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 glyphosate herbicide (*cp4 epsps*, *gox*, *Brassica napus* L.) (RT73, OECD UI : MON-ØØØ73-7)
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 a recipient organism or the species to which the recipient organism belongs

(1) Taxonomical position and state of distribution in natural environment

i) The common name and the scientific name

Oilseed rape is an annual plant which belongs to the genus *Brassica* of the family *Brassicaceae*, and the scientific name is *Brassica napus* L.

ii) Type of the recipient organism

The recipient organism is Wester, an ordinary species of oilseed rape sown in spring (*Brassica napus* L.) which belongs to the genus *Brassica* of the family *Brassicaceae*.

iii) Wild habitat in natural environment in Japan and abroad

Oilseed rape (*Brassica napus*) is an amphidiploid species produced as a result of crossing between *B. rapa* [referring to the whole of the plants known as conventional rapeseed (*Brassica rapa* var. *nippo-oleifera*), turnip, Chinese cabbage, Komatsuna, Nozawana, greens for pickling, bok choy, pak choi, etc.] which belongs to the genus *Brassica* of the family *Brassicaceae*, and *B. oleracea* to which cabbage and relevant plants belong (Reference 1). The origin of oilseed rape is considered to be the northern region of Europe, where the distribution areas of the parents of crossing, *B. rapa* and *B. oleracea*, overlap. At present, however, oilseed rape is distributed worldwide (Reference 2). In Japan, among the genus *Brassica*, *Brassica napus* (oilseed rape), *B. rapa* (conventional rapeseed), *B. juncea* (mustard) and *B. nigra* (known as black mustard) are growing voluntarily (Reference 3). The *B. juncea* includes seisai, zasai and other vegetables, which were introduced into Japan before the Meiji period (before 1868) and have been cultivated in various districts, in addition to the mustard. However, among the species, mustard is growing voluntarily at present in wastelands, roadsides, flood plains of rivers and other places (Reference 4; Reference 3; Reference 5).

It is known that oilseed rape could become self-seeding at the roadsides, abandoned plant sites and other places maintained with regular care (Reference 6), and there are reports showing that oilseed rape is cultivated in embankments along rivers in Japan (Reference 7, Annex 4, and Reference 8) and that oilseed rape is growing around the off-loading harbors for use as raw material for cooking oil (Annex 6). However, it is known that, in the natural environment left out from human access, oilseed rape is difficult to become self-seeding because it could not survive competition with perennial weeds, pteridophyte and other plants (Reference 6).

(2) History and present state of Use

i) History of Type 1 Use in Japan and abroad

Oilseed rape has been long used and it is referred to in the literature in the ancient India in 2,000 to 1,500 B.C. and in Greece, Rome and China in 500 to 200 B.C. (Reference 9). The cultivation in the European fields is said to start in Belgium in 13th Century (Reference 10).

In both Asia and Europe, oilseed rape has been widely used for lamplight since old times through oil extraction from the seeds (Reference 11). In addition, in Europe, it became used for lubricating oil for steam locomotives, which accelerated the cultivation of oilseed rape in Europe. Furthermore, during the World War II, cultivation of oilseed rape was started in Canada for the purpose of supplying lubricating oil for warships (Reference 10).

Primarily it is known that the oil extracted from the seeds of oilseed rape contains erucic acid, glucosinolate and other harmful substances. It is reported that high doses of erucic acid to experiment animals can induce pathologic changes in the myocardium or skeletal muscle. In addition, glucosinolate is known to cause the enlargement of thyroid gland of livestock (Reference 11). Therefore, it has been considered that oilseed rape is not suitable for use as food or feed. However, the selective breeding in Canada created the Canola cultivar featuring reduced contents of erucic acid and glucosinolate. As a result, it is widely applied as cooking oil such as vegetable oil, shortening and margarine. In addition, the rape cake produced after oil extraction is utilized for animal feed (Reference 10).

The cultivar, which has been long cultivated in Japan, is *B. rapa*. The cultivation of this species is considered to come from the eastern Mediterranean area to China through Siberia and western regions of China and then reach Japan in the ancient times from China and Korea. This species is referenced in the “Engishiki” (905), documentaion in the Heian period (794-1185), as a vegetable, flower buds of which were used as food. In the Edo period (1603-1867), extensive cultivation was started to provide raw material for lamp oil and cooking oil. On the other hand, cultivation of oilseed rape was started in the Meiji period (1868-1912) and became widespread throughout country, while *B. rapa* (conventional rapeseed) became on the decline (Reference 10).

The total planted area and production of oilseed rape in Japan amounted to 110,000 ha and 120,000 to 130,000 ton respectively in 1937 to 1938, though taking a sudden drop during World War II. After the war, they shot up again to some 200,000 ha and 300,000 ton respectively in 1952 to 1958. Then, however, the total planted area decreased sharply because the planting period came to fall on that for rice due to the adoption of the early season culture of rice and also the rapid economic development in Japan starting around 1955. Since then, commercial cultivation of oilseed rape in Japan has been little undergone, and the cultivation is primarily intended as a tourist attraction (Reference 2; Reference 12).

The total planted area of oilseed rape in Canada in 2003 was around 4.69 million ha (Reference 13). According to the investigations by Monsanto Canada, it is found

that the present commercial cultivation of oilseed rape in Canada involves about 92% of oilseed rape conferred with the herbicide tolerant traits, 77% of which are given the herbicide tolerant traits by the genetic recombination technology, 15% by the breeding of mutation, and 7.5% by the traditional breeding method. In addition, the planted area of *B. rapa* (conventional rapeseed) is about 3.75% of that of oilseed rape, and the planting district is localized primarily in the northwestern Alberta and northeastern British Columbia. For the *B. rapa* (conventional rapeseed) cultivated commercially at present, there is no cultivar that has been developed by the genetic recombination technology.

ii) Main cultivation area, cultivation method, current status of distribution and use

The major production districts of oilseed rape include China (11.32 million ton), EU (8.88 million ton), Canada (5.06 million ton), and India (4.80 million ton). In addition, production in Australia is increasing, amounting to 1.6 million ton in FY 2002 (Reference 14).

The major exporters are Canada (2.73 million ton) and Australia (1.23 million ton), which account for 68% of the total exports (Reference 14). Japan imported a total of 2.08 million ton in FY 2002 primarily from Canada (1.58 million ton) and Australia (0.45 million ton) (Reference 15). In addition, rapeseed oil was imported 17,000 ton in 2003, and rape cake for feed was imported 42,000 ton (Reference 16).

(3) Physiological and ecological properties

i) Basic properties

Oilseed rape is an annual plant propagating by seed.

ii) Environmental conditions allowing inhabiting or growth

Oilseed rape is available in autumn sowing varieties, which need low temperatures for breaking dormancy, starting bolting and flower bud differentiation; and spring sowing varieties, which do not need any low temperatures (Reference 2). Oilseed rape grows well in the temperature range from 15 to 20℃, and it could survive acidity and humidity with relative ease, though it could not survive in the heavy clay or significantly dried sandy soils. The germination rejects excessive humidity, but the growth requires a plenty of water. In addition, oilseed rape could be cultivated elsewhere across the country in Japan (Reference 11).

iii) Mode of propagation or reproduction

(a) Mode of dispersion, shedding habit, dormancy and longevity of the seeds

Oilseed rape produces a number of seeds in a pod, and the pod with the matured and dried seeds cleave at the pod stalk, thereby dispersing the seeds (Reference 11).

