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	Cotton tolerant to glyphosate herbcide (*cp4 epsps*, *Gossypium hirsutum* L.) (1445, OECD UI : MON-Ø1445-2)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, 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

a) The common name: Cotton; The academic name: *Gossypium hirsutum* L.; Upland cotton

b) The recipient organism is a tetraploid cultivar of cotton (*Gossypium hirsutum*), Coker 312, which belongs to the genus *Gossypium* of the family Malvaceae.

c) The wild species of the genus *Gossypium* are distributed in arid, tropical and subtropical regions. Based on its geographical distribution, diploid wild species are classified into 3 categories: an Australian group (11 species), an African/Arabian group (8 species), and an American group (12 species).

In addition to the diploid wild species, there are tetraploid wild species growing in the Americas, which are classified into the following species: *G. tomentosum* (Hawaii), *G. mustelinium* (northwest Brazil), *G. darwinii* (Galapagos), *G. lanceolatum* (Mexico), *G. barbadense* (Antilles Islands, Central and South America), and *G. hirsutum* (Central America). Spontaneous *G.hirsutum* is rarely found in clumps, and in many cases they grow dispersed along the coastline or on islets. In Japan, natural distribution of *G. hirsutum* or any plant of the genus *Gossypium* that can hybridize with tetraploid cultivars of cotton has not been reported.

(2) History and present state of Use

a) The genus *Gossypium* consists of 41 species and subspecies. The wild species of the genus is found in the Old and New Continent, Africa, and Australia, and its place of origin is believed to be India, Mexico and Peru. It is regarded that cotton was first introduced into Japan in 799 from India. However this cotton seems to have disappeared soon after. Cotton seeds were then introduced to Japan again through Kyushu between 1592 and 1595, and cultivation of cotton spread in the Kanto and southward area of Japan. In 1882-1887, cotton cultivation expanded to 100,000 hectares and 24,000 tons were produced. However, the industry waned after that due to pressures from imported cotton. At present, commercial cultivation of cotton is scarcely carried out in Japan; it is mainly cultivated only as an ornamental plant. The cultivar that has long been cultivated in Japan is considered to be Asian cotton, *G. arboreum.*

b) The genus *Gossypium* consists of 41 species. Among them, cultivated species are classified into 2 categories: diploid species (n=13) generically called “Asian cotton”, *G. herbaceum* and *G. arboreum*; tetraploid species (n=26) generically called “Upland cotton”, *G. hirsutum* and *G. barbadense*. At present, the cultivation

of “Asian cotton” is practiced only in certain limited areas in India, Africa and Asia. On the other hand, “Upland cotton” accounts for about 98% of cotton produced in the world, of which *G. hirsutum* represents 90%.

Since collected seed cotton contains seeds, it is ginned using a cotton ginning machine to separate lint, and is called cotton or raw cotton. Cotton is used for cotton products including such as cotton thread and cotton fabric, for guncotton, and for filling material. The remains after the separation of lint from seed cotton are seeds. On their surface, the seeds have short fiber of 3-5 mm in length on average (called short fuzz or original fuzz). Such fiber is scratched using a linter removing machine, and are called linters. Linters are produced as secondary product at oil mills, and used to make artificial fiber and guncotton. Relatively long linter can also be used for coarse yarn. Seeds without linter have 17-23% of oil content; these seeds are expressed, or extracted in solvent, to obtain cottonseed oil. About 130 kilograms of cottonseed oil is obtained from 1 ton of cottonseed, and is used as a raw material for margarine and soap, in addition to cooking oil. The oil-cake that remains after oil extraction is purified and used mainly for feed and manure.

According to statistical information of the US Department of Agriculture, the total area of cotton cultivation in the world in 2002 was 29.43 million hectares. Top cotton producing countries include India (7.60 million hectares), the US (5.03 million hectares), China (4.18 million hectares) and Pakistan (2.80 million hectares).

