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

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Marunouchi Chiyoda-ku, Tokyo

Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Oilseed rape tolerant to glufosinate herbicide (*pat*, *Brassica napus* L.) (Topas 19/2, OECD UI :ACS-BNØØ7-1)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

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

1. Information concerning preparation of living modified organisms

(1) Information the concerning donor nucleic acid

i) 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.,) (hereinafter referred to as "the recombinant oilseed rape Topas 19/2") and the origins of component elements are shown in Table 1.

Table 1 , Sizes, 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 45).
*pat**	552	Derived from *Streptomyces viridochromogenes*, encoding the enzyme phosphinothricin acetyl transferase (PAT) and conferring the glufosinate tolerance (Reference 21). This gene is a modified type of wild *pat* gene to adapt to the frequently-used codon in plants.
T-35S	220	35S RNA terminator derived from Cauliflower Mosaic Virus. It terminates transcription and induces polyadenylation of transcripts (Reference 56).
npt II gene expression cassette		
P-nos	293	Nopaline synthase gene promoter derived from *Agrobacterium tumefaciens*, permitting the transcription of *npt II* gene in plants (Reference 17).
npt II	795	A gene derived from *Escherichia coli* transposon Tn5, encoding the neomycin phosphotransferase Ⅱ (NPT Ⅱ) and conferring the resistance to aminoglycoside antibiotics including kanamycin and neomycin (Reference 4).
T-ocs	612	3'-terminal regulated region of octopine synthase gene derived from *Agrobacterium tumefaciens*, terminating transcription and inducing polyadenylation of transcripts (Reference 16).
Others		
RB	25	The right border repeated sequence of T-DNA derived from *Agrobacterium tumefaciens*.
LB	25	The left border repeated sequence of T-DNA 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 recombinant oilseed rape Topas 19/2 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 21, 47, and 82). Nucleotide sequences of wild *pat* gene and the modified *pat* gene are shown in Figure 1.

Figure 1 Comparison of nucleotide sequences between wild *pat* gene and the modified *pat* gene transferred into the recombinant oilseed rape Topas 19/2

ii) 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 recombinant oilseed rape Topas 19/2 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

[PAT protein]

In the process of nitrogen metabolism, crops produce ammonia by nitrate reduction, amino acid degradation, photorespiration, and so on. Glutamate synthase plays an important role in detoxification of the ammonia produced, though the glutamate synthase 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 synthase by the glufosinate, ammonia does not accumulate in the plant body, and the crop does not die even if it is sprayed with glufosinate (Figure 2).

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 47). 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 83).

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, this protein did not show any homology with known allergens.

[NPT II protein]

The *npt II* gene produces neomycin phosphotransferase (NPT II) which phosphorylates the 3'-hydroxyl group of amino-hexose portion of aminoglycoside antibiotics including neomycin and kanamycin in the presence of ATP (Reference 14). Phosphorylated neomycin or kanamycin is prevented from introduction and binding to bacterial ribosome and as a result, the cells exhibit the resistance. This permits selection of transformed cells on the medium containing neomycin or kanamycin.

The NPT II protein catalyzes the phosphorylation reaction specifically with aminoglycoside antibiotics such as neomycin and kanamycin (References 14, 15, and 57). In addition, in order to identify the allergenicity of this protein, search for homology with known allergens in the databases Swiss-Prot, PIR and HIV-AA was conducted and as a result, no homology with any of the known allergens examined was observed.

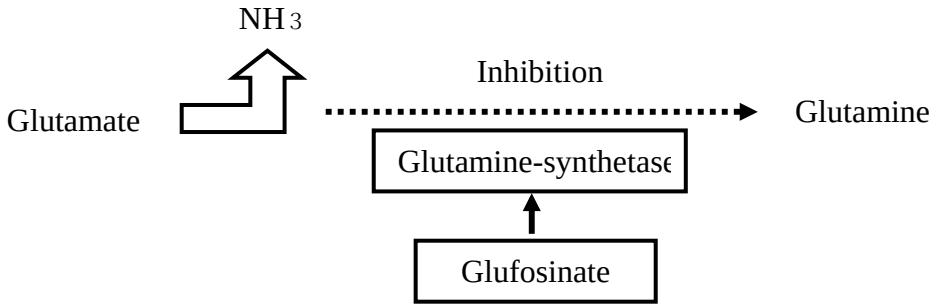
(c) Contents of any change caused to the metabolic system of recipient organism

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 78). 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 (Reference 85). 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.