The dried pod easily cleaves in response to only a small physical impact and disperses the seeds (Reference 2). Therefore, shedding habit is considered relatively high.

Dormancy of the seeds is known relatively low for both autumn and spring sowing varieties, though it is known that dormancy is acquired when significant temperature change, shortage of water, or dark condition persists for a long period of time (Reference 17). In addition, some cultivars reportedly could acquire dormancy even under high-temperature conditions above 20°C depending on the genetic type (Reference 18). It is known that the acquired dormancy may be broken in the low-temperature conditions (2 to 4°C) (Reference 18) or when exposed again to any varying temperatures (Reference 17).

The longevity of the seeds of oilseed rape depends on the conditions of seed harvesting or storage. Even six (6) years after storage in a dry condition following ripening, 80% of the seeds were successfully germinated, though the germination rate after three (3) years when placed in a room was about 30% (Reference 19).

(b) Mode of vegetative propagation and tissues or organs capable of regenerating the plant body under natural condition

Oilseed rape propagates by seeds and there is no observed reproduction from any other organ under natural condition.

(c) Degree of selfing and out-crossing, presence or absence of self-incompatibility, and crossability with related plants

Oilseed rape has no self-incompatibility but often produces the seeds by self-pollination. It is reported that the crossability with related plants by wind- and insect-pollination is 5 to 30% (Reference 20; Reference 21).

Relatives which may be possibly crossed with oilseed rape include *B. rapa*, utilized as cultivar in Japan from ancient times, *B. nigra*, naturalized after the Meiji period, *Raphanus raphanistrum* (known as radish), naturalized in the early Showa period (1926-1989), and *B. juncea*, naturalized after the war (Reference 4; Reference 3; Reference 1).

B. rapa includes not only conventional rapeseed but also its variants, turnip, Chinese cabbage, Komatsuna, Nozawana, greens for pickling, bok choy, and pak choi, though only the conventional rapeseed is growing voluntarily in Japan in the wastelands and roadsides (Reference 1). For the crossability of *B. rapa* with oilseed rape, it is reported that probability of crossing was low, ranging from 0.4% to 1.5%, when the population of *B. rapa* (conventional rapeseed) was planted in the fields outside the border of the fields for oilseed rape (Reference 22) though probability of crossing was 13% when *B. rapa* (conventional rapeseed) was planted in the fields for oilseed rape in the same rate of population (Reference 23). In addition, when a single individual of *B. rapa* (conventional rapeseed) was planted in the oilseed rape field, the

crossability was found 93% (Reference 23). This may be attributed to the possible reason that *B. rapa* (conventional rapeseed) possesses the trait of self-incompatibility and thus allows only cross pollination (Reference 24).

B. nigra is a naturalized weed which is growing voluntarily in the wastelands and roadsides in Japan, and it is considered to be naturalized after Meiji period (Reference 4; Reference 3; Reference 5). For the crossability with oilseed rape, there is an investigation, which addressed the probability of crossing after rearing the both plants adjacent to each other and concluded that there was no crossability (Reference 25). Artificial pollination was also attempted for possible crossing, though it failed in most cases (Reference 26; Reference 27) and the probability was extremely low even if the artificial pollination was successful (Reference 28; Reference 29).

Raphanus raphanistrum is a naturalized plant growing voluntarily in wastelands and roadsides in Japan, and it is estimated naturalized in the early Showa period (Reference 4; Reference 3). For the crossability with oilseed rape, there is a report showing the crossing at 0.2% when oilseed rape and *Raphanus raphanistrum* were raised in combination (Reference 30). However, as a result of five-year monitoring of *Raphanus raphanistrum* in UK on a regular basis raised near the field for glufosinate herbicide tolerant oilseed rape, no crossbreed was observed (Reference 31).

B. juncea includes not only mustard but also seisai, zasai and other vegetables which were introduced into Japan before Meiji period and cultivated in various areas. However, growing voluntarily at present in wastelands, roadsides and river-terraces is mustard, a naturalized plant introduced after the war from Europe and North America (Reference 4; Reference 3; Reference 5). For the crossability with oilseed rape, it has been observed that crossbreds are growing near the oilseed rape field in foreign country (Reference 29; Reference 32; Reference 33; Reference 34; Reference 35). The crossability depends on the proportion between the growing oilseed rape and *B. juncea* (mustard), though there is a report showing crossing of about 3% at maximum of the total (Reference 29; Reference 23). Crossing can occur irrespective of which variety acts as pollen parent, though it is known that the number of crossbreds produced is smaller when *B. juncea* (mustard) acts as pollen parent (Reference 29; Reference 23).

(d) Production, fertility, shape, mode of pollination, dispersion distance and longevity of pollen

Oilseed rape produces around 60,000 to 70,000 grains of pollen per flower.

The pollen of oilseed rape is yellow in color, and oval in shape with three vertical constrictions. The pollen measures 37 to 39 μm in major axis and 20 to 22 μm in minor axis (Reference 10). In addition, pollen of oilseed rape is heavy and sticky (Reference 6).

Mode of pollination is wind or insect (primarily honey bee) (Reference 6).

Oilseed rape pollen is heavy and sticky but small in size and then, there is a report showing that the pollens are dispersed by wind (Reference 36). According to the report in Reference 37, 0 to 22 grains of pollens were dispersed per 1 m^2 at a distance of 1.5 km from the source of pollens, though the density of pollens in air decreases sharply with increased distance from the source of pollens. The investigation in Reference 38 indicates that the density of pollens in air at a distance of 20 m from the field was reduced by about 90% compared to the inside of the field. In addition, it is reported that insects play an important role in long-distance transmission of pollens (Reference 39; Reference 40). According to Reference 39, a stock of honey bees can travel as far as 2 km from the beehive for sucking nectar, therefore it is estimated in theory that pollens could be dispersed up to 4 km (Reference 39).

Fertility of the pollen is maintained 24 hours to one week, and starts to be lost gradually after 4 days have elapsed (Reference 41).

iv) Productivity of harmful substances

It is known that the seed of non-recombinant oilseed rape produces erucic acid and glucosinolate as harmful substances to human and other mammals. It is reported that erucic acid can induce pathological changes in the myocardium and/or skeletal muscle when administered in large doses to experiment animals. In addition, it is known that glucosinolate can cause enlargement of thyroid gland of livestock (Reference 11). However, today, as a result of long-standing breed improvement of oilseed rape and successful development of those cultivars of low contents of erucic acid and glucosinolate, the oil has become used as food for human and rape cake as feed. Such less erucic acid (less than 2% in refined oil) and less glucosinolate (less than 30 μmol in 1 g rape cake) oilseed rape is generally known as Canola, one variety of which is Wester, the mother line of this recombinant oilseed rape.

