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

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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Oilseed rape tolerant to glufosinate herbicide (*pat, Brassica napus* L.) (T45, OECD UI: ACS-BNØØ8-2)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

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

1. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

Composition of the donor nucleic acid that was used for the production of glufosinate tolerant oilseed rape (*pat, Brassica napus* L., T45, OECD UI: ACS-BN008-2) (hereinafter referred to as "T45") and the origins of component elements are shown in Table 1.

Table 1 Origins and functions of component elements in the donor nucleic acid

Component elements	Size (bp)	Origin and function
pat gene expression cassette		
P-35S	533	35S RNA promoter derived from Cauliflower Mosaic Virus. It expresses *pat* genes in plants constitutively (Reference 40).
pat	552	Derived from *Streptomyces viridochromogenes*, encoding the enzyme phosphinothricin acetyl transferase (PAT) and conferring the glufosinate tolerance (Reference 12). This gene is a modified type of wild *pat* gene to adapt to the frequently-used codon in plants. The amino acid sequence of the protein which is produced by this modification remains unchanged.
T-35S	220	35S RNA terminator derived from Cauliflower Mosaic Virus. It terminates transcription and induces polyadenylation of transcripts (Reference 50).
Others		
RB	25	The right border terminal region sequence of nopaline type Ti plasmid derived from *Agrobacterium tumefaciens*.
LB	25	The left border terminal region sequence of octopine type Ti plasmid derived from *Agrobacterium tumefaciens.*

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

The *pat* gene transferred into the T45 is a modified type of wild *pat* gene obtained from *Streptomyces viridochromogenes* to adapt to the frequently-used codon in plants. The amino acid sequence of the enzyme which is produced by this modification remains unchanged (References 12, 42, and 74). Nucleotide sequences of wild *pat* gene and the modified *pat* gene are shown in Figure 1.

Figure 1 Comparison between the transferred *pat* gene in the T45 and the wild type *pat* gene

2) Function of component elements

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

Functions of component elements of donor nucleic acid that was used for the development of the T45 are shown in Table 1.

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

In the process of nitrogen metabolism, crops produce ammonia by nitrate reduction, amino acid degradation, photorespiration, and so on. Glutamate synthetase plays an important role in detoxification of the ammonia produced, though the glutamate synthetase is inhibited if crops are sprayed with glufosinate herbicide, ammonia accumulates, and the crops wither and die.

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

It is reported that the PAT protein is not toxic to humans and other animals, and it shows no significant homology except with PAT proteins derived from various other species as a result of search for any homology with amino acid sequence of all proteins registered in the GENBANK database (Reference 42). In addition, as a result of comparison of physico-chemical and biochemical characteristics of PAT protein with known allergen, it was observed that this protein has no possibility to possess allergenicity (Reference 75). Moreover, based on the nucleotide sequence and amino acid sequence of this protein, overall homology search (EMBL and Swiss-Prot) was conducted. As a result, it was confirmed that this protein shows no significant homology except with PAT proteins derived from various other species and that it has no homology with any known toxin or allergen.

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

The PAT protein exhibits a high affinity to glufosinate classified into *L*-amino acid, though it does not cause any acetyl group transfer to the other various amino acids and it has little affinity for the glutamic acid which has specifically high structural similarity and it causes virtually no transfer reaction in vivo (Reference 70). In addition, even in the presence of excessive amount of various amino acids, the acetyl group transfer reaction to glufosinate by PAT protein was never inhibited (References 42 and 77). As a result, it is considered that the PAT protein possesses high substrate specificity and it does not affect the metabolic system of the recipient organism.

(2) Information concerning vector

1) Name and origin

The vector used for the production of the T45 is pHOE4Ac (II), which is constructed based on the plasmid pUC18 derived from *E. coli* (Reference 30).

2) Properties

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

The total number of base pairs of pHOE4Ac(II) is 6,652bp. The entire nucleotide sequence of the vector is shown in Annex 1, and the physical map of the plasmid and the restriction enzyme cleavage site are shown in Figure 2.