The NPT II protein possesses high substrate specificity (References 14, 15, and 57) and thus it is considered not to catalyze phosphrylation to any substance other than aminoglycoside antibiotics including neomycin, kanamycin and so on. In addition, aminoglycoside antibiotics are not present in any plants. Therefore, this protein is considered not to affect the metabolic pathway of the recipient organism.

A) Normal Plant

Since glufosinate herbicide inhibits glutamine synthetase, ammonia accumulates in the plant body, causing the plant to die.



B) Recombinant Plant

Glufosinate herbicide is acetylated and becomes N-acetylglufosinate by action of the PAT protein, and the inhibition of the glutamine-synthetase does not occur. Therefore, ammonia does not accumulate in the plant body, and the plant can grow.

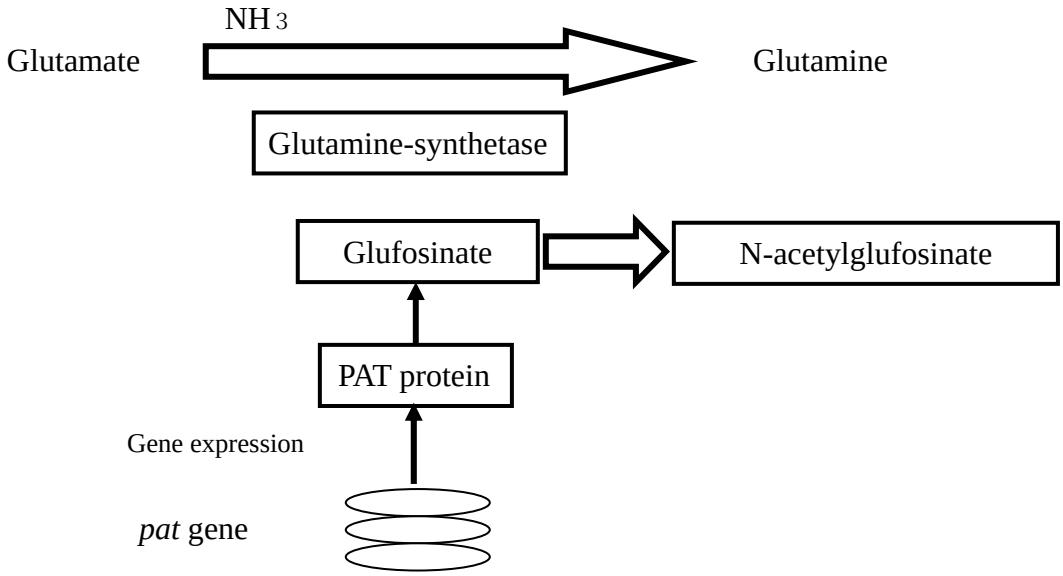


Figure 2 Mechanism of tolerance to glufosinate herbicide by the product of *pat* gene

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

(2) Information concerning vector

i) Name and origin

The vector used for the production of the recombinant oilseed rape Topas 19/2 is the vector pOCA18/Ac, which is derived from *Escherichia coli* plasmid pRK290 (Reference 51).

ii) Properties

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

The total number of base pairs of the vector pOCA18/Ac is 24,285bp. In addition, the genes present in this vector have been all clarified.