In the process of assessment of components of this recombinant oilseed rape, analysis was made on the erucic acid and glucosinolate, and it was confirmed that these components in this recombinant oilseed rape do not exceed the allowable threshold quantities for the Canola cultivar but fall within or close to the analytical values of the non-recombinant control variety Wester (Annex 4).

2. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

i) Composition and origins of component elements

Composition of the donor nucleic acid that was used for the development of glyphosate torelant oilseed rape (*cp4 epsps*, *gox*, *Brassica napus* L.) (RT73, OECD UI : MON-ØØØ73-7) (hereinafter referred to as “this recombinant oilseed rape”) and the origins of component elements are shown in Table 1.

In this recombinant oilseed rape, *cp4 epsps* gene which was the modified gene of the wild-type *cp4 epsps* gene and *gox* gene which was the modified gene of the wild-type *gox* gene were introduced, and hereinafter these genes are referred to as "modified *cp4 epsps* gene" and "*gox v247* gene", respectively. In addition, the proteins being expressed are referred to as "modified CP4 EPSPS protein" and "GOX v247 protein", respectively.

The nucleotide sequences of all the component elements of this recombinant oilseed rape are shown in Annex 1.

ii) Function of component elements

Functions of component elements of donor nucleic acid that was used for the development of this recombinant oilseed rape are shown in Table 1.

[Modified *cp4 epsps* gene]

(a) Glyphosate herbicide is the active ingredient of Roundup, a nonselective herbicide, and inhibits the activity of 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) (E.C.2.5.1.19), one of the enzyme in the shikimate pathway for aromatic amino acid biosynthesis, by specifically binding to the enzyme (Reference 42; Reference 43). As a result, plants treated with glyphosate cannot synthesize aromatic amino acid essential for protein synthesis due to the inhibition of EPSPS, and die. The modified *cp4 epsps* gene, a target gene of this recombinant oilseed rape, expresses the modified CP4 EPSPS protein which has high tolerance to the herbicide glyphosate. The activity of the modified CP4 EPSPS protein to be produced by the modified *cp4 epsps* gene is not inhibited even under the presence of glyphosate, thus the recombinant plants that express this protein have normal functions of shikimate pathway and can grow.

EPSPS is one of the enzyme to catalyze the shikimate pathway for aromatic amino acid biosynthesis, which is unique to plant or microorganism, and EPSPS is located in chloroplasts or plastids in plants (Reference 44). The shikimate pathway is an important metabolic pathway that is considered to be involved in one fifth of carbon fixation by plants (Reference 45; Reference 43). This pathway is regulated by 3-deoxy-D-arabino-heptulonate-7-phosphate (DAHP) synthase, which is involved in the first step of the pathway, but it has been clarified to be extremely unlikely that the stages from DAHP to the synthesis of chorismic acid through the production of 5-enol-pyruvylshikimate-3-phosphate (EPSP) which is catalyzed by the EPSPS are inhibited or suppressed by metabolic intermediates or end products of this pathway (Reference 46; Reference 47). This suggests that EPSPS is not a rate-determining enzyme in this pathway, and as such it is not considered that enhanced EPSPS activity will increase the concentration of aromatic amino acids, the end products of this pathway. In practice, it is reported that plant cells that produce 40 times as much EPSPS as compared to normal do not synthesize excessive aromatic amino acids (Reference 48). In addition, Monsanto Co. examined amino acid content in the seeds of the recombinant crops in the process of food/feed safety assessment of crop plants (soybean,

cotton and maize) that have been commercialized with the feature of tolerance to the Roundup herbicide, and confirmed that there is no difference in the aromatic amino acid content between the original non-recombinant plants and recombinant plants. These facts support the theory that EPSPS is not the rate-determining enzyme in this pathway. Besides, EPSPS is the enzyme that catalyzes a reversible reaction to produce EPSP and inorganic phosphate (Pi) from phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Reference 49), and it is known to specifically react with these substrates (Reference 50). The only substance that is known to react with EPSPS other than these is shikimate, an analogue of S3P, but the reactivity with shikimate is only one two millionth of the reactivity with S3P, and it is unlikely that shikimate exerts any significant influence on the metabolic system of plant body even if it reacts.

Compared with the wild-type CP4 EPSPS protein, the modified CP4 EPSPS protein expressed in this recombinant oilseed rape contains the leusine, the 2nd amino acid in the N-terminal sequence, replaced by serine. This modification is conducted in order to produce restriction enzyme of *Nde*I in the N-terminal side.

(b) In order to investigate whether the modified CP4 EPSPS protein shares functionally important amino acid sequence with known allergens, the CP4 EPSPS protein was compared with known allergens in the database (GenBank, EMBL, PIR, NRL3D, SwissProt). As a result, the CP4 EPSPS protein did not share structurally related homologous sequences with any of the known allergens examined.

[*gox v247* gene]

(a) Glyphosate is degraded and inactivated by microorganisms in soil. This is attained based on the process that the enzyme for degradation of glyphosate (Glyphosate Oxidoreductase; GOX) in microorganisms degrades the herbicide glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate, both of which offer no herbicidal activity and are commonly found in a number of gram-negative and gram-positive bacteria degrading the herbicide glyphosate (Reference 51; Reference 52). Then, among the organisms estimated to offer the capability of degrading the glyphosate into AMPA and glyoxylate, *Ochrobacterium anthropi* (designation based on former classification: *Achromobactor* sp.) LBAA strain was selected which showed the highest capability of glyphosate degradation, and the *gox* gene was isolated (Reference 53; Reference 54). The *Ochrobacterium anthropi* LBAA strain is reportedly one of the organisms existing most in number in the rhizosphere of plants (Reference 55), and it is known that Ochrobacterium anthropi LBAA strain could utilize glyphosate as carbon and/or phosphorus sources (Reference 54). In addition, the GOX v247 protein is an enzyme which catalyzes the degradation of glyphosate into AMPA and glyoxylate, though it has high substrate specificity and then it does not affect the metabolic system in plants (Reference 56).

The GOX v247 protein expressed in this recombinant oilseed rape contains, i)

the glycine, the 84th amino acid in the N-terminal sequence, replaced by serine; ii) the arginine, the 153rd amino acid replaced by lysine; and iii) the arginine, the 334th amino acid, replaced by histidine; in order to enhance the capability of degrading the herbicide glyphosate. The *gox* gene and this *gox v247* gene are 99% or more homologous with each other in the nucleotide sequence.

(b) In order to investigate whether the GOX v247 protein shares functionally important amino acid sequences with known allergens, the GOX 247 protein was compared with allergens in the database (GenBank, EMBL, PIR, NRL3D, SwissProt). As a result, the GOX v247 did not share structurally related homologous sequences with any of the known allergens examined.