In 2002, Japan imported about 150,000 tons of cottonseed, 96% of which was from Australia. Among the imported cottonseed, about 40,000 tons were used for oil expression, and most of the rest was used for cattle feed. In Japan, only one oil company, in Osaka Prefecture, obtains oil by expressing cottonseeds imported from overseas. At present, cottonseeds for cultivation are mostly imported from the US, and are mainly cultivated as ornamental plants. These cottonseeds for cultivation are imported by a certain nursery company. According to this company, it is ensured by PCR method that the cottonseeds for cultivation imported through third parties from the US are those of non-recombinant cotton.

(3) Physiological and ecological properties

i) Basic properties

Cotton is a perennial plant of the family Malvaceae, that reproduce by seed propagation. It grows to a plant height of 90-120 cm, having 15-20 nodes with a leaf and 2 buds each, and forms vegetative and bearing shoots. It is perennial only in tropical regions in principle, and is annual in Japan.

ii) Environmental conditions allowing inhabiting or growth

The average temperature of 25ºC is optimum and 20-28ºC is suitable for inhabitation and growth of cotton. A considerable amount of rainfall is required during the growing season, and a precipitation of 1,000-1,500 mm/year is suitable. A lot of rain after the flowering season enhances abscission of flowers and bolls, while insufficient rain reduces the lint percentage. In North America, cotton is grown in the range latitude 37-

39 degrees north. Cotton is distributed up to latitude 42 degrees north in Europe, latitude 44.3 degrees north in Central Asia, and latitude 43 degrees north is the northern limit of the hemisphere for cotton growing in general. In Japan, the southern end of Ouu District (latitude 37.5 degrees north) is regarded as the northern most limit. Well-drained sandy loam being the most suitable, cotton prefers alkaline soil over acidic soil. The crop also grows on reclaimed land containing considerably high salt concentrations.

iii) Mode of propagation or reproduction

a) Ripe seeds appear when a boll opens, and usually do not easily shed because they are covered with lint. The level of seed dormancy is extremely low.

b) The propagation of cotton is based on seed propagation, not on vegetative propagation. So far, there were no reports that tissues or organs have budding property that can regenerate the plant body under natural conditions.

c) The mode of pollination of cotton is basically self-pollination, though it is known that crossability is also possible. In Japan, wild relatives that can hybridize with cotton are not known.

d) Since the pollen of cotton is relatively heavy and sticky, crossability by wind pollination is unlikely to occur. Instead, the pollen is sometimes transferred by bumble bees (*Bombus* sp.) or honey bees (*Apis mellifera*). However t, it is only in a limited range that pollens are dispersed by insect pollination. According to a study where fluorescent particles were applied to pollens so as to trace pollens dispersed to neighbor flowers, dispersed pollens were observed in only about 1.6% of flowers in flower fields 45-60 m away from a cotton field around which beehives were arranged. Besides, it was reported that another test showed that the percentage of crossability was under 0.4% in flowers planted 1 m away from a cotton field, while it was down to under 0.3% in flowers 16 m away. Moreover, according to a result of crossability tests using a marker gene of genetically recombinant cotton, the percentage of crossability was 5% in flowers planted 1 m away from a cotton field of 30×136 m, while it was under 1% in flowers 7 m away. However, the percentage of crossability under 1% was also found in some flowers planted farthest away from – or 25 m away from – the cotton field.

iv) Productivity of harmful substances

Productivity of substances that may have an effect on the inhabitation or growth of wild animals or plants, such as allelo chemicals, is not known.

v) Other information

Cotton contains gossypol, a kind of terpenoid. This physiologically active substance exists in secretory organs in all plant tissues including the seeds. Gossypol causes inflammation in the visceral organs and lungs of mammals, and is known as one of the toxic substances that causes dyspnea and paralysis in

experimental animals. However, wild mammals that eat cottonseeds are not reported.

By the way, there has been no report stating that seeds of cotton spilled on the ground during transportation germinated and became self-seeding in Japan.