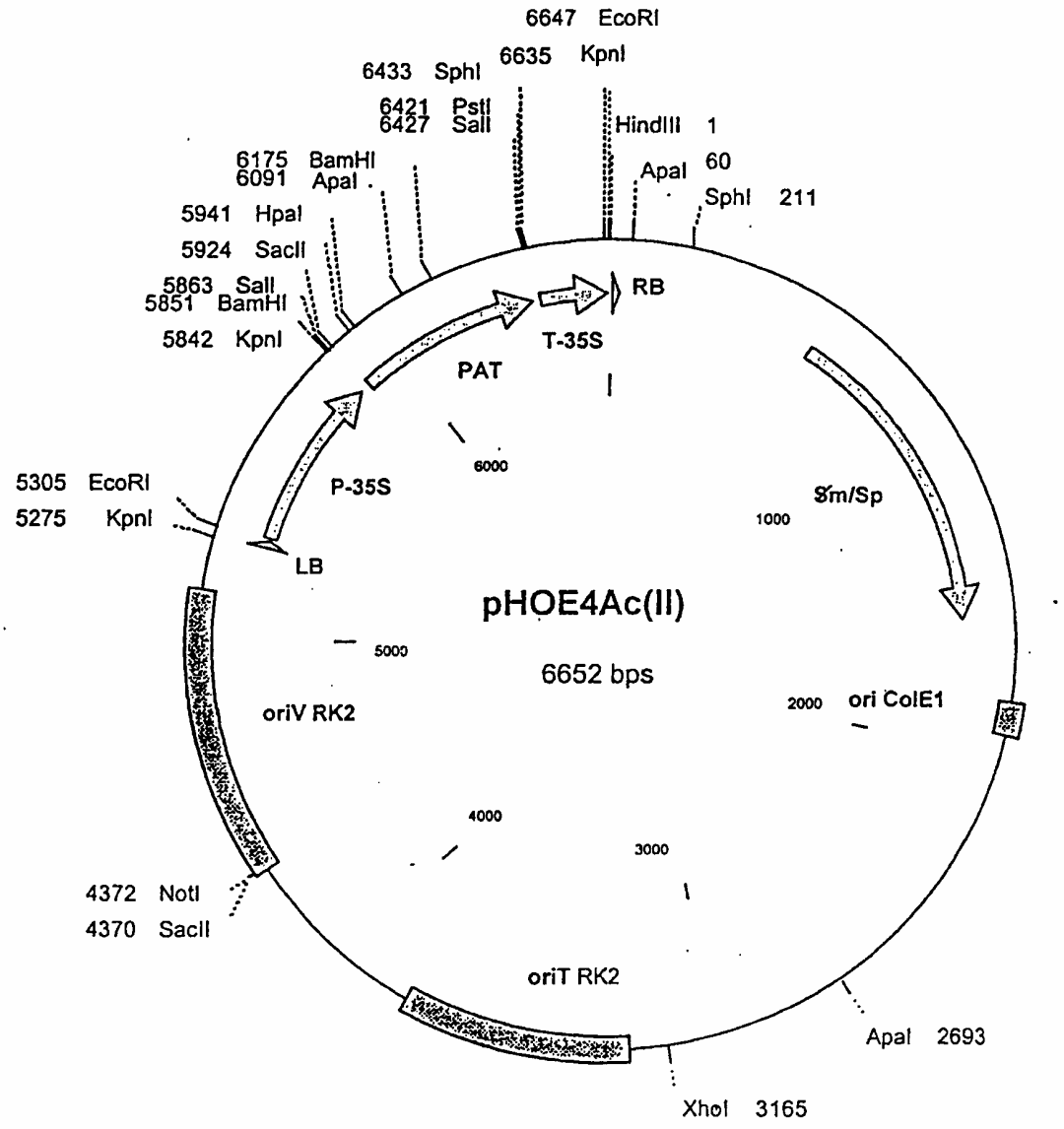

Figure 2 Physical map of plasmid pHOE4Ac(II) and the restriction enzyme cleavage site