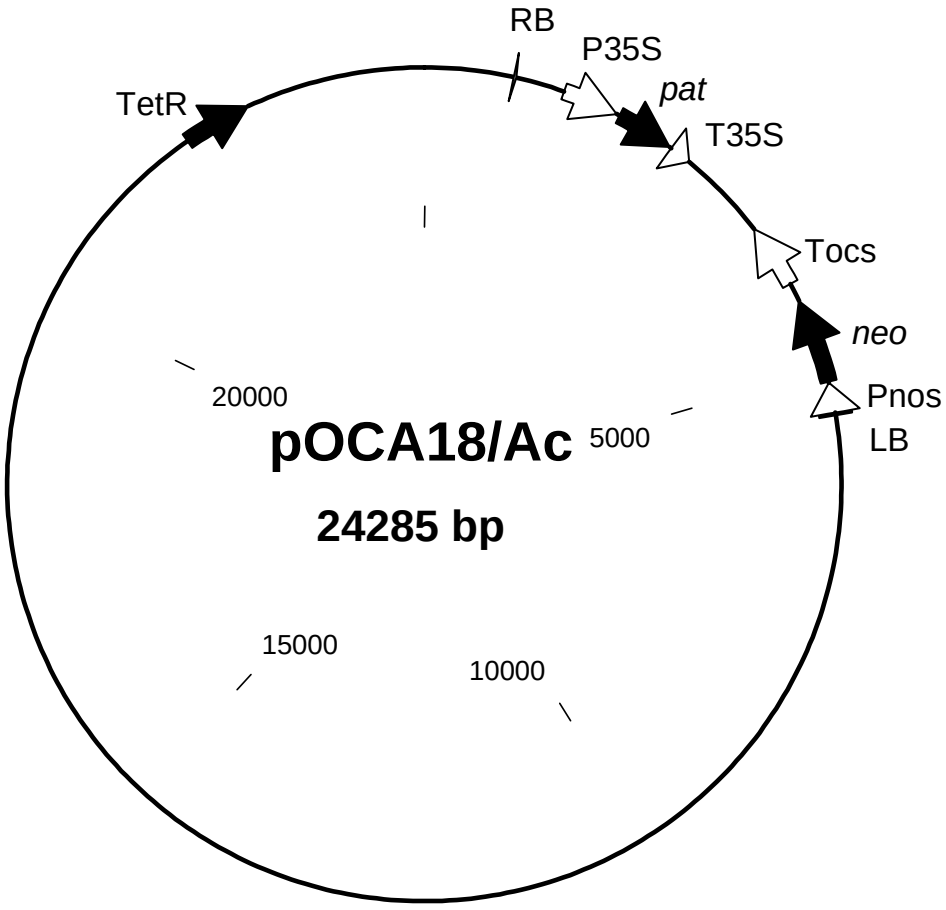


Figure 3 Vector pOCA18/Ac

The "*neo*" refers to the *npt II* gene.

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

The vector pOCA18/Ac possesses the tetracycline resistant gene (TetR) derived from pRK290 as a selective marker in *E. coli* outside the T-DNA region. However, this vector is under the control of a promoter derived from *E. coli* and thus, it only expresses in the procaryotic cell and does not express in any plant cells. In addition, it has been confirmed that this gene is not transferred into any plant cells (Annex 9).

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

The vector pOCA18/Ac does not replicate itself autonomously in plant cells and thus it does not possess any infectious characteristics.

(3) Method of preparing living modified organisms

i) 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 RB and LB on the vector pOCA18/Ac (Figure 3). The nucleotide sequence of the T-DNA region is shown in Annex 1.

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

The *Agrobacterium* method was used for transferring the nucleic acid to the recipient organism. *E.coli* DH1 to which the plasmid pOCA18/Ac was transferred and the *Agrobacterium tumefasiens* C58C1 Rif^R (EHA101) were co-cultured. Then *E. coli* was removed, and the *Agrobacterium tumefasiens* C58C1 strain and the dedifferentiated culture cells derived from immature pollens of Topas 4079 were co-cultured for transformation.

iii) Processes of rearing of living modified organisms

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

After transformation, callus derived from microspore was transferred to a selection plate containing kanamycin for incubation. Then the callus was additionally transferred to a regeneration medium containing glufosinate for regeneration of plant body, and clone collection was conducted several times.

(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

Plant body was regenerated and treated with colchicine to double the chromosome number. Then the seedlings were planted in soil and the posterity plants were examined for the level of glufosinate herbicide tolerance (glufosinate spraying test) and the contents of the harmful biological active substances glucosinolate and erucic acid for selective breeding of elite lines, and the recombinant oilseed rape Topas 19/2 was obtained. The pedigree tree of the recombinant oilseed rape Topas 19/2 is shown in Figure 4.

The approvals of the recombinant oilseed rape Topas 19/2 received from organizations in Japan are as follows.

