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	Maize tolerant to glufosinate herbicide (*pat*, *Zea mays* subsp.*mays* (L.) Iltis) (T14, OECD UI :ACS-ZMØØ2-1)
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 preparation of living modified organisms

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of donor nucleic acid and the origins of component elements used for the production of maize tolerant to glufosinate herbicide (*pat, Zea mays* subsp. *mays* (L.) Iltis, T14, OECD UI :ACS-ZMØØ2-1) (hereinafter referred to as "the recombinant maize T14") are shown in Table 1.

Table 1 Component elements of donor nucleic acid in vector pUC/Ac, and their position, size, origin and functions

Component elements (abbreviation)	Position in vector	Size (bps)	Origin and function
Modified *pat* cassette			
P-35S	1746～1217	531	35S RNA promoter derived from Cauliflower Mosaic Virus. It expresses modified *pat* genes in plants constitutively (Odell *et al*.,1985).
Modified *pat*	1188～637	552	It encodes PAT protein and gives tolerance to glufosinate herbicide, derived from *Streptomyces viridochromogenes* (Eckes *et al*.,1989). This gene is a modified type of natural *pat* gene to adapt to plants*.
T-35S	618～412	207	35S RNA terminator derived from Cauliflower Mosaic Virus. It terminates transcription and induces polyadenylation of transcripts (Pietrzak *et al*.,1986).
Others			
bla	3783～2923	861	It is an ampicillin resistant gene derived from *E.coli*. It expresses β-lactamase only in bacteria (Sutcliffe, 1978).
ori-pUC	2714～2164	551	It is the replication origin (ColE1) of pUC18, and initiates replication of plasmid (Yanisch-Perron *et al.*,1985).

※ Since the natural *pat* gene obtained from *Streptomyces viridochromogenes* contains G:C (Guanine: Cytosine) in such a large amount as not observed frequently in plants, the introduced modified *pat* gene is a modified type of the natural *pat* gene whose sequence is modified to fit codons used in the plant. The amino acid sequence of the enzyme which is produced by this modification remains unchanged (Eckes *et al*.,1989; USDA 1995). Structure of nucleotide sequence of the modified *pat* gene is shown in Figure 1.

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Figure 1 Comparison between the nucleotide sequences of modified *pat* gene and natural *pat* gene

2) Function of component elements

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

Functions of component elements of donor nucleic acid which were used for the productionof the recombinant maize T14 are shown in Table 1.

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

[Modified *pat* gene]

In the process of nitrogen metabolism, plants produce ammonia by nitrate reduction, amino acid degradation, photorespiration, and other chemical actions. Glutamine-synthetase plays an important role in detoxification of the ammonia produced, though the glutamine-synthetase is inhibited if plants are sprayed with glufosinate herbicide, ammonia accumulates, and the plants die.

On the other hand, in the plant body to which the modified *pat* gene is introduced, phosphinothricin acetyl transferase (PAT) is produced, and this enzyme acetylates the glufosinate to transform it to N-acetylglufosinate. This action prevents the inhibition of glutamine-synthetase by the glufosinate, ammonia is not accumulated in the plant body, and the plant does not die even if it is sprayed with glufosinate (Figure 2).

It is reported that the PAT protein is not toxic to humans and other animals, and it shows no significant homology except with PAT proteins derived from various other species as a result of search for any homology with amino acid sequence of all proteins registered in the GENBANK database (OECD, 1999). In addition, as a result of comparison of physico-chemical and biochemical characteristics of PAT protein with known allergen, it was considered that the possibility of this protein to possess allergenicity is extremely low (USEPA, 1997).

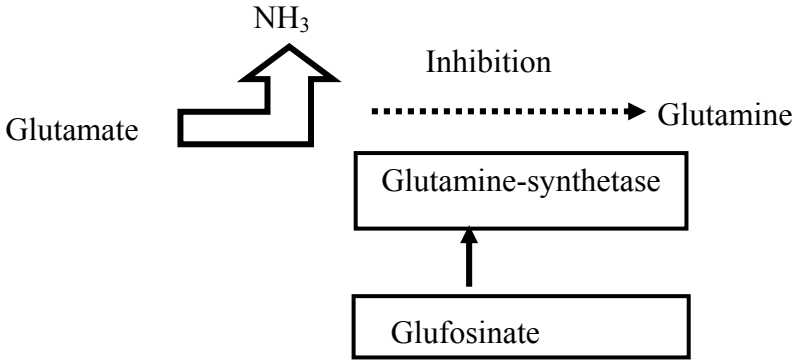
Moreover, the modified *pat* gene sequence was compared with all nucleotide sequences which were published in the EMBL database (European Molecular Biology Laboratory, Germany, Release 40.0, September 1994). In addition, regarding PAT protein, homology search was performed by SWISSPROT database (Geneva, Switzerland, Release 30.0 from September 1994). The result in both cases shows no significant homology except with PAT proteins derived from various other species. Consequently, no homology with known allergen was observed.