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

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

The plasmid pHOE4Ac(II) possesses the streptomycin/spectinomycin tolerant gene (*Sm/Sp*) derived from the plasmid R538-1 of *E. coli* (Reference 19), the replication origin ori ColE1 derived from the plasmid piAN7 of *E. coli* (Reference 20), and the oriV and oriT derived from the plasmid RK2 of *E. coli* (Reference 13). The *Sm/Sp* gene was used as a selectable marker for the vector in *E. coli*. The ori ColE1, and the oriV and oriT have functions to cause autonomous replication in the *E. coli* and the *Agrobacterium*, respectively, though they do not work in any plant. In addition, it has been confirmed that these sequences are all located outside the T-DNA region and they are not transferred in the genome of oilseed rape (Annex 2, Figure 4).

(c) Presence or absence of infectious characteristics of vector and the information concerning the region of recipient organism if the infectivity of vector is found present

The range of recipient organisms for the autonomous replication of plasmid pHOE4Ac(II) is limited to *Escherichia coli* and a few gram-negative bacteria and thus, it is considered that the plasmid pHOE4Ac(II) does not possess the infectious characteristics in plant bodies.

(3) Method of preparing living modified organisms

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

In the recipient organism, the T-DNA region was transferred which is located between LB and RB on the pHOE4Ac(II) (Figure 2).

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

The *Agrobacterium* method was used for transferring the nucleic acid to the recipient organism. In the co-existence of *Escherichia coli* (*E. coli*) which possesses the helper plasmid pRK2013, the plasmid pHOE4Ac(II) transferred to *E. coli* DH1 was transferred to the *Agrobacterium tumefaciens* C58C1 Rif^R (EHA101) and then co-cultured with the protoplast of the recipient species for transformation.

3) Processes of rearing of living modified organisms

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

After transformation, the protoplast was cultured for 5 to 6 weeks, and the generated micro colonies were cultivated on the selective culture plate containing glufosinate. Then, the surviving colonies were transferred to a regeneration medium for regeneration of plant body.

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

After transformation by the *Agrobacterium*, 200 mg/L of carbenicillin and 200 mg/L of cefotaxime were added to the medium and thus the remaining *Agrobacterium* was removed.

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

Regenerated seedlings were planted in soil and the posterity plants were repeatedly self-pollinated and back-crossed for screening with glufosinate herbicide spraying and examination for the traits including the level of glufosinate herbicide tolerance and the contents of glucosinolate and erucic acid for selective breeding of elite lines, and the T45 was obtained. The commercial line of the T45 was produced from selective breeding of T3 or BC1F1 generation and its posterity. In addition, this application for approval is intended for the individuals segregated in the T1 generation to have the tolerance to glufosinate herbicide and their derived progeny.

The pedigree tree is shown in Figure 3. The approvals received from organizations in Japan are as follows.

[Food safety]

In May 1997, based on the “Guideline for food safety Assessment of food and food additives derived from Recombinant-DNA technology”, safety of use for food was approved by the Ministry of Health and Welfare (The Ministry of Health, Labour and Welfare, currently). In addition, along with legislating, passing through the “Procedures for food safety Assessment of food and food additives derived from Recombinant-DNA technology”, safety of use for food was approved by the Ministry of Health, Labour and Welfare in March 2001.

[Feed safety]

In June 1997, based on the “Guideline for feed safety Assessment of recombinant feed”, the compatibility to the guideline was confirmed by the Ministry of Agriculture, Forestry and Fisheries. In addition, along with legislating, passing through the “Procedures for feed safety Assessment of feed and feed additives derived from Recombinant-DNA technology”, safety of use for feed was approved by the Ministry of Agriculture, Forestry and Fisheries in March 2003.

[Environmental safety]

In 1995, based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", conducting isolated field test was approved by the Ministry of Agriculture, Forestry and Fisheries. In addition, in April 1997, based on the Guideline, the compatibility to the guideline regarding recombinant being imported to Japan (used for processing and feed) was confirmed by the Ministry of Agriculture, Forestry and Fisheries.