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 cotton tolerant to glyphosate herbicide (*cp4 epsps, Gossypium hirsutum*) (1445, OECD UI: MON-Ø1445-2) (hereinafter referred to as "this recombinant cotton") and the origin of components are shown in Table 1 (Page 7).

ii) Functions of component elements

a) Functions of component elements of donor nucleic acid that was used for the development of this recombinant cotton are as shown in Table 1 (Page 7).

Glyphosate is the active ingredient in 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 enzymes in the shikimate pathway for aromatic amino acid biosynthesis, by specifically binding to the enzyme. As a result, plants treated with glyphosate cannot synthesize aromatic amino acids essential for protein synthesis due to the inhibition of EPSPS, and die. The target gene, *cp4 epsps* gene, expresses the CP4 EPSPS protein which has high tolerance to the herbicide glyphosate. The activity of the CP4 EPSPS protein that is produced by *cp4 epsps* gene is not inhibited even under the presence of glyphosate, thus, recombinant plants that express this protein have normal functions of shikimate synthesis and can grow.

In plants, EPSPS exists in chloroplasts or plastids. The shikimate pathyway is an important metabolic pathway that is considered to be involved in one fifth of carbon fixation by plants. This pathway is regulated by 3-deoxy-D-arabino-heptulosonate-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 are inhibited or suppressed by metabolic intermediates or end products of this pathway. This suggests that EPSPS is not the rate-determining enzyme, 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. In addition, Monsanto Co. examined amino acid contents in the seeds of the recombinant crops in the process of food/feed safety assessment of crop plants (soybean, coleseed, cotton, and maize) that are tolerant to the herbicide Roundup, 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 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 phosphates (Pi) from phosphoenolpyruviate (PEP) and shikimate-3-phosphate (S3P), and is known to specifically react with these substrates. 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 reacts as the substrate of EPSPS in the living body.

This recombinant cotton has tolerance to the herbicide glyphosate, as a result of the expression of CP4 EPSPS proteins. Therefore, after the first application of glyphosate to a non-plowed field before seeding, weed control is achieved by additional applications of glyphosate in consideration of weed growth, through the fourth leaf of growing cotton. It has saved cotton farmers in the US 3,000 tons of herbicide per year as well as labor for weed control such as herbicide application and the others.

The *nptII* gene was used as a selectable marker in the development of Roundup Ready cotton 1445. An *nptII* gene encodes an enzyme protein, neomycin phosphotransferase type II (NPTII), which transfers the terminal phosphate group of adenosine 5'-triphosphate (ATP), to a hydroxyl group at the aminoglycoside region of antibiotics. As a result, aminoglycoside antibiotics such as paromomycin and kanamycin become inactivated. In general, these aminoglycoside antibiotics bind specifically with a protein on ribosome in a cell; then protein synthesis is inhibited, and the cell is killed. However, when these antibiotics are phosphorylated by NPTII protein, they cannot bind with the target protein on ribosome any longer. Consequently, they cannot kill cells due to the absence of the ability to inhibit protein synthesis.

b) In order to investigate whether the CP4 EPSPS protein and NPT Ⅱ protein share functionally important amino acid sequences with known allergens, the CP4 EPSPS protein and NPT Ⅱ protein were compared with allergens in the database (GenBank, EMBL, PIR, NRL3D, Swiss Prot). As a result, the CP4 EPSPS protein and NPT Ⅱ protein did not share structurally related homologous sequences with any of the known allergens examined.