[Environmental safety]

In 1995, based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries," the compatibility to the guideline regarding the isolated field test on the recombinant oilseed rape (HCN92) was confirmed by the Ministry of Agriculture, Forestry and Fisheries. In addition, in May 1996, the compatibility to the guideline regarding the recombinant oilseed rape (HCN92) being imported to Japan as glufosinate herbicide tolerant (used for processing and feed) was confirmed by the Ministry of Agriculture, Forestry and Fisheries.

[Feed safety]

In September 1996, based on the "Guideline for feed safety assessment of recombinant feed," the compatibility to the guideline regarding the glufosinate herbicide tolerant oilseed rape HCN92 was confirmed by the Ministry of Agriculture, Forestry and Fisheries. In addition, in January 1998, based on the guideline, the compatibility to the guideline regarding the glufosinate herbicide tolerant oilseed rape HCN10 was confirmed by the Ministry. Moreover, along with legislating, passing through the "Procedures for feed safety assessment of feed and feed additives derived from recombinant-DNA technology," safety of use of glufosinate herbicide tolerant canola (HCN92) and glufosinate herbicide tolerant canola (HCN10) for feed was approved by the Ministry of Agriculture, Forestry and Fisheries in March 2003.

[Food safety]

In September 1996 and December 1997, based on the "Guideline for food safety assessment for food and food additives derived from recombinant-DNA technology," safety of use of glufosinate herbicide tolerant oilseed rape HCN92 and glufosinate herbicide tolerant oilseed rape HCN10 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 of HCN92 and HCN10 for food was approved by the Ministry of Health, Labour and Welfare in March 2001.

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Figure 4 Pedigree tree of the recombinant oilseed rape Topas 19/2

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

i) Place where the replication product of transferred nucleic acid exists

As a result of examination for the segregation ratio between glufosinate-tolerant and glufosinate-sensitive individuals of recombinant oilseed rape Topas 19/2, the F1 individuals all showed glufosinate tolerance, and the F2 individuals were segregated at a ratio of 3-to-1 the number of glufosinate tolerant individuals-to-the number of glufosinate sensitive individuals. In addition, the BC1F1, BC2F1 and BC3F1 generations all showed a segregation ratio of 1:1 (Annex 2). These results show a good agreement with typical segregation pattern of single dominant inheritance and then, it is considered that the T-DNA region transferred into the recombinant oilseed rape Topas 19/2 exists in a chromosome of the oilseed rape genome.

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

In order to identify the number of copies of transferred T-DNA region, the Southern blot analysis was conducted with the genome DNA of the recombinant oilseed rape Topas 19/2 (Annex 1). As a result, it was confirmed that two (2) copies of T-DNA region were transferred in the recombinant oilseed rape Topas 19/2 and that the *npt II* region in one of the two copies was partly deleted. In addition, it was also confirmed that the 2 copies of T-DNA region are arranged in the inverted orientation as shown in Figure 5.