Confidential: Not made available or disclosed to unauthorized person

Figure 3 Pedigree tree of the T45

(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

It is expected that in the F1 obtained by crossing the recipient species AC Excel with the individual considered to be homozygote for the transferred gene locus among the T1 generation individuals obtained from self-fertilization of the original gene transformant (T0) of the T45, a segregation ratio of 3:1 would be obtained in theory between glufosinate-tolerant and glufosinate-sensitive individuals in the F2 generation obtained by self-fertilization of F1 under the control of single gene locus, while in the BC1F1 generation obtained by additional back-crossing of AC Excel with F1, the segregation ratio would become 1:1. In fact, as a result of examination on the segregation ratio between glufosinate-tolerant and glufosinate-sensitive individuals in the F2 and BC1F1 generations of the T45, it was confirmed that the segregation ratio showed a good agreement with the theoretical segregation ratio {Annex 3, Table 1 (Reference 29)}. Consequently, the replication products of transferred nucleic acid in the T45 are all considered to exist on the genome of oilseed rape at one site.

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

As a result of Southern blotting analysis for the genome DNA extracted from F5 generation of the T45, it was confirmed that one copy of T-DNA region was transferred (Annex 4, pp.15 - 17, Figures 2 to 4). In addition, as a result of sequence analysis for the transferred T-DNA in the T45, it was confirmed that the identical sequence as in the T-DNA region on the plasmid was transferred (Annex 5, pp.10 - 12). Composition of the entire nucleic acid transferred to the T45 is shown in Figure 4.

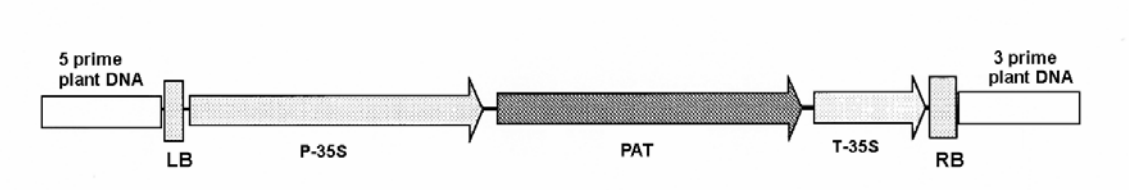

Figure 4 Genetic map of transferred genes

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

Moreover, as a result of Southern blotting analysis for the genome DNA of the T45 of the generations T2, F5 and F7, the identical band pattern was detected in all generations. As a result, it was confirmed that the replication products of transferred nucleic acid in the T45 are inherited stably through the multiple generations (Annex 6, Figure 2).

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

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

As a result of Northern blotting analysis for the RNA extracted from leaves, stems, roots and seeds of the T45 of F5 generation, the transcripts of the *pat* gene were detected in leaves, stems and roots, but not detected in seeds (detection limit: 12.5 pg/5μg total RNAs) (Annex 7, Figure 1).

In addition, as a result of determination of the content of PAT protein in leaves and seeds of the T45 of two generations based on the ELISA method, the PAT protein was detected in all the generations. As a result, it was confirmed that the *pat* gene is stably expressed (Annex 8, Tables 2 and 3).

Moreover, as a result of the special screened greenhouse test conducted in 2006, in which the seeds of the T45 and its next generation obtained from open pollination of the T45 were sown and the germinated seedlings were sprayed with glufosinate herbicide, the individuals in all the generations all exhibited the tolerance to glufosinate. Consequently, it was confirmed that this trait is stably expressed in the multiple generations (Annex 10, Table 21).

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

The T45 contains no DNA sequence which possesses transferring factor and therefore, it is considered unlikely that the transferred nucleic acid would be transmitted to wild animals and wild plants under a natural environment.

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

Specific identification of this event is available by PCR method using the flanking sequences of DNA transferred in the T45. This PCR method is utilized effectively for cultivation management of the T45 (Annex 13).

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

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

The T45 exhibits the trait to be tolerant to glufosinate herbicide due to the expression of the *pat* gene.