Table 1 Component elements of the expression vector PV-GHGT07

Component elements	Origin and Function
Cp4 epsps gene expression cassette	
CmoVb	35S promoter of figwort mosaic virus. Involved in the constant expression of the target gene in all tissues.
ctp2	A sequence in the *epsps* gene of *Arabidopsis thaliana* that encodes chloroplast transit peptide located at N-terminal region of EPSPS protein. Transports CP4 EPSPS protein to the chloroplast where aromatic amino acids are synthesized.
cp4 epsps	5-enol-pyruvyl-shikimate-3-phosphate synthase gene derived from *Agrobacterium* CP4 strain.
E9 3'	3' untranslated region of pea ribulose-1, 5-bisphosphate carboxylase E9 gene. Terminates transcription of mRNA and induces polyadenylation.
nptII gene expression cassette	
P-35S	35S promoter region of cauliflower mosaic virus (CaMV). Involved in the constant expression of the target gene in all tissues.
nptII(Kan)	A gene that was isolated from Tn5 transposon of *E. coli.* Encodes neomycin phosphotransferase type II (NPTII) enzyme protein.
NOS 3'	3'untranslated region of nopaline synthase (NOS) gene from T-DNA of *Agrobacterium tumefaciens*. Terminates transcription of mRNA and induces polyadenylation.
gox gene expression cassette	
CmoVb	35S promoter of figwort mosaic virus. Involved in the constant expression of the target gene in all tissues.
ctp1	A sequence that encodes chloroplast transit peptide located at N-terminal region of small subunit 1A of rubisco derived from *A. thaliana.* Transports GOX protein to chloroplast where aromatic amino acids are synthesized.
gox	A sequence that encodes C-terminal of v247, a variant derived from glyphosate oxidoreductase (gox) of *Achromobacter* sp. strain LBAA. GOX protein dissolves glyphosate
NOS 3'	3'untranslated region of nopaline synthase (NOS) gene from T-DNA of *Agrobacterium tumefaciens*. Terminates transcription of mRNA and induces polyadenylation.
Other component elements	
ori-V	The replication origin isolated from the broad-recipient range plasmid RK2. Permits autonomous replication of vectors in *Agrobacterium tumefaciens.*
aad	The gene encoding the Tn7 adenyltransferase (AAD). Confers resistance to spectinomycin or streptomycin.
Right border sequence	A DNA sequence containing right border sequence (25bp) of nopaline type T-DNA derived from Ti plasmid pTiT37. Used as the initiation point of T-DNA transfer from

(Right Border)	*Agrobacterium tumefaciens* to plant genome.
ori322	Replication origin region isolated from pBR322, a plasmid derived from *E. coli*. Permits autonomous replication of vectors in *E. coli.*
rop	Derived from *E. coli*. Regulates the number of plasmids to be replicated in *E. coli.*

(2) Information concerning vector

i) Name and origin

The plasmid vector used to generate this recombinant cotton is constructed from several vectors such as pBR322 derived from *E.coli.*

ii) Properties

The total number of base pairs of this plasmid vector is 12,032 bp.

The plasmid vector pBR322 is a double strand circular DNA which has tetracyclin/ampicillin resistance as a selectable marker for construction vector in *E.coli*, and ori sequence, the origin of DNA replication.

The infectivity of this plasmid vector is not known.

(3) Method of preparing living modified organisms

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

The component elements which were transferred into the recipient organism include the *cp4 epsps* gene expression cassette, the nptII gene expression cassette and the additional genetic elements CmoVb, ori-V, aad and the Right Border sequence. All of these elements are shown in Table 1 (Page 7) .

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

The T-DNA of plasmid vector PV-GHGT07 was introduced into a current cotton cultivar, Coker 312, by *Agrobacterium* transformation.

iii) Processes of rearing of living modified organisms

a) The T-DNA of plasmid vector PV-GHGT07 was introduced into the hypocotyls of Coker 312 by *Agrobacterium* transformation, and then regenerated individuals were obtained by culturing them in media containing kanamycin.

b) The transformants were cultured in media containing carbenicillin and cefotaxime. The transformants were then cultured embryo germination media containing no antibiotics. Thus, it was ensured that there was no remaining

Agrobacterium.

c) Regarding the obtained regenerated individuals, further selection was carried out based on the analysis of inserted genes and expression level of CP4 EPSPS. Tests in climate chamber and greenhouse were then carried out, and actual glyphosate tolerance and agronomic characters were examined in outdoor field tests. This recombinant cotton was selected by comprehensive evaluation of these results.