Table 1 Component elements of plasmid PV-BNGT04[1]

Component elements	Origin and function
gox v247 gene expression cassette	
P-FMV	35S promoter of *Figwort mosaic virus* (Reference 57; Reference 58; Reference 59). Involved in the constant expression in all organs of the target gene. It is known that among virus of the genus *Caulimovirus* to which FMV belongs, cauliflower mosaic virus (CaMV) takes the plant of the genus *Brassica* to which oilseed rape belongs as the recipient organism. However, the homology of 35S promoters of FMV and CaMV is low as 10% or less. Therefore, it was considered that the possibility to come in a new virus due to the recombination is extremely low (Annex 7).
Arab-SSU1A /CTP1	N-terminal chloroplast transit peptide sequence of small subunit 1A of ribulose-1,5-bisphosphate carboxylase of *Arabidopsis* (Reference 60). Transfers target proteins to chloroplast.
gox v247	A variant of glyphosate degradation enzyme (glyphosate oxidoreductase; GOX) derived from *Ochrobacterium anthropi* LBAA strain (Reference 54; Reference 61). It degrades glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate. The GOX v247 protein expressed in this recombinant oilseed rape contains the glycine, the 84th amino acid in the N-terminal sequence, replaced by serine, the 153rd amino acid arginine by lysine, and the 334th amino acid arginine by histidine, in order to enhance the capability of glyphosate degradation. The wild-type *gox* gene and this *gox v247* gene are 99% or more homologous with each other in the nucleotide sequence.
E9 3'	3' untranslated region of *rbcS E9* gene of pea. Terminates polyadenylation of *gox v247* gene and modified *cp4 epsps* gene (Reference 62; Reference 63).
Modified *cp4 epsps* gene expression cassette	
P-FMV	35S promoter of *Figwort mosaic virus* (Reference 57; Reference 58; Reference 59). Involved in the constant expression in all organs of the target gene. It is known that among virus of the genus *Caulimovirus* to which FMV belongs, *cauliflower mosaic virus* (CaMV) takes the plant of the genus *Brassica* to which oilseed rape belongs as the recipient organism. However, the homology of 35S promoters of FMV and CaMV is low as 10% or less. Therefore, it was considered that the possibility to come in a new virus due to the recombination is extremely low (Annex 7).
AEPSPS/CTP2	N-terminal chloroplast transit peptide sequence of *epsps* gene of *Arabidopsis* (Reference 57; Reference 58; Reference 64). Transfers target proteins to chloroplast.
Modified *cp4 epsps*	5-enol-pyrovylshikimate-3-phosphate (*epsps*) gene form *Agrobacterium* sp. strain CP4 (Reference 65). Expresses modified CP4 EPSPS protein which shows high tolerance to glyphosate herbicide.
E9 3'	3' untranslated region of *rbcS E9* gene of pea. Terminates polyadenylation of *gox v247* gene and modified *cp4 epsps* gene (Reference 62; Reference 63).

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

Table 1 Component elements of plasmid PV-BNGT04 (continued) [2]

Component elements	Origin and function
Other component elements	
Right Border (RB)	It is the restriction fragment from pTiT37 plasmid. Initiates the T-DNA transferred from *Agrobacterium tumefaciens* to plant genome (Reference 66).
Left Border (LB)	It is the restriction fragment from Octopine Ti plasmid pTiA6, containing the left border sequence (25 bp) of T-DNA(Reference 67).
ori-V	The replication origin segment in *Agrobacterium* derived from the broad-recipient range plasmid RK2 (Reference 68).
ori-322	The replication origin of PV-BNGT04 in *E. coli* derived from pBR322. (Reference 69).
Aad	A bacterial gene enconding Tn7 AAD 3' adenylyltransferase. It confers spectinomycin/streptomycin tolerance to bacterial cells (Reference 70).

[2] All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

(2) Information concerning vector

i) Name and origin

The synthetic plasmid vector PV-BNGT04 to be used for developing this recombinant oilseed rape is the plasmid vector PV-BNGT04 which was composed of the plasmid pBR322 derived from *Escherichia coli* and others. The T-DNA region in this vector transferred to the recipient organism is composed of *gox v247* gene expression cassette [P-FMV]-[Arab-SSU1A/CTP1]-[*gox v247*]-[E9 3'] and modified *cp4 epsps* gene gene expression cassette [P-FMV]-[AEPSPS/CTP2]-[modified *cp4 epsps*]-[E9 3'].

ii) Properties

The total number of base pairs of this vector is 11,479bp, and it possesses modified *cp4 epsps* gene expression cassette and *gox v247* gene expression cassette (Figure 1). The infectivity of this vector is not known.

(3) Method of preparing living modified organisms

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

The T-DNA region of the synthetic plasmid vector PV-BNGT04 to be used for developing this recombinant oilseed rape is composed of *gox v247* gene expression cassette ([P-FMV]-[Arab-SSU1A/CTP1]-[*gox v247*]-[E93']) and modified *cp4 epsps* gene expression cassette ([P-FMV]-[AEPSPS/CTP2]-[*cp4 epsps*]-[E93']), which are both controled by the FMV promoter. The structure of the nucleic acid and the origin of component elements are shown in Table 1.

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

The T-DNA region of the plasmid vector PV-BNGT04 was introduced to the non-recombinant oilseed rape cultivar, Wester, by the *Agrobacterium* method.