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

The PAT protein produced by the modified *pat* gene exhibits a high affinity to glufosinate classified into *L*-amino acid, though it does not cause any acetyl group transfer reaction to the other various amino acids and it has little affinity for the glutamic acid which has specifically high structural similarity to glufosinate and it causes virtually no transfer reaction *in vivo*(Thompson *et al*., 1987). In addition, even in the presence of excessive amount of various amino acids, the acetyl group transfer reaction to glufosinate by PAT protein was never inhibited (Wehrmann *et al*., 1996; OECD, 1999). As a result, it is considered that the PAT protein possesses high substrate specificity and it does not affect the metabolic system of the recipient organism.

A) Normal Plant

The plant dies if ammonia accumulates in the plant body due to the inhibition of glutamine-synthetase caused by the effect of glufosinate herbicide.



B) Recombinant Plant

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

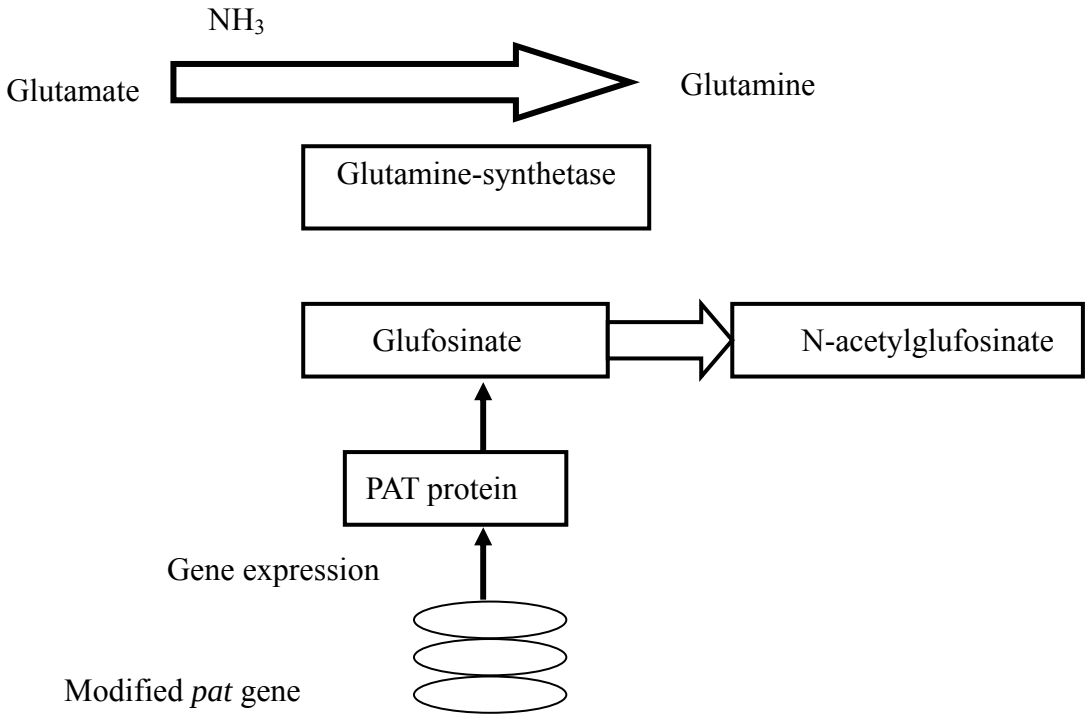


Figure 2 Mechanism of tolerance to glufosinate herbicide by the product of modified *pat* gene

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

(2) Information concerning vectors

1) Name and origin

The vector used for the production of the recombinant maize T14 is pUC/Ac (Pietrzak *et al.*, 1986). This vector is composed of the modified *pat* gene inserted at the section of Sal I between the 35S promoter of pDH51 produced from pUC18 derived from K12 strain of *Escherichia coli* and the terminator.

2) Properties

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

The total number of base pairs of the pUC/Ac used for the production of the recombinant maize T14 is 3,983bp. The nucleotide sequence of pUC/Ac is shown in Annex 1.

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

[*bla* gene]
The pUC/Ac contains the *bla* gene to confer the resistance to ampicillin and the ori-pUC to serve as the region of the autonomous replication origin (Table 1).

The *bla* gene was used as a selective marker for construction of vector using the *Escherichia coli*, though this gene does not possess any promoter which functions in plants, and therefore, it never be expressed in any plant body.

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

The pUC/Ac does not possess a transferring ability, the infectious characteristics of this vector is not known.. In addition, it is known that the range of recipient organisms for the autonomous replication of pUC/Ac is limited to *Escherichia coli* and a few gram-negative bacterias.

(3) Method of preparing living modified organisms

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

The region other than the modified *pat* gene cassette【〔P-35S〕-〔PAT〕-〔T-35S〕】between the two sections of EcoR I (405bp and 1747bp) on the pUC/Ac constitutes pUC18 which served as the origin of pDH51. Structure of the entire pUC/Ac is shown in Figure 3.