The following shows the approvals received from organizations abroad.

July 11, 1995: The United States Department of Agriculture (USDA) approved unlimited cultivation of Roundup Ready cotton 1445.

September 11, 1995: The US Food and Drug Administration (FDA) approved the safety of Roundup Ready cotton 1445 as food and feed.

February 21, 1996: The US Environmental Protection Agency (EPA) approved the use of herbicide glyphosate for growing cotton.

September 14, 2000: The Australian Interim Office of Gene Technology Regulator (IOGTR) approved Roundup Ready cotton 1445 safe as feed and for the environment.

November 24, 2000: Food Standards Australia New Zealand (FSANZ) approved the safety of Roundup Ready cotton 1445 as food.

June 19, 2003: The Australian Office of Gene Technology Regulator (OGTR) approved Roundup Ready cotton 1445 being safe as feed and for the environment.

The following shows the approvals received from organizations in Japan.

December 9, 1997: 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.

December 16, 1997: Based on the “Guideline for the conduct of Food Safety Assessment of Food and Additives derived from Recombinant-DNA Plants, Chapter 4”, safety of use for food was approved by the Ministry of Health, Labour and Welfare.

January 12, 1998: 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)”.

March 30, 2001: The Ministry of Health, Labour and Welfare ensured the safety of use of the cultivar for food, in accordance with “Safety Evaluation Criteria for Food and Additives derived from Recombinant-DNA techniques”.

March 27, 2003: The Ministry of Agriculture, Forestry and Fisheries ensured 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”.

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

Based on Southern blotting analysis, it was confirmed that one set of the copy of genes was inserted at one site in the genome of this recombinant cotton. Also, Southern blotting analyses where CMoVb promoter, *gox* gene, *cp4 epsps* gene, *aad* gene, and *nptII* gene were used as probes, indicated that CMoVb promoter, *cp4 epsps* gene, *aad* gene, and *nptII* gene were inserted in the genome of this recombinant cotton, but *gox* gene was not. By these analyses, it was confirmed that one copy of the T-DNA region (*cp4 epsps* gene, *aad* gene, *nptII* gene) excluding *gox* gene was inserted in the genome of this recombinant cotton. Anyway, a simple screening was conducted by PCR method for R0 generation (this indicates the first generation of the recombinant cotton. Hereinafter, the number of generation advance by selfing or other forms is shown as a suffix to R). It was confirmed that *gox* gene had not been inserted into the cotton genome from the beginning. Also, the genetic map of the inserted genes was finalized by determining both neighbor sequences of the inserted genes. An *aad* gene, which were used as a marker to perform the screening of *E. coli* and *Agrobacterium tumefaciens*, was also inserted to the genome of this recombinant cotton. However, the *aad* gene is not expressed in plants, because it fails to have a promoter that functions in plants. In actual analysis, the amount of AAD protein, the product of *aad* gene, was under the detectable limit of ELISA assay (0.025 ng per 1 mg of the fresh weight of seed tissue, 0.013 ng per 1 mg of the fresh weight of leaf tissue).

In addition, as a result of Southern blotting analysis of R3 and R5, the inserted genes were stably inherited in offspring. Besides, ELISA analysis of R4 and R5 seeds indicated that CP4 EPSPS protein and NPTII protein are stably expressed.

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

For the detection and identification of this recombinant cotton, a qualitative PCR method has been developed where the DNA sequences of the inserted genes and neighboring areas of plant genome are used as primers. This method makes it possible to specifically detect this recombinant cotton.

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

i) The CP4 EPSPS protein that is encoded by the *cp4 epsps* gene is constantly expressed in all tested tissues of this recombinant cotton.

ii) To compare R5 generation of this recombinant cotton and recipient Coker312 as the control, isolated field tests were carried out at an isolated field in Kyusyu National Agricultural Experiment Station from May 1997 to October 1997.