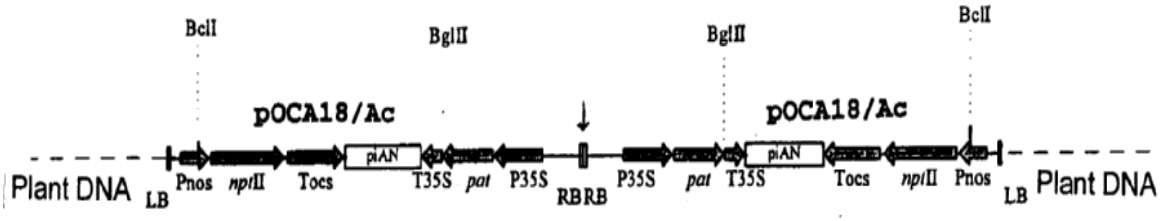


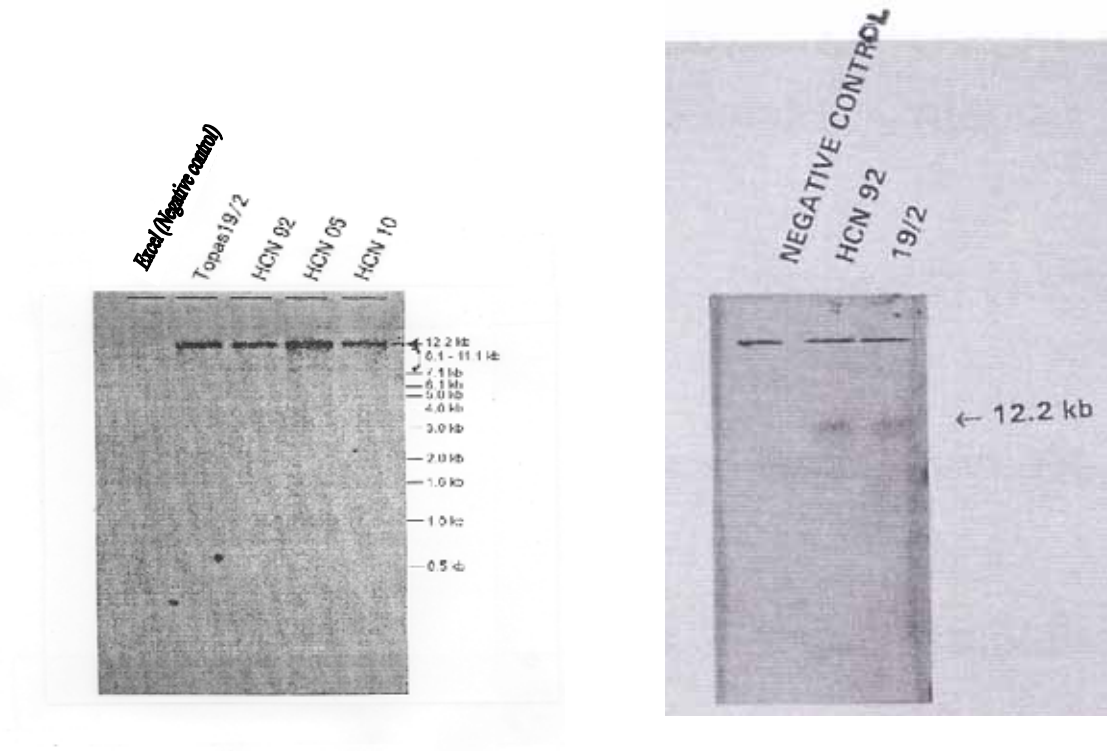
Figure 5 Genetic map of transferred genes

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

Moreover, the Southern blot analysis was additionally conducted for the genome DNA extracted from three individuals of hybrid offspring of the recombinant oilseed rape Topas 19/2, obtained by crossing with different varieties respectively and treated with restriction enzyme, using the radiolabeled *pat* gene as a probe. As a result, the single band of 12.2kbp was observed in all the individuals (Figure 6a).

Furthermore, the Southern blot analysis was conducted for the genome DNA extracted from the inbred offspring and the hybrid offspring of the recombinant oilseed rape Topas 19/2, treated with restriction enzyme, using the radiolabeled *npt II* gene as a probe. As a result, the band of 12.2kb was observed (Figure 6b).

Based on the above results, it was confirmed that the *pat* gene and the *npt II* gene are both inherited stably through the multiple generations.



a. *pat* probe

b. *npt II* probe

Figure 6 Southern blot analysis to identify the stability 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.)

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

As mentioned above, it was confirmed that 2 copies of T-DNA region are transferred adjacent to each other in the inverted orientation in the recombinant oilseed rape Topas 19/2.

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

[PAT protein]

In 2005, in the special screened greenhouse in Japan, test seeds (F2) and the next generation seeds (F3) were sown in conjunction with the control variety of recipient organisms Topas 4079 (hereinafter referred to as “the non-recombinant control oilseed rape”), and the seedlings germinated were sprayed with glufosinate herbicide. As a result, it was confirmed that, for the non-recombinant control oilseed rape, all the individuals in all the generations examined exhibited the glufosinate sensitivity, whereas for the recombinant oilseed rape Topas 19/2, all the individuals exhibited the glufosinate tolerance (Annex 6). Therefore, it is considered that the transferred genes are stably expressed across the individuals and generations.

