hybrid Derivatives between Wild Soybean and Cultivated Soybean: Based on Field Surveys at Akita, Ibaraki, Aichi, Hiroshima and Saga Prefectures. Annual Report on Exploration and Introduction of Plant Genetic Resources. Vol. 21, National Institute of Agrobiological Sciences, 2005, p.73-95.

31. Kuroda, Yosuke; Kaga, Akito; Joe, Guaf; Vaughan, Duncan A.; Tomooka, Norihiko. Exploration, Collection and Monitoring of Wild Soybean, Cultivated Soybean and Hybrid

Derivatives between Wild Soybean and Cultivated Soybean: Based on Field Surveys at Akita, Ibaraki, Kochi and Saga Prefectures. Annual Report on Exploration and Introduction of Plant Genetic Resources. Vol. 22, National Institute of Agrobiological Sciences, 2006, p.1-12.

32. Kuroda, Yosuke; Kaga, Akito; Poafa, Janet; Vaughan, Duncan A.; Tomooka, Norihiko; Yano, Hiroshi. Exploration, Collection and Monitoring of Wild Soybean, Cultivated Soybean and Hybrid Derivatives between Wild Soybean and Cultivated Soybean: Based on Field Surveys at Akita, Hyogo and Saga Prefectures. Annual Report on Exploration and Introduction of Plant Genetic Resources. Vol. 23, National Institute of Agrobiological Sciences, 2007, p.9-27.

33. Ministry of Finance Japan. "Principal commodity by country". Trade Statistics of Japan. http://www.customs.go.jp/toukei/srch/index.htm?M=13&P=0, (see 2013-2-12).

34. Food Safety Commission of Japan. Risk Assessment Report Glufosinate. 2010. http://www.fsc.go.jp/fsciis/evaluationDocument/show/kya20070717010, (see 2013-2-12).

35. Zheng, Shauhui. "Soybean". Introduction to Agronomy. Daimon, Hiroyuki (author and editor). Asakura Publishing CO., Ltd., 2008, p.132-146.

36. National Agriculture and Food Research Organization. Agronomic Characteristics and Yieldability of Soybean Seeds after Long-term Low-temperature Low-humidity Storage. FY2003 Kyushu Okinawa Agricultural Research Result Information. http://www.naro.affrc.go.jp/org/karc/seika/kyushu_seika/2003/2003015.html, (see 2013-2-12).

37. Numata, Makoto; Okuda, Shigetoshi; Kuwabara, Yoshiharu; Asano, Sadao; Iwase, Toru. Weed flora of Japan illustrated by colour (New edition). Numata, Makoto; Yoshizawa, Nagato (eds.). National Rural Education Association, Inc. 1978, p.107.

38. Ministry of Agriculture, Forestry and Fisheries. "Screening Standards for Soybeans". Screening Standards by Agricultural, Forestry and Fishery Plant Species. 2012, http://www.hinsyu.maff.go.jp/info/sinsakijun/kijun/1307.pdf, (see 2012-5-1).

39. Furuya, Yoshihito. "Soybeans". Agriculture Dictionary -1977 Revision-. Noguchi, Yakichi (editor-in-chief). Yokendo Co.Ltd. 1977, p.501-508.

Attachment List

Confidential and Non-disclosed

1. Monitoring Result Report
2. Isolated Field Test Results Report of Soybean Tolerant to Aryloxyalkanoate Herbicide and Glufosinate Herbicide (modified *aad-12*, *pat*, *Glycine max* (L.) Merr.) (DAS68416, OECD UI: DAS-68416-4)
3. Attachment 1: Herbicides to which the modified AAD-12 protein exhibits activity
4. Attachment 2: Substrate Specificity of Aryloxyalkanoate Dioxygenase-12 (AAD-12)
5. Attachment 3: Risk Assessment Overview of 2,4-D Treated Soybean Containing DAS AAD-12 Trait
6. Attachment 4: Nucleotide Sequence of pDAB4468
7. Attachment 5: Number of Copies of Introduced Gene and Inter- and Intra-generation Stability
8. Attachment 6: Production Process of pDAB4468
9. Attachment 7: Cloning and Characterization of DNA Sequence for the Insert and Flanking Border Regions of AAD-12 Soybean Event DAS-68416-4
10. Attachment 8: Detection Method of Modified Soybean