(a) Morphological and growth characteristics

Differences in the following 19 items of morphological and growth characteristics were examined between this recombinant cotton and non-recombinant control cotton: the uniformity of germination; germination rate; plant type; stem height; flowering time; flower color; leaf shape; the number of effective flower buds; the number of bearing shoots; boll opening time; the color of fiber (lint); the shape of bolls (fruits of cotton); the number of bolls per plant; the number of segments of a boll; the number of seeds per boll; the color of seeds; harvest time; the dry weight of a boll; and the weights of above- and under-ground parts at the harvest time .

For the statistical analysis of the following items among those above, 5 individual plants were selected from each row of each plot: plant type; stem height; the number of effective flower buds; the number of bearing shoots; the color of fiber (lint); the shape of bolls (fruits of cotton); the number of bolls per plant; the number of segments of a boll; the number of seeds per boll; the color of seeds; the dry weight of a boll; and the weights of above- and under-ground parts at the harvest time. As for the analysis of bolls among these items, 2 bolls were selected from each individual plant. With regard to the analysis of the following items, including uniformity of germination, germination rate, flowering time, boll opening time, and harvest time, all individuals were analyzed. As a result, statistically significant differences ($p<0.05$) between this recombinant cotton and non-recombinant control cotton were found in germination rate, leaf length, and the minor axis of a boll, but not in other items.

In the analysis of germination rate, in which a statistical difference was observed, the germination rate of non-recombinant control cotton was 95% on average, while that of this recombinant cotton was as low as 55% on the average of three replications. However, it was proved that there was no difference in germination rate between this recombinant cotton and non-recombinant control cotton, through field tests conducted at about 65 fields, mainly in the US and Puerto Rico for 3 years between 1992 and 1994. Besides, using the 1445 line and Coker 312 which were cultivated in the Dominican Republic, germination tests were conducted where seeds were sown in petri dishes under 2 conditions – warm condition (31°C and 24°C, day and night) and cool condition (19°C) – and 7 days after, germination rates were examined. Also in these tests, no difference was found in germination rates between them. Also, we made contact with the Head Quarters of Monsanto Co. in the US, and received information as follows: both the seeds were collected at the same experimental plot in 1996; first, non-recombinant control cotton seeds were collected; however, when the recombinant cotton seeds were collected, there was a heavy rain, causing the cracking of seed coat in many seeds; therefore, the quality of this recombinant cotton seeds was poor compared to those of non-recombinant control cotton. Based on the above, it was concluded that the differences in germination rate observed in the isolated field was caused by the poor quality of seed samples of this recombinant cotton owing to the cracking of seed coat, not by the inserted genes.

Statistically significant differences were observed in leaf length, and the minor axis of bolls. In this recombinant cotton and non-recombinant control cotton, the average values of leaf length were 17.8 cm and 17.1 cm respectively, and the average values of minor axis of bolls were 3.5 cm and 3.2 cm respectively.

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

A chilling-tolerance test was not conducted during the isolated field tests. Instead, concerning whether seedlings after germination can overwinter, the germination rate and overwintering ability were examined by seeds collected in 1994 from R5 generation cultivated in 3 fields in the US (Tifton, Georgia [GA]; Starkville, Mississippi [MS]; and Loxley, Alabama [AL]), sowing directly to the ground in all three locations. All of these 3 regions are famous for cotton cultivation in the South of the US. Besides, compared to average climatic conditions in Japan, the winter coldness in these regions is relatively mild. Therefore, it is considered that these regions provide better climatic conditions for cotton growth than Japan.

The results of the examination show that only a few (0.3%) of the seeds, which were sown in the field in Loxley, AL on October 18, germinated on December 15. However, all of them had died by January 17 of the following year. After that, seeds showed no germination while observed up to April 27. In the other 2 fields, no germination was observed in sown seeds.

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

Basically cotton is a perennial plant, but only in tropical regions. In other cotton cultivation regions in the world, including Japan, cotton naturally dies in winter seasons after fruit-bearing. In practice, it was observed that the plants were partly dead when the isolated field tests of this recombinant cotton were completed. Based on the above, the wintering ability test was not conducted for adult plants.