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

In FY 1996, the isolated field tests were conducted at the Hokkaido Agricultural Research Center of the Ministry of Agriculture, Forestry and Fisheries to make comparison between the T45 and the recipient species AC Excel (hereinafter referred to as "non-recombinant oilseed rape") (Annex 9). In addition, in 2006, in the special screened greenhouse in Japan, comparison was made between the T45 and the non-recombinant oilseed rape (Annex 10). For references of evaluation, results of comparison between the T45 and the non-recombinant oilseed rape conducted in Canada at two areas in 1995 were used (Annex 11).

(a) Morphological and growth characteristics

In isolated field tests, comparison was made between the T45 and the non-recombinant oilseed rape for the plant height, the number of primary branches, dry weight of aerial parts (stems and leaves), plant shape, color of leaves, time of bolting, flowering period, the number of days required for maturation, rate of pods formation, length of pod, the number of seed setting (seeds/pod), property of open pods and color of seed coat. As a result, for the plant height, the number of primary branches, leaf shape, color of leaves, time of bolting, the number of days required for maturation, rate of pods formation, property of open pods and color of seed coat, the T45 and the non-recombinant oilseed rape were found almost equivalent to each other. On the other hand, compared to the non-recombinant oilseed rape, the T45 showed 4-day delayed flowering period, 17 g lower weight of aerial parts (stems and leaves), 5 mm shorter length of pod, and 5-seed larger number of seed setting (seeds/pod) (Annex 9, Tables 1 and 2).

In addition, in the special screened greenhouse tests, examination was made on the uniformity of germination, time of bolting, time of flower initiation, flowering period, maturation period, plant height, the number of primary branches, plant shape, color of leaves, the number of pods formed, the number

of pods unformed, rate of pods formation, length of pod, the number of seeds per pod, color of seed coat, uniformity of the seeds and dry weight of aerial parts (stems and leaves). As a result, for the uniformity of germination, time of bolting, time of flower initiation, maturation period, plant shape, color of leaves and color of seed coat, the T45 and the non-recombinant oilseed rape were found almost equivalent to each other. The T45 showed one week earlier flowering period compared to the non-recombinant oilseed rape. For the plant height, the number of primary branches, the number of pods formed, the number of pods unformed, rate of pods formation, length of pod, the number of seed setting (seeds/pod), uniformity of the seeds and dry weight of aerial parts, there was no statistically significant difference between the T45 and the non-recombinant oilseed rape (Annex 10, Tables 2 and 3).

As mentioned above, the T45 showed 4-day delayed flowering period in the isolated field tests, while one week earlier in the special screened greenhouse tests, and there was no fixed tendency observed. In addition, for the weight of stems and leaves, length of pod and the number of seed setting (seeds/pod), a difference was observed in the isolated field tests, though in the special screened greenhouse tests, in all the items examined, no statistically significant difference was observed between the T45 and the non-recombinant oilseed rape.

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

In order to evaluate the heat-tolerance at the early stage of growth, the seedlings of the T45 and the non-recombinant oilseed rape at one week after germination were raised under the conditions (35℃ and 12-hour day length and 12-hour night length) and the survival rate was examined one month later. As a result, it was confirmed that all the individuals were found completely dead at the time of examination (Annex 10, Table 20). Therefore, it is considered that the T45 exhibits no heat-tolerance at the early stage of growth similarly as the non-recombinant oilseed rape.

The oilseed rape varieties sown in autumn in Japan are generally known to grow even in winter in both warm and cold districts, though the rate of growth varies (Reference 62).

(c) Wintering ability and summer survival of the matured plant

As a result of observation for the summer survival in the isolated field tests, no difference was observed between the T45 and the non-recombinant oilseed rape that had been sown in spring. In addition, at the time of harvesting in September, the both lines were found similarly turned yellow and showing the sign of withering (Annex 9).