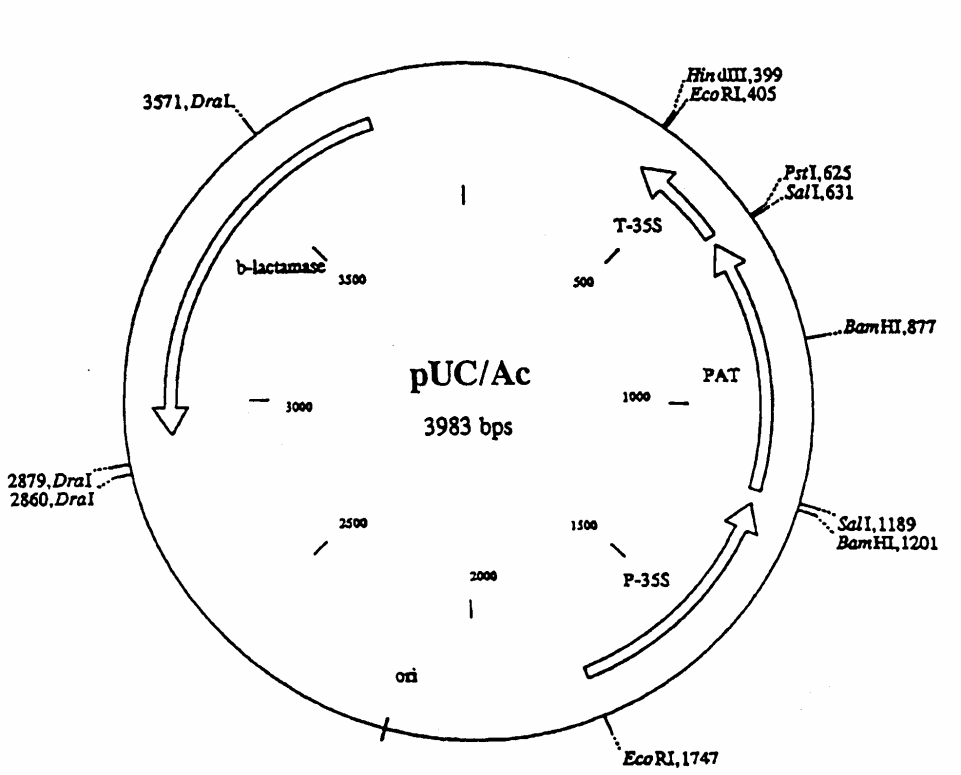


Figure 3 Vector pUC/Ac used for the production of transgenic plant of the recombinant maize T14

PAT refers to the modified *pat* gene, and b-lactamase refers to the *bla* gene.

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

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

pUC/Ac was mixed with the protoplast culture fluid of He/89 derived from tissue culture, and transformed by Polyethylene-glycol method.

3) Processes of rearing of living modified organisms

(a) Mode of selection of the cell in which nucleic acid is transferred

After transformation, and after forming micro-colonies from 20 to 50 protoplasts, they were moved to a solid medium containing 0.5mM glufosinate. Then, the glufosinate-tolerant callus that survives among the transformed cells was selected. The selected callus was regenerated to the plant body according to the method of Mórocz *et al.* (1990).

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

This item is not applicable.

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

The regenerated young plant body was acclimatized, planted in a pot, and raised in an phytotron . The recombinant maize T14 obtained in this way was crossed with non-recombinant elite inbred lines as a pollen parent and then, a group of lines, which is comparable to the elite inbred line in genetic background except the introduced gene, was raised for rearing.

The pedigree tree is shown in Figure 4.

[The approvals received from organizations in Japan]

－ 1996: Based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries," conducting isolated field test was approved by the Ministry of Agriculture, Forestry and Fisheries.

- March 7, 1997: Based on the "Procedure to confirm the safety of feed and feed additives derived from recombinant-DNA technology", safety of use for feed was approved by the Ministry of Agriculture, Forestry and Fisheries.
- May 26, 1997: Based on the "Guideline for food safety Assessment of food and food additives derived from Recombinant-DNA technology", safety of use for food was approved by the Ministry of Health and Welfare (The Ministry of Health, Labour and Welfare, currently).
- 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 confirmed by the Ministry of Agriculture, Forestry and Fisheries.

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Figure 4 Process of rearing of the recombinant maize T14

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

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

As a result of glufosinate herbicide-spraying tests using the BC1 generation raised by backcrossing of the modified *pat* gene-introduced generation T14 (the original transformant), a segregation ratio of 1 : 1 was obtained between glufosinate-torelant and glufosinate-sensitive individuals (Annex 3). In addition, as a result of Southern blotting analysis using the genome DNA of the recombinant maize T14, it was confirmed that the transferred nucleic acid was inserted into the chromosome (Annex 3).

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

In order to identify the number of copies of transferred nucleic acid, Southern blotting analysis using the genome DNA isolated from the recombinant maize T14 and PCR analysis with various primers were conducted. As a result, it was suggested that 3 copies of DNA derived from vector pUC/A were inserted in the chromosome of the recombinant maize T14 (Annex 2).

Between the 3 copies, no difference is found in the gene composition from 