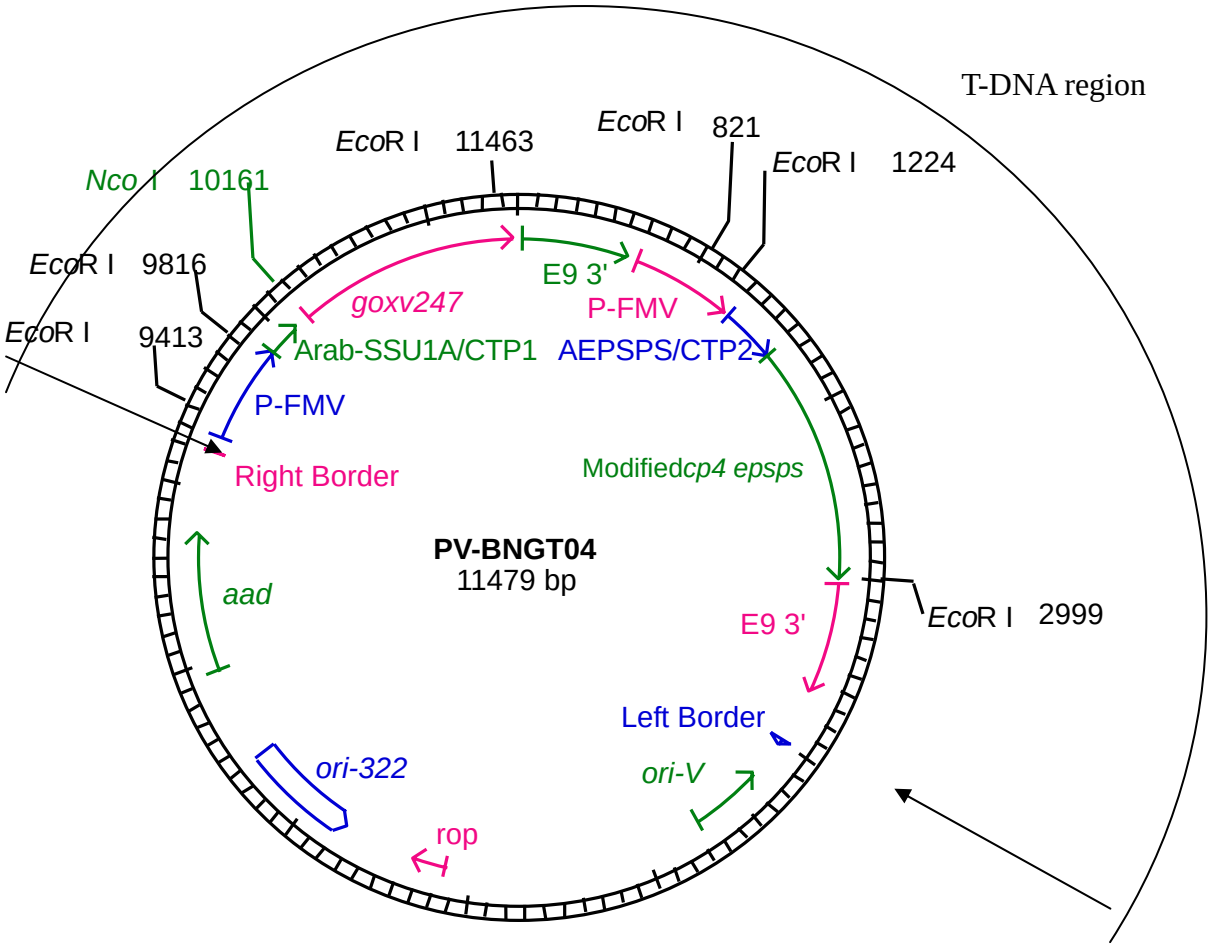

Figure 1 Map of plasmid PV-BNGT04 [3]

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

iii) Processes of rearing of living modified organisms

T-DNA region of plasmid vector PV-BNGT04 was introduced into the explants (leaves or flower buds) of Wester by the *Agrobacterium* method, and then regenerated individuals were obtained by culturing them in media containing glyphosate. In order to eliminate *Agrobacterium* from the regenerated plant, the regenerated plant was cultivated in media containing carbenicillin and paromomycin.

Regarding the obtained regenerated individuals, further selection was carried out based on the analysis of inserted genes and the expression level of the modified CP4 EPSPS protein. Tests in climate chamber and greenhouse were then carried out, and glyphosate tolerance and agronomic characters were examined in field tests. This recombinant oilseed rape was selected upon the comprehensive evaluation of these results (Figure 2).

The following shows the approvals received from organizations in Japan.

February, 1996:	Based on the "Guideline for the conduct of Food Safety Assessment of Food and Additives derived from Recombinant-DNA Plants, Chapter 4", the safety of use for food was approved by the Ministry of Health, Labor and Welfare (Ministry of Health and Welfare, at the time).
March, 1996:	Based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", the compatibility to the guideline regarding recombinant being imported to Japan (used for processing and feed) was certified by the Ministry of Agriculture, Forestry and Fisheries.
September, 1996:	The safety of use of the cultivar for feed was approved in accordance with "Guideline for the safety evaluation of feed derived from recombinant-DNA plants, 6-(2)" by the Ministry of Agriculture, Forestry and Fisheries.
March, 2001:	The safety of use of the cultivar for food, in accordance with "Safety Evaluation Criteria for Food and Additives derived from Recombinant-DNA Techniques" was ensured by the Ministry of Health, Labour and Welfare.
March, 2003:	The safety of the use of the cultivar as feed, following "Procedure to Check the Safety of Feed and Additives Produced by Recombinant-DNA Techniques" was ensured by the Ministry of Agriculture, Forestry and Fisheries.

[Not made available or disclosed to unauthorized person]

Figure 2 The process of rearing the oilseed rape RT73 tolerant to glyphosate herbicide

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

i) Location of copies of the introduced nucleic acids

On the chromosome

ii) Number of copies of the introduced nucleic acids and inter-generational inheritance stability of copies of the introduced nucleic acids

The inserted genes were analyzed by Southern blotting analysis and PCR analysis using the genome DNA extracted from the leaf tissues of R3 generation. As a result, it was confirmed that one copy of T-DNA region consisting of the modified *cp4 epsps* gene expression cassette and *gox v247* gene expression cassette was inserted at one site in the genome DNA of this recombinant oilseed rape, and no other components were inserted in the genome DNA of this recombinant oilseed rape (Figure 3). The details on the analysis of inserted genes for this recombinant oilseed rape are described in Annex 2 and Annex 3. The result showing the site of insertion is only one is supported by the gene data indicating that the phenotype of glyphosate herbicide tolerance represents single dominant Mendel character (Reference 71).

iii) Inter-individual or inter-generational expression stability under natural conditions

As a result of Southern blotting analysis using multiple generations (R3 and R5), it was confirmed that the inserted genes were stably inherited to posterity (Annex 2). The hereditary stability of the inserted gene among this recombinant oilseed rape was confirmed by the tolerance test to glyphosate herbicide in each generation.

iv) Existence of transmission routes and its scale, if it is possible that nucleic acids introduced via viral infection or other routes might be transmitted to wild animals and plants

It is only in gram-negative bacteria, such as *E.coli* and *A. tumefaciens*, that the plasmid PV-BNGT04 can sustain autonomous replication. Therefore, there is no possibility that the introduced nucleic acids might be transmitted to wild animals and plants that are not sexually compatable under natural conditions.

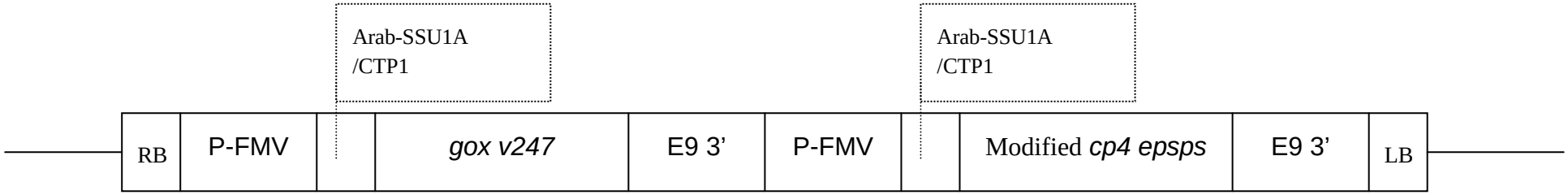

Figure 3 Genetic map of inserted genes in the oilseed rape RT73 tolerant to glyphosate herbicide [4]

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

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

For the detection and identification of this recombinant oilseed rape, a qualitative PCR method has been developed where the DNA sequences of the inserted genes and the nearby regions of the plant genome are used as primers. This method makes it possible to specifically detect this recombinant oilseed rape (Annex 3).

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

i) As a result of ELISA method, it was confirmed that the modified CP4 EPSPS protein, which is encoded by the modified *cp4 epsps* gene, and the GOX v247 protein, which is encoded by the *gox v247* gene, are expressed in this recombinant oilseed rape (Annex 2).

ii)[5] Difference of this recombinant oilseed rape from the recipient organism of non-recombinant control oilseed rape was examined primarily based on the results of isolated field tests conducted between May 1995 and March 1996 at the National Institute for Agro-Environmental Science (NIAES), though it was also evaluated based on comprehensive consideration on the results of greenhouse tests in non-closed system greenhouse carried out from July 1994 to February 1995 at Monsanto Japan Limited. In the isolated field tests conducted at the National Institute for Agro-Environmental Science (NIAES), the tests regarding morphological and growth characteristics and weediness were carried out in the No.9 isolated field (Annex 5), and the tests regarding reproduction were carried out in the No.10 isolated field (Annex 5).

(a) Morphological and growth characteristics

As described in Annex 5, differences in the following 21 items of morphological and growth characteristics were examined between this recombinant oilseed rape and the non-recombinant control oilseed rape in the isolated field tests: the uniformity of germination; germination rate; time of flower initiation; plant height; time of flower completion; plant shape or plant type; the number of primary branches; the number of open flowers; harvest time; the number of pods formed; rate of opened pods; length of pod; width of pod; the number of seeds per pod; color of grain; uniform excellence of grains (uniformity of grain size); shape of hilum; weights of above and under-ground parts at the harvest time; fresh weight of under-ground part at harvest time; dry weight of above-ground part; and dry weight of under-ground part. In addition, regarding the pods obtained from artificial pollination of four (4) flowers each for 4 individuals selected in each repetition of plants in the planting area, additional examination was conducted for the following characteristics: rate of opened pods; length of pod; width of pod; the number of seeds per pod; color of grain; uniform excellence of grains; and shape of hilum. Furthermore, also regarding the plants in the area for testing of reproduction characteristics (No.10 isolated field), examination was carried out for the

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

number of open flowers, the number of pods formed and the number of opened pods. As a result, in all of the items examined, no difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5).

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

In order to evaluate the cold-tolerance at the early stage of growth of this recombinant oilseed rape, this recombinant oilseed rape and the non-recombinant control oilseed rape around at the 1.2 leaf stage were examined for plant height and leaf age, and then raised in an artificial climate control room maintained at 5℃ (humidity 35%, 3,500 lx, 12-hour day length) (5 replications). Then, 30 days later, cold-tolerance of this recombinant oilseed rape and the non-recombinant control oilseed rape was evaluated again based on the plant height and leaf age. As a result, no statistically significant difference was observed in the growth between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 4).

Also in the isolated field tests, regarding the growth in summer and winter seasons, comparison was made between this recombinant oilseed rape and the non-recombinant control oilseed rape. In the examination of growth in summer, seedlings at the 5th to 6th leaf stage were planted on July 25 for evaluation of subsequent growth and seeds were sown on August 1 for evaluation of germination rate and other characteristics. In the examination of growth in winter, seeds were sown in autumn (date of sowing: October 6) to examine the germination rate and the growth at the early stage (Annex 5). As a result, in the planting test and sowing test in summer, regarding the survival rate, plant height, germination rate and other characteristics, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5). Also regarding the germination rate and the growth at the early stage in the autumn sowing test, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5).

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

Regarding the individuals of third replication of this recombinant oilseed rape and the non-recombinant control oilseed rape planted on May 31, overwintering test was carried out for the matured plant without harvesting (Annex 5). As a result, this recombinant oilseed rape and the non-recombinant control oilseed rape both turned brown and died out on November 22 and no difference was observed between them (Annex 5).

(d) Fertility and size of the pollen

To examine the fertility (maturity) and size of the pollens, pollens were collected from long stamen and short stamen of this recombinant oilseed rape and the non-recombinant control oilseed rape (10 flowers for each), stained with iodine potassium-iodine solution (3% potassium iodine solution + 1% iodine) and then observed under a microscope. As a result, no difference was

observed in the fertility between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 4), and also no difference was observed in the size between them (Annex 4).

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

Regarding seed production, the differences between this recombinant oilseed rape and the non-recombinant control oilseed rape have been examined for the number of pods formed and the number of seeds per pod as mentioned in (a) Morphological and growth characteristics. As a result, in all items examined, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5). Also regarding the number of seeds per pod obtained from artificial pollination and natural crossing (Annex 5), the number of pods formed, and rate of formation of pods (Annex 5), no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape.

Regarding shedding habit, the differences between this recombinant oilseed rape and the non-recombinant control oilseed rape have been examined for the rate of opened pods as mentioned in (a) Morphological and growth characteristics. As a result, regarding the rate of opened pods, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5). In addition, regarding the rate of opened pods and the number of opened pods obtained from artificial pollination and natural crossing, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5).

To evaluate dormancy and germination rate, artificial pollination was conducted with this recombinant oilseed rape and the non-recombinant control oilseed rape, and the seeds were harvested 69 days after the pollination. Germination rate of the seeds was examined under the condition at 20℃. The harvested seeds were sown in a greenhouse 25 grains each for 3 repetitions, and germination rate was examined 18 days after the sowing. As a result, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape which exhibited the germination rate of 100% and 99% respectively, and thus it was confirmed that dormancy of the seed is extremely low (Annex 4).

(f) Crossability

Crossability of this recombinant oilseed rape with non-recombinant oilseed rape (Name of cultivar; Wester), *B. juncea* (mustard, Name of cultivar; leaf mustard, Sakata Seed Co.) and *B. rapa* (conventional rapeseed, Name of cultivar; rapeseed, Sakata Seed Co.), which were all set in the isolated field (No.10) to produce flowers at almost same timing, was examined. The examination was conducted for the crossability with non-recombinant oilseed rape and *B. juncea* (mustard) in the period from June 2, 1995 to June 26 (Annex 5), and the crossability with non-recombinant oilseed rape and *B. rapa* (conventional rapeseed) in the period from October 6, 1995 to October 31,

1995 (Annex 5). The details on the method of examination of crossability are described in Annex 5. Also in the period from June 27, 1995 to August 9, 1995, additional examination was conducted for the crossability of this recombinant oilseed rape with non-recombinant oilseed rape, *B. juncea* (mustard) and *B. rapa* (conventional rapeseed) (Annex 5), though the period of this examination felled on the rainy season and plants died out in many fields, and then the results of this examination were not used as data.

As a result of the examination, crossability of this recombinant oilseed rape with *B. juncea* (mustard) in the period from June 2, 1995 to June 26, 1995 was 2.3%, 2.1%, 0.1% and 0% on average respectively in the east, west, south and north 0, 2, 5 and 10 m areas, and the crossability of this recombinant oilseed rape with non-recombinant oilseed rape was 11.2%, 4.5%, 1.7% and 0.1%, respectively (Annex 5).

In addition, crossability of this recombinant oilseed rape with *B. rapa* (conventional rapeseed) in the period from October 6, 1995 to October 31, 1995 was 3.1%, 1.6%, 0.3% and 0% on average respectively in the east, west, south and north 0, 2, 5 and 10 m distant zones, and the crossability of this recombinant oilseed rape with non-recombinant oilseed rape was 17.8%, 3.5%, 0.8% and 0.4%, respectively (Annex 5).

Rounding up the above results regarding the crossability from the isolated field tests, crossability of this recombinant oilseed rape with non-recombinant oilseed rape is found highest in the 0 m distant zone, and 1% or less in the 10 m distant zone. Among all the results of crossability tests, the highest crossability obtained from the 0 m distant zone is 21% (Annex 5), and this value provides no difference from the natural crossability (around 20%, Reference 72) obtained in the case when non-recombinant oilseed rape is cultivated adjacent to each other. The crossability of this recombinant oilseed rape with *B. juncea* (mustard) and *B. rapa* (conventional rapeseed) was also found highest in the 0 m distant zone, and 0% in the 10 m distant zone.

In 1989, experiment was carried out in the field in Canada to examine the dispersion of pollens (Annex 4). As a result, incidence of crossbred individuals was found 0.19% (average for 3 zones) in the zones of non-recombinant oilseed rape at a distance of 50 m from the cultivation zone of this recombinant oilseed rape, 0.12% (average for 3 zones) in the zones at a distance of 100 m, and 0.08% (average for 4 zones) in the zones at a distance of 150 to 225 m) (Annex 4).

To check in a non-closed system greenhouse that the wind- and insect-pollination characteristics of this recombinant oilseed rape remain unchanged, pollen dispersion distance by artificial wind (Annex 4) and the flower-visiting behavior of honey bees (Annex 4) were examined. As a result, regarding the pollen dispersion distance, this recombinant oilseed rape and the non-recombinant control oilseed rape are very similar to each other in the pollen dispersion pattern, and for the amount of pollens dispersed, no statistically significant difference was observed between them (Annex 4). Also regarding the total number of flower-visiting times of honey bees and the

number of flow-visiting times and time length of one honey bee, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 4).

In addition, to examine in a non-closed system greenhouse the affinity for crossability of *B. juncea* (mustard) and *B. rapa* (conventional rapeseed) used in the crossability tests with this recombinant oilseed rape and the non-recombinant control oilseed rape, the number of ripened seeds in pods obtained following artificial pollination was identified. As a result, *B. juncea* (mustard) exhibited high affinity of crossability with both the recombinant oilseed rape and non-recombinant oilseed rape without any significant difference observed in the affinity with the both plants (Annex 5). Similarly, *B. rapa* (conventional rapeseed) also exhibited high affinity of crossability with both the recombinant oilseed rape and non-recombinant oilseed rape without any significant difference observed in the affinity with the both plants (Annex 5).

Then, germination rate of seeds obtained from crossing of all combinations between *B. juncea* (mustard), *B. rapa* (conventional rapeseed), this recombinant oilseed rape and the non-recombinant control oilseed rape was examined. As a result, the germination rate of self-pollinated seeds of *B. juncea* (mustard), which has been known to possess very high dormancy, was very low, showing dormancy as expected (Annex 5), while the germination rates of the seeds obtained from crossing between this recombinant oilseed rape and non-recombinant control oilseed rape were similarly high, showing no dormancy (Annex 5). In addition, the germination rate of self-pollinated seeds of low-dormancy *B. rapa* (conventional rapeseed) was as high as the germination rate of the seeds obtained from crossing between this recombinant oilseed rape and the non-recombinant control oilseed rape, showing no dormancy (Annex 5).

(g) Productivity of harmful substances

In order to confirm that this recombinant oilseed rape does not produce any substances which can affect other plants and/or microorganisms in soil, soil microflora test was conducted in the isolated field tests, and succeeding crop test, plow-in test, assay by reaching liquid from leaves, and soil microflora test were carried out in the non-closed system greenhouse test. For all items tested, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5).

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 was introduced to Japan in early Meiji period, and it is reportedly growing on river banks, along roadsides, in the surroundings of seed off-loading harbors, and in other such areas. It is generally known that oilseed rape could become self-seeding in the environments such as roadsides and abandoned plant sites maintained with regular care though it could not survive the competition with perennial wild plants and thus it is difficult to become self-seeding under natural conditions left almost uncontrolled, therefore it is not regarded as an invasive species which could exterminate Japanese native species of plants and cause Adverse Effect on Biological Diversity.

This recombinant oilseed rape has the tolerance to glyphosate herbicide conferred by the transferred modified *cp4 epsps* and *gox v247*, but it is hard to consider that the glyphosate will function as a selection pressure in the natural environment. In addition, various traits relating to the competitiveness of this recombinant oilseed rape have been investigated in the isolated fields in Japan and as a result, no significant difference was observed between this recombinant oilseed rape and non-recombinant oilseed rape.

Even if this recombinant oilseed rape, growing at roadsides or in other areas where herbicide glyphosate is used for the purpose of weed control, could survive the herbicide sprayed, such areas are in general exposed to non-selective weed control, and this recombinant oilseed rape could be removed by use of any weed killers other than the glyphosate and/or mowing. In addition, as mentioned above, it is considered that herbicide tolerance does not act on selection pressure more dominantly under natural condition than the non-recombinant oilseed rape does. Consequently, it is considered to be extremely low that, even if this recombinant oilseed rape could survive, it propagates beyond the roadsides and becomes dominant in the natural conditions kept away from human intervention.

Based on the above understanding, no wild species can be specified as having some effects, the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to competitiveness is valid.

(2) Productivity of harmful substances

Conventional oilseed rape is known to produce erucic acid and glucosinolate in the seeds as harmful substances to human and other mammals. Wester, the recipient

organism of this recombinant oilseed rape, is one of the cultivars known as Canola which features reduced contents of erucic acid and glucosinolate achieved by selective breeding. In fact, the component analysis of this recombinant oilseed rape has confirmed that erucic acid and glucosinolate contents fall within the threshold specified for Canola cultivars.

This recombinant oilseed rape produces the modified CP4 EPSPS protein and GOX v247 protein which contribute to confer the tolerance to glyphosate, though there is no report showing these proteins function as harmful substances. EPSPS protein is an enzyme which catalyzes the shikimate pathway for aromatic amino acid biosynthesis, but it is known that EPSPS is not a rate-determining enzyme in this pathway, and as such it is not considered that enhanced EPSPS activity will increase the concentration of aromatic amino acids, the end products of this pathway. In addition, EPSPS protein is an enzyme which specifically reacts with the phosphoenolypyruvate (PEP) and shikimate-3-phosphate (S3P), therefore it is not considered that CP4 EPSPS protein catalyzes reaction of any other substances and produces different substances. GOX protein is an enzyme which catalyzes the reaction to degrade glyphosate into aminomethylphosphonic acid and glyoxylate, but it has high substrate specificity, and it is considered that its modified type GOX v247 protein does not act on any other metabolic systems of plants.

In addition, in Japan, the productivity of harmful substances (including secretion from roots to affect the other plants, secretion from roots to affect microorganisms in soil, and substances in the plant body to affect the other plants after dying out) has been investigated in the isolated fields, but there is no significant difference between recombinant oilseed rape and non-recombinant oilseed rape.

Based on the above understanding, no wild species can be specified as having some effects, the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to productivity of harmful substances is valid.

(3) Crossability

In 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* L.) include *B. rapa* L. (turnip, Komatsuna, conventional rapeseed, etc.) of the genus *Brassica*; *B. juncea* (L.) Czern (mustard, leaf mustard, etc.); *B. nigra* (L.) W.D.J.Koch (black mustard) and *Raphanus raphanistrum* L. (radish) in addition to oilseed rape itself. Oilseed rape, *B. juncea*, *B. nigra*, and *R. raphanistrum* are all introduced species reportedly brought into Japan artificially after Meiji period. In addition, *B. rapa* is also a cultivar-derived introduced species though it was introduced to Japan in olden times. As such, these are not specified as wild specifies as to be possibly affected.

Based on the above understanding, no wild species can be specified as having some effects, the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to crossability is valid.

(4) Additional information

i) Possibility of indirect Adverse Effect on Biological Diversity attributable to

crossing

Regarding the possibility of indirect Adverse Effect on Biological Diversity caused by crossing with related species including this recombinant oilseed rape and oilseed rape in natural environment in Japan, the following (a) and (b) were evaluated.

(a) Crossbreds produced by crossing become competitive and destroy population of other wild animals and plants.

(b) Transferred genes from crossing act to reduce population of related species, thereby compromising the preservation of population of insects and other wild animals and plants which rely on the related species.

1) Possibility of indirect Adverse Effect on Biological Diversity caused by crossing with oilseed rape (*B. napus*)

Cross pollination of oilseed rape by wind- or insect-pollination is reported 5 to 30%.

As a result of crossability tests in isolated fields in Japan and tests in non-closed system greenhouse for fertility of pollen, dispersion of pollen, flower-visiting behavior of honey bees and other such reproduction characteristics, it is suggested that this recombinant oilseed rape is comparable to non-recombinant oilseed rape in the crossability.

Based on above understanding, there is a possibility that this recombinant oilseed rape could grow at roadsides or other places from fallen seeds or pollens dispersed from cultivated areas and cross with oilseed rape growing at roadsides or riverbanks, thereby causing crossbreds.

However, as discussed in (1), it is unlikely that the glyphosate herbicide tolerance conferred by the modified *cp4 epsps* and *gox v247* transferred into this recombinant oilseed rape offers competitiveness under natural environment, therefore, it is considered extremely low that the crossbreds between this recombinant oilseed rape and non-recombinant oilseed rape become more competitive compared to non-recombinant oilseed rape and exterminate population of other wild animal and plant species.

In addition, it is hard to consider that penetration of herbicide tolerant genes rapidly reduces the population of oilseed rape.

2) Possibility of indirect Adverse Effect on Biological Diversity attributable to crossability with *B. rapa, B. juncea, B. nigra* and *R. raphanistrum*

B. rapa, B. juncea, B. nigra, and *R. raphanistrum* are all growing voluntarily in wastelands and roadsides in Japan. Overseas literature search indicates that crossability with oilseed rape is 0.4 to 13% for *B. rapa*, 3% for *B. juncea*, and very low for *B. nigra* and *R. raphanistrum*. In addition, even in the event of crossing, these related species are different from each other in the number of chromosomes and compositions, and as

such it is suggested that there is a mechanism of crossbred breakdown causing significant decreased fertility of pollens or seeds of inter-specific crossbreds produced by crossing.

The isolated field tests in Japan have shown that the natural crossability of this recombinant oilseed rape with *B. rapa* and *B. juncea* is highest in the zone at an adjoining distance of 0 m, 8.5% with *B. rapa*, 3.4% with *B. juncea*, and 0% with the both species in the zone at a distance of 10 m. In addition, as mentioned in 1), it is shown that crossability of this recombinant oilseed rape is similar to that of non-recombinant oilseed rape. Based on the results, it is considered that the crossability character of this recombinant oilseed rape with related species including *B. nigra* and *R. raphanistrum*, for which no test has been conducted, is not significantly different from that of non-recombinant oilseed rape.

Consequently, it is unlikely that this recombinant oilseed rape could cross with *B. rapa*, *B. juncea*, *B. nigra*, and *R. raphanistrum* and even in the event of crossing, it is considered extremely low that the transferred genes could penetrate into the population of these species.

Based on the above understanding, the conclusion made by the applicant that there is no risk of indirect Adverse Effect on Biological Diversity attributable to crossability is valid.

ii) Possibility of Adverse Effect on Biological Diversity attributable to occurrence of recombinant virus

For this recombinant oilseed rape, 35S promoter of *Figwort mosaic virus*（FMV） of the genus *Caulimovirus* is used. In Japan, FMV is not distributed, but as the virus belonging to the genus *Caulimovirus*, *Carnation etched ring virus*（CERV）, *Cauliflower mosaic virus*（CaMV）, *Dahlia mosaic virus*（DMV）, and *Strawberry vein banding virus*（SVBV） are distributed, and among these, CaMV is known to make the genus *Brassica* the recipient organism. In response to this fact, a possibility was examined whether recombinant virus could emerge through homologous recombination in which 35S promoter of FMV is caught into CaMV.

However, the homology of nucleotide sequences between FMV35S promoter used in this recombinant oilseed rape and CaMV35S is 10% or less for the 553bp nucleotide sequence equivalent almost to the total length, though there exists 56bp region showing the homology of 68%, therefore, it is considered extremely low that homologous recombination could occur. Consequently, it is also considered extremely low that recombinant virus will emerge.

Based on the above discussion, it was judged that the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to occurrence of recombinant virus is valid.

2. Conclusion

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 valid.

Annex List

Annex 1 Sequence of the Genetic Elements in PV-BNGT04

[Not made available or disclosed to unauthorized person]

Annex 2 Excerpts from "Part 1 - Safety Assessment of Roundup Ready® Canola - Executive Summary -" submitted in February 1996 to the Ministry of Health, Labor and Welfare (former Ministry of Welfare and Labor)

[Not made available or disclosed to unauthorized person]

Annex 3 Confirmation of the Genomic DNA Sequences Flanking the 5' and 3' Ends of the Insert in Roundup Ready Canola Event RT73

[Not made available or disclosed to unauthorized person]

Annex 4 Program for application of recombinant plant: Recombinant rapeseed tolerant to glyphosate herbicide (Application in a simulated environment)

[Not made available or disclosed to unauthorized person]

Annex 5 Program for application of recombinant plant: Recombinant rapeseed tolerant to glyphosate herbicide (Application in an open system)

[Not made available or disclosed to unauthorized person]

Annex 6 Factual investigation on falling oilseed rape imported for active ingredient

(Reference address) http://www.s.affrc.go.jp/docs/press/2004/0629.htm

Annex 7 *Figwort mosaic virus* 35S promoter used in glyphosate herbicide tolerant rapeseed (RT73)

[Not made available or disclosed to unauthorized person]