In addition, the phosphinothricin acetyl transferase enzyme activity shown by the PAT protein was measured for the leaves, stems, flower buds, roots and seeds of the inbred offspring of the recombinant oilseed rape Topas 19/2 and the HCN92 and also the honey and pollens collected from the honeybees visiting the HCN92 for collection of nectar. As a result, the activity was observed in the leaves, roots, flower buds and seeds of the inbred offspring of the recombinant oilseed rape Topas 19/2 and the HCN92, though the activity was not detected in the stems (detection limit of 10 ng PAT/mg total protein). On the other hand, the activity was not observed in the honey and pollens. In all the negative controls, the activity was not detected (Annex 3, Table 1).

[NPT II protein]

The NPT II acitivity was measured based on the dot blotting method for the leaves, stems, flower buds, roots and seeds of the inbred offspring of the recombinant oilseed rape Topas 19/2 and the HCN92 and also the honey and pollens collected from the honeybees visiting the HCN92 for collection of nectar. As a result, the activity was observed in the leaves, stems, flower buds, roots and seeds of the inbred offspring of the recombinant oilseed rape Topas 19/2 and the HCN92. On the other hand, the activity was not observed in the honey and pollens (detection limit 25 ng NPTⅡ/mg total protein). In all the negative controls, the activity was not detected in any of the sites (Annex 4, Table 1).

v) 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 recombinant oilseed rape Topas 19/2 contains no DNA sequence which possesses transferring factor and therefore, there is no possibility of transmission of nucleic acid transferred 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 detection method for the recombinant oilseed rape Topas 19/2 is available by the PCR using its flanking sequences of transferred DNA as primers. In addition, high-sensitivity identification is available with use of 20 to 50 ng DNA. This PCR is utilized effectively for cultivation management of the recombinant oilseed rape Topas 19/2 in general (Annex 8).

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

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

With the expression of the *pat gene*, the recombinant oilseed rape Topas 19/2 exhibits tolerance to glufosinate herbicide.. In addition, the *npt II* gene confers resistance to aminoglycoside antibiotics including neomycin,kanamycin and so on.

ii) 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 1995, the isolated field tests were conducted at the Hokkaido Agricultural Research Center to compare morphological and growth characteristics, summer survival of matured plant, and production, shedding habit and germination rate of the seed between the HCN92, hybrid offspring of the recombinant oilseed rape Topas 19/2, and the controls, Drakkar, a commercial variety of oilseed rape sown in spring similarly as the recipient organism Topas 4079 (hereinafter referred to as “Drakkar”), and Karafuto, which has been cultivated in Hokkaido as a spring sowing variety (hereinafter referred to as “Karafuto”) (Annex 5). In addition, in 2006, in the special screened greenhouse in Japan, regarding the heat-tolerance of young seedlings, fertility and size of the pollen, dormancy of the seed, and productivity of harmful substances, comparison was made between the recombinant oilseed rape Topas 19/2 (F2) and the non-recombinant control oilseed rape (Annex 6). Crossability was examined based on the comparison between the oilseed rape Karafuto and the *B.juncea* (Cutlass and leaf mustard) in the above mentioned isolated field tests (Annex 5), and between *B.rapa* and *R.raphanistrum* in 1994 in Canada (Annex 7).

(a) Morphological and growth characteristics

The comparison was made between the recombinant oilseed rape Topas 19/2 and non-recombinant control oilseed rape, Drakkar and Karafuto, for the uniformity of germination, plant height, the number of primary branches, dry weight of aerial part (stems and leaves), plant shape, color of leaves, time of bolting, flowering period, maturation period, rate of pods formation, length of pod, the number of seeds per pod, color of seed and shape of seed.

As a result, the uniformity of germination was found on the same day. The time of bolting, flowering period and maturation period were all found 2 to 6 days earlier for the recombinant oilseed rape Topas 19/2 than Drakkar and Karafuto. The plant height was found shorter for the recombinant oil seed rape Topas 19/2 (101 cm) than Drakkar (136 cm) and Karafuto (126 cm), though the weight of stems and leaves for the recombinant oilseed rape Topas 19/2 (284 g) was between Drakkar (332 g) and Karafuto (229 g). For the color of leaves, the recombinant oilseed rape Topas 19/2 was found equivalent to Karafuto. The length of pod was shorter for the recombinant oilseed rape Topas 19/2 (69.8 mm) than Drakkar (92.0 mm) and Karafuto (77.3 mm), though the number of seed setting per pod was larger for the recombinant oilseed rape Topas 19/2 (23.5) than Drakkar (20.3) and Karafuto (15.7). On the other hand, the number of primary branches, plant shape, rate of pods formation, color of seed and shape of seed were found equivalent between the recombinant oilseed rape Topas 19/2 and Drakkar (Annex 5, Tables 1, 2 and 3).