(d) Fertility and size of the pollen

In Japan, there are no plans for this recombinant cotton to be sold, and commercial cultivation of cotton itself is not carried out. In addition there have been no reports concerning the natural distribution of plants of the genus *Gossypium* which can hybridize with this recombinant cotton. Therefore, if the recombinant cotton cause Adverse Effect on Biological Diversity in Japan, it would be in the following way: cotton seeds imported for oil extraction and feed are spilled during transportation; after that, such spilled seeds grow or become self-seeding at places which are not under human control, and expel other plants from the area. Hence, in the isolated field tests of this recombinant cotton, the main point of examination was to see the possibility that cotton seeds spilled during transportation could germinate right there, and grow or become self-seeding. On the other hand, the fertility and the size of pollen were not examined, because pollen is not formed until spilled cotton seeds mature after germination, and because there have been no reports that seeds

spilled during transportation grow and become self-seeding under natural conditions in Japan.

(e) Productivity, dormancy, and germination rate of the seeds

Regarding seed production, the differences between this recombinant cotton and non-recombinant control cotton were examined in "a) Morphological and growth characteristics", as the number of bolls, the number of segments of a boll, the number of seeds per boll. As a result, no difference was observed between this recombinant cotton and non-recombinant control cotton.

It is known that the level of seed dormancy of cotton is extremely low. Also, it is known that the longevity of cotton seeds is short under natural conditions, and most of them decay in soil if sown before a period when soil temperature reaches 15-16°C. As described in b), seeds collected in autumn were soon sown in 3 fields in the US (Tifton, GA; Starkville, MS; and Loxley, AL), and observed until the next spring; as a result, no difference was observed between this recombinant cotton and non-recombinant control cotton. Based on the result, this recombinant cotton seeds decayed in soil because of low soil temperature, like the non-recombinant control cotton seeds. Therefore, it was considered that this recombinant cotton seeds have extremely poor survival ability in low temperature and extremely low overwintering ability, so the test concerning dormancy was not conducted.

Germination rate was examined in "a) Morphological and growth characteristics". As a result, statistical difference was observed between this recombinant cotton and non-recombinant control cotton: the average germination rate of this recombinant cotton was 55%; while that of the non-recombinant control cotton was 95%. However, as described in "a) Morphological and growth characteristics", it was concluded that the differences were caused by the poor quality of seed samples of this recombinant cotton owing to the cracking of seed coat, not by the inserted genes.

(f) Crossability

In Japan, no wild relatives exist that belong to *Gossypium* which is hybridized with tetraploid cotton cultivar (*Gossypium hirsutum)* to which this recombinant cotton belongs. Thus, crossability was not assessed.

(g) Productivity of harmful substances

As described in d), if this recombinant cotton produces harmful substances and cause Adverse Effect on Biological Diversity in Japan, it would be in the following way: cotton seeds imported for oil extraction and feed are spilled during transportation; after that, such spilled seeds grow or become self-seeding at places which are not under human control, and expel other plants from the area. Hence, in the isolated field tests of this recombinant cotton, the main point of examination was to see the possibility that cotton seeds spilled during transportation could germinate right there, and grow or become self-

seeding. On the other hand, the productivity of harmful substances was not examined, because harmful substances are not produced in the root or aerial parts of the plant until spilled cotton seeds germinate and become matured to a certain extent, and because there are no reports that seeds spilled during transportation grow and become self-seeding under natural conditions in Japan.

3. Information concerning the Use of living modified organisms

(1) Content of the Use

Provision as food, provision as feed, processing, storage, transportation, disposal and acts incidental to them.

(2) Information obtained from Use abroad

Between 1992 and 1994, field tests were conducted for this recombinant cotton at 65 fields in the US. In the tests, the cotton was evaluated by observing the following items: characteristics regarding weediness; characteristics regarding morphology and growth; characteristics regarding yields; pest sensitivity; and ability to become self-seeding (volunteers). However, no difference was observed between this recombinant cotton and non-recombinant control cotton.