(d) Fertility and size of the pollen

In the special screened greenhouse, pollens were collected from the T45 and the non-recombinant oilseed rape, and stained with an acetocarmine solution and observed under a microscope. As a result, 100% of the pollens from the

both lines were found stained, showing the equivalent fertility of the pollens (Annex 10). In addition, as a result of comparison of size of pollen, no statistically significant difference was observed between the T45 and the non-recombinant control oilseed rape (Annex 10, Table 5).

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

Regarding seed production, comparison was made in the isolated field tests for the seed yield and 1000-seed weight between the T45 and the non-recombinant oilseed rape. As a result, the seed yield was found 19.2 g for the T45 and 16.3 g for the non-recombinant oilseed rape, showing about 3 g heavier value in the T45, and the 1000-seed weight was found 2.00 g for the T45 and 1.86 g for the non-recombinant oilseed rape, showing a 0.14 g higher value in the T45 (Annex 9, Table 2).

In addition, also in the special screened greenhouse tests, comparison was made for the seed yield and 1000-seed weight. As a result, no statistically significant difference was observed between the T45 and the non-recombinant oilseed rape (Annex 10, Table 3).

As mentioned above, in the isolated field tests, a difference was observed between the T45 and the non-recombinant oilseed rape regarding the seed yield and 1000-seed weight, though the difference is judged as insignificant for the varietal characteristics of oilseed rape (References 39 and 73). Moreover, in the special screened greenhouse tests, no statistically significant difference was observed in all the items examined. Based on the above understanding, it is considered that there is no difference in the production of the seed between the T45 and the non-recombinant oilseed rape.

In the examination conducted in Canada in two areas in 1995, comparison was made for the seed yield (g/m^2) between the T45 and the non-recombinant oilseed rape. As a result, in one area, the T45 showed a higher value (Annex 11, Table 1), though in the other area, the both lines showed almost same values (Annex 11, Table 2). In addition, for the 1000-seed weight, in the both areas, almost no difference was observed (Annex 11, Tables 1 and 2).

For the property of open pods, comparison was made between the T45 and the non-recombinant oilseed rape based on the five-scale evaluation (1 for difficult opening of pods to 5 for easy opening of pods) and as a result, the both lines showed the relatively easy property of open pods (Scale 4) and no difference was observed between the both plants (Annex 9, Table 2).

To evaluate the germination rate, 50 seeds each obtained from the T45 and the non-recombinant oilseed rape, which were cultivated and harvested in the special screened greenhouse, were sown. As a result, one week after sowing, the germination rate was found 98% (49/50 seeds) for the both plants. Therefore, the T45 and the non-recombinant oilseed rape are considered not to possess any dormancy (Annex 10, Table 21).

(f) Crossability

Crossability tests were not performed for this recombinant oilseed rape. However, the T45 and the non-recombinant oilseed rape are found equivalent to each other in terms of the fertility of the pollen (Annex 10), and no statistically significant difference has been observed regarding the size of the pollen (Annex 10, Table 4). In addition, as mentioned in (e) Production, shedding habit, dormancy and germination rate of the seed, the T45 is considered not to differ from the non-recombinant oilseed rape in the production of the seed, therefore also regarding the crossability showing an ability of receiving pollens, it is considered that there is no difference between the both plants. Consequently, it is estimated that there is no difference in crossability between the T45 and the non-recombinant oilseed rape.

(g) Productivity of harmful substances

In order to check whether the substances are excreted from the roots of the T45 which can affect other plants, exist in the plant body which can affect other plants after dying, and are excreted from the roots which can affect microorganisms in soil, succeeding crop test, plow-in test and soil microflora test were carried out respectively in the special screened greenhouse.

Succeeding crop test： After cultivating the T45 and the non-recombinant control oilseed rape in the Wagner pots for about 5 months, radishes were cultivated as test plants in the remaining soil respectively, and the comparison was made for germination rate, plant height, root length, fresh weight and dry weight of radishes. As a result, in all the items examined, no statistically significant difference was observed (Annex 10, Tables 7, 9, and 11). Therefore, it is considered that the T45 has not acquired any productivity of the substances excreted from the roots which can affect other plants.

Plow-in test: The dried powder of plant body of the T45 and the non-recombinant control oilseed rape was mixed with soil (1%), respectively, and seeds of radish were sowed in the soil and cultivated. Then the comparison was made for germination rate, plant height, root length, fresh weight and dry weight. As a result, there was no significant difference confirmed in any items (Annex 10, Tables 13, 15 and 17). Therefore, it is considered that the T45 has not acquired any productivity of the substances which can affect other plants after dying.

Soil microflora test: The soil was obtained after cultivating the T45 and the non-recombinant control oilseed rape in the Wagner pots for about 5 months, and was diluted by adding sterilized phosphate buffer solution. Bacteria and Actinomyces were incubated in PTYG medium, and mold fungi were incubated in Rose Bengal medium, and the comparison was made for the number of each microorganisms. As a result, there was no significant difference confirmed in any items (Annex 10, Tables 19). Therefore, it is considered that the T45 has not acquired any productivity of the substances excreted from the roots which can affect microorganisms in soil.

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

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

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

(1) Competitiveness

Oilseed rape (*Brassica napus* L.) to which the recipient organism belongs is reportedly growing on river beaches, along railroad tracks, in the surroundings of seed off-loading harbors, and in other such areas. It is generally known that oilseed rape would be eventually replaced with perennial plants and shrub in the environmental conditions without any regular disturbance such as roadsides, cliffs and riverside areas.

This recombinant oilseed rape is given a trait to be tolerant to glufosinate herbicide, though it is generally considered that the glufosinate does not exert selective pressure under a natural environment. Therefore, it is considered unlikely that the trait can cause the recombinant oilseed rape to become competitive under a natural environment.

In the isolated field and special screened greenhouse in Japan, the traits relating to the competitiveness of this recombinant oilseed rape were examined based on the comparison with the non-recombinant control oilseed rape (AC Excel). As a result, in the isolated field tests, a difference from the control was observed in the flowering period, weight of stems and leaves, length of pod, the number of seeds per pod, seed yield and 1000-seed weight among the items examined. For the flowering period, the recombinant oilseed rape showed a 4-day later value in the isolated field tests, while one week earlier value in the special screened greenhouse. In addition, for the weight of stems and leaves, the recombinant oilseed rape showed a lighter value compared to the non-recombinant control oilseed rape, and the difference observed in the length of pod and the number of seeds per pod was found falling within the varietal characteristics of oilseed rape. Moreover, the difference observed in the seed yield and 1000-seed weight was judged as insignificant for the varietal characteristics of oilseed rape. For the items in which a difference was observed from the non-recombinant control oilseed rape in the isolated field tests except the flowering period, no statistically significant difference was observed in the special screened greenhouse tests. In addition, in the other items examined, there was no morphological and growth characteristics observed that could cause the recombinant oilseed rape to become competitive. Consequently, it is considered unlikely that this recombinant oilseed rape could become competitive.

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

(2) Productivity of harmful substances

Conventional oilseed rape is known to produce erucic acid and glucosinolate in the seeds recognized as harmful substances to human and other mammals. The cultivar of the recipient organism of this recombinant oilseed rape is one of the canola cultivar, in which the erucic acid and glucosinolate content was reduced by selective breeding. In the componential analysis of this recombinant oilseed rape, it was confirmed that the erucic acid and glucosinolate content falls within the ranges for several commercial canola cultivars.

This recombinant oilseed rape produces the PAT protein which confers the trait to be tolerant to glufosinate. There is no report that the PAT protein possesses adverse effect on wild animals and wild plants. In addition, it is suggested that the PAT protein possesses high substrate specificity. Therefore, it is considered that the PAT protein does not affect the metabolic system of the recipient organism. Moreover, for the amino acid sequence of PAT protein, homology search was conducted and as a result, no homology with any known allergen was observed.

In order to check the productivity of harmful substances of this recombinant oilseed rape (the substances excreted from the roots which can affect other plants, the substances excreted from the roots which can affect microorganisms in soil, and the substances existing in the plant body which can affect other plants after dying), succeeding crop test, soil microflora test and plow-in test were conducted in the special screened greenhouse in Japan. As a result, in all the tests conducted, no significant difference was observed in comparison with the test plots for the non-recombinant control oilseed rape.