The number of seed setting per pod was larger for the recombinant oilseed rape Topas 19/2 (23.5) than Drakkar and Karafuto, though it is considered to fall within the variable ranges for the varietal characteristics since there are some varieties including the Japanese canola Bansai (24) and Chikusi (25) which show the larger number of seed setting per pod (Reference 81). Consequently, it is considered unlikely that the differences observed in the above results cause the recombinant oilseed rape Topas 19/2 to become competitive.

(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 plants of the recombinant oilseed rape Topas 19/2 and the non-recombinant control oilseed rape at one week after germination were raised under the conditions (35℃ and 12-hours day length and 12-hours night length). As a result, it was confirmed 6 weeks later that all the individuals had died (Annex 6). Therefore, it is considered that neither of the plants exhibits heat-tolerance at the early stage of growth.

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 70).

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

As a result of observation for the summer survival in the isolated fields, no difference was observed between the recombinant oilseed rape Topas 19/2 and the other varieties examined (Annex 5).

It is generally known that oilseed rape shows high cold tolerance and high snow endurance (Reference 70).

(d) Fertility and size of the pollen

Pollens were collected from the recombinant oilseed rape Topas 19/2 and the non-recombinant control oilseed rape cultivated in the special screened greenhouse, and stained with acetocarmine solution and observed under a microscope. As a result, 99% of the pollens from the both plants were found stained, showing a high fertility of the pollens. In addition, as a result of comparison of size of pollen, no statistically significant difference was observed between the recombinant oilseed rape Topas 19/2 and the non-recombinant control oilseed rape (Annex 6, pp.17 - 18).

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

The recombinant oilseed rape Topas 19/2 showed smaller seed yield per plant (46.0 g) than Drakkar (50.2 g) and Karafuto (49.4 g) and smaller 1,000-seeds weight (2.11 g) than Drakkar (2.48 g) and Karafuto (3.00 g) (Annex 5, Table 2). From the results, the recombinant oilseed rape Topas 19/2 is found to provide the number of seeds per plant (2.18×10^4 seeds), near 10% higher than Drakkar (2.02×10^4 seeds) and 30% or so higher than Karafuto (1.65×10^4 seeds). However, varieties like as Yokkaichi Black and Korean are reported to produce 2.50×10^4 or more seeds (Reference 81). Consequently, the number of seeds per plant for the recombinant oilseed rape Topas 19/2 is considered to fall within the variable ranges for the varietal characteristics.

Regarding the shedding habit, comparison was made for the rate of open pods formed and as a result, the recombinant oilseed rape Topas 19/2 was found relatively difficult to open pods compared to Drakkar (Annex 5, Table 2).

Regarding the germination rate and dormancy of the seed, the seeds harvested from the recombinant oilseed rape Topas 19/2 and the non-recombinant control oilseed rape cultivated in the special screened greenhouse were sown in pots, 20 grains each, to examine the germination rate. As a result, one week after sowing, the germination rate was found 100% (20/20) and 85% (17/20) respectively for the recombinant oilseed rape Topas 19/2 and the non-recombinant control oilseed rape and thus, it is considered that the seeds of the recombinant oilseed rape Topas 19/2 have not acquired any high dormancy (Annex 6). The seeds of the non-recombinant control oilseed rape that were found not germinated were evaluated for life or dead based on the tetrazolium method and they were confirmed to be dead.