The *cp4 epsps* gene, which contributes to the tolerance to herbicide glyphosate, was introduced to such crops as soy bean, coleseed, maize and cotton. Since 1996, these crops have been commercially cultivated in many countries, especially in the US. It is estimated that, by 2002, the cultivation area of crops tolerant to herbicide glyphosate will have reached 48.6 million hectares, equivalent to about 1.3 times as large as the total area of Japan. Of such area, it is estimated that this recombinant cotton will be cultivated in about 2.2 million hectares of fields mainly in the US. So far, there is no report that crops tolerant to herbicide glyphosate, including this recombinant cotton, caused Adverse Effect on Biological Diversity.

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

1. Competitiveness

In Japan, cotton (*Gossypium hirsutum* L), to which the recipient organism belongs, has been used under Type I Use Regulation. However there is no report that it becomes self-seeding in Japan.

Thanks to the introduction of *cp4 epsps* gene, this recombinant cotton has tolerance to herbicide glyphosate. But it is unlikely that glyphosate will serve as selection pressure under the natural environment. Also, studies at the isolated field in Japan proved that there are no differences in character concerning competitiveness between this recombinant cotton and non-recombinant control cotton, except small differences found in the leaf length and the minor axis of a boll. Therefore it is unlikely that this recombinant cotton will have better propagating and survival ability than non-recombinant control cotton.

Based on the above understanding, no wild plants and wild animals possibly affected by the competitiveness of this recombinant cotton are identified. Therefore, it was judged that the conclusion by the applicant that the use of this recombinant cotton poses no significant risk of Adverse Effect on Biological Diversity attributable to c**ompetitiveness** is reasonable.

2. Productivity of harmful substances

Regarding cotton, to which the recipient organism belongs, there is no report that it produces harmful substances that adversely affects wild plants or wild animals.

This recombinant cotton produces CP4 EPSPS protein, which contributes to the tolerance to glyphosate. But it is not reported that this protein is a harmful substance. EPSPS protein is an enzyme that serves as a catalyst in shikimate pathway where aromatic amino acids are synthesized. However, it is known that EPSPS protein is not a rate-determining factor in the pathway. Moreover, it is proved that there is no change in the amount of aromatic amino acids produced in other recombinant cotton to which *cp4 epsps* was introduced. Hence, it is unlikely that aromatic amino acids are produced excessively in the recombinant cotton. In addition, EPSPS is an enzyme that specifically reacts with phosphoenolpyruvate and shikimate-3-phosphate. Therefore, it is unlikely that CP4 EPSPS protein catalyzes a reaction of other substances to produce different substances.

Content of the Type 1 Use of this recombinant cotton does not include “cultivation”. As discussed in the section of “competitiveness”, there is no report that cotton becomes self-seeding in Japan. Therefore, it is unlikely that this recombinant cotton will be more competitive than non-recombinant control cotton.

Based on the above, no wild plants and wild animals possibly affected by the productivity of harmful substances of this recombinant cotton are identified. Therefore, it was judged that the conclusion by the applicant that the use of this recombinant cotton poses no significant risk of Adverse Effect on Biological Diversity attributable to the productivity of harmful substances is reasonable.

3. Crossability

In the Japanese natural environment, there are no wild species which can hybridize with cotton. Therefore, it was judged that there are no specific wild plants or wild animals that are possibly affected by the crossability of this recombinant cotton, and that the conclusion by the applicant that the use of this recombinant cotton poses no significant risk of Adverse Effect on Biological Diversity attributable to crossability.

III. Comprehensive assessment of Adverse Effect on Biological Diversity

Consequently, it was judged that there is no risk of Adverse Effect on Biological Diversity in Japan attributable to the use of this recombinant cotton for provision as food, for provision as feed, processing, storage, transportation, disposal and acts incidental to them.