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

(3) Crossability

In a natural environment in Japan, a number of plants of the family *Brassicaceae* are growing, though known species that can be crossed with oilseed rape (*Brassica napus*) include *B. rapa* (turnip, Komatsuna, conventional rapeseed, etc.) of the genus *Brassica*; *B. juncea* (mustard, leaf mustard, etc.); *B. nigra* (black mustard) and *Raphanus raphanistrum* (wild radish) in addition to oilseed rape itself. However, they are all regarded as foreign species and are not specified as wild species as to be possibly affected.

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

(4) Additional information

The possible indirect Adverse Effect on Biological Diversity attributable to crossing of recombinant oilseed rape with the non-recombinant oilseed rape and the related species described on the above was evaluated. The possible indirect Adverse Effect on Biological Diversity refers to that; i) hybrid produced by crossing would become competitive and exterminate species population of the other wild animals and wild plants, and ii) related species population would decrease due to the effect of transferred gene spread by crossing, and wild animals and wild plants such as insects which are dependent on the related species would be affected for maintenance of their population.

1) Crossability of this recombinant oilseed rape with the non-recombinant oilseed rape, *B. rapa*, *B. juncea*, *B. nigra* or *R. raphanistrum* was not examined, though this recombinant oilseed rape is considered not to differ from the non-recombinant oilseed rape in the crossability as a receiver of pollens due to the findings listed below:

   a. The fertility of pollens is found equivalent and also regarding the size of pollens, no statistically significant difference is observed.

   b. It is considered that there is no difference in the production of the seed.

2) Regarding the crossability with related species, it is reported that it would be hard to produce hybrid, and even if hybrid is produced, the progeny would possess low fertility. Consequently, it is judged that the possibility of hybrid obtained by crossing would become competitive and exterminate the population of the other wild animals and wild plants is extremely low.

   In addition, it has been confirmed that this recombinant oilseed rape does not differ from the non-recombinant oilseed rape regarding the competitiveness, the productivity of harmful substances and the crossability and thus, it is considered unlikely that the transferred gene to this recombinant oilseed rape would affect the maintenance of the related species population.

   Based on the above understanding, it was judged that the conclusion by the applicant that there is no risk of indirect 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, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this recombinant oilseed rape in accordance with Type I Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion above made by the applicant is reasonable.

Reference

Confidential: Not made available or disclosed to unauthorized person

Annex List

Annex 1: Nucleotide sequence of vector pHOE4Ac (II)

Confidential: Not made available or disclosed to unauthorized person

Annex 2: Identification of vector sequences outside the T-DNA region in T45

Confidential: Not made available or disclosed to unauthorized person

Annex 3: Genetic characterization of glufosinate-ammonium tolerant summer rape lines (Kumar et al., 1998)

Confidential: Not made available or disclosed to unauthorized person

Annex 4: Characteristics of transferred gene in T45

Confidential: Not made available or disclosed to unauthorized person

Annex 5: Determination of transferred gene in T45 based on sequence analysis

Confidential: Not made available or disclosed to unauthorized person

Annex 6: Verification of stability of transferred genes across generations

Confidential: Not made available or disclosed to unauthorized person

Annex 7: Analysis on the expression of transferred gene

Confidential: Not made available or disclosed to unauthorized person

Annex 8: Detection of PAT protein

Confidential: Not made available or disclosed to unauthorized person

Annex 9: Isolated field test report in 1996

Confidential: Not made available or disclosed to unauthorized person

Annex 10: Report on the tests in the special screened greenhouse in 2006

Confidential: Not made available or disclosed to unauthorized person

Annex 11: Results of the cultivation tests in Canada

Confidential: Not made available or disclosed to unauthorized person

Annex 12: Fatty acid profile and glucosinolate contents

Confidential: Not made available or disclosed to unauthorized person

Annex 13: Event Identifying Method

Confidential: Not made available or disclosed to unauthorized person