(f) Crossability

In the isolated field, the recombinant oilseed rape Topas 19/2, two varieties of oilseed rape (Drakkar and Karafuto) and two varieties of *B.juncea* (Cutlass and leaf mustard) were cultivated adjacent to each other (Annex 5, row planting plan). The test plots were entirely shielded with insect screening frames and honeybees were released. The seeds harvested from the individual varieties were set to germinate and then sprayed with glufosinate herbicide to examine the glufosinate tolerance. As a result, the glufosinate tolerance was observed in 7.4% of Drakkar, 2.5% of Karafuto, and 0.1% of Cutluss and leaf mustard (Annex 5, Table 4). Crossability with Drakkar and Karafuto was found not to exceed the existing findings that the outcrossing rate of oilseed rape through wind-pollination or insect-pollination is between 5 and 30% (References 29 and 58), and crossability with Cutlass and leaf mustard was also found not to exceed the known crossability of 0.3 to 1.1% between *B.juncea* and oilseed rape (planting rate 1:1) (Reference 6).

In 1994, in Canada, crossability of the recombinant oilseed rape Topas 19/2 with *B.rapa* and *R.raphanistrum* was investigated. As a result of examination for the herbicide tolerance of seedlings derived from the seeds harvested from *B.rapa* and *R.raphanistrum* cultivated adjacent to the recombinant oilseed rape Topas 19/2, the individuals showing the herbicide tolerance was found 3.3% on the average for *B.rapa* and 0% for *R.raphanistrum* (Annex 7, Table 2). Crossability between *B.napus* and *B.rapa* is reportedly to be 6.5 to 7.1% in the case of mixed planting (Reference 84), or 2% in the case of alternated planting on ridges between fields (Reference 50), but the above investigational results were found to fall within the variable ranges in the existing findings. In addition, it is reported that crossing between *B.napus* and *R.raphanistrum* is very rare and in fact, the crossing is not confirmed in this test, showing the results that support the existing findings.

(g) Productivity of harmful substances

In order to check whether the substances are excreted from the roots of the recombinant oilseed rape Topas 19/2 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 in 2006.

Succeeding crop test： After cultivating the recombinant oilseed rape Topas 19/2 and the non-recombinant control oilseed rape for about 3 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 6, Tables 4, 6, and 8). Therefore, it is considered that the recombinant oilseed rape Topas 19/2 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 recombinant oilseed rape Topas 19/2 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 6, Tables 10, 12, and 14). Therefore, it is considered that the recombinant oilseed rape Topas 19/2 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 recombinant oilseed rape Topas 19/2 and the non-recombinant control oilseed rape for about 3 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 6, Tables 16, 17). Therefore, it is considered that the recombinant oilseed rape Topas 19/2 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 fields in Japan, various traits relating to the competitiveness of this recombinant oilseed rape were examined based on the comparison with the non-recombinant control oilseed rape (Drakkar and Karafuto). As a result, this recombinant oilseed rape was found to show larger values for the number of seed setting per pod and also the number of seeds per plant culculated on the basis of the seed yield per plant and 1,000-seeds weight. However, these values were within the variable ranges for the varietal characteristics and thus, they are considered not to cause this recombinant oilseed rape to become competitive. 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 (Topas 4079).

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 introduced 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 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.

In concrete, (i) it has been confirmed that crossability of this recombinant oilseed rape

with the non-recombinant oilseed rape, *B. rapa*, *B. juncea* or *R. raphanistrum* does not exceed the existing findings; and

(ii) 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 that 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： Molecular analysis of T-DNA region in HCN92

Confidential: Not made available or disclosed to unauthorized person

Annex 2： Study on the segregation from Hoechst transgenic strain Mic19/2

Confidential: Not made available or disclosed to unauthorized person

Annex 3： PAT activity in HCN92 and Topas 19/2

Confidential: Not made available or disclosed to unauthorized person

Annex 4： NPT II activity in HCN92

Confidential: Not made available or disclosed to unauthorized person

Annex 5： Isolated field test report

Confidential: Not made available or disclosed to unauthorized person

Annex 6：Report on the test in the special screened greenhouse

Confidential: Not made available or disclosed to unauthorized person

Annex 7： Evaluation on the crossability between glufosinate herbicide tolerant oilseed rape and related species

Confidential: Not made available or disclosed to unauthorized person

Annex 8： Event Identifying Method

Confidential: Not made available or disclosed to unauthorized person

Annex 9： Southern blot analysis for the outside of T-DNA region in the recombinant oilseed rape Topas 19/2

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Annex 10： Glucosinolate and erucic acid contents in the recombinant oilseed rape Topas 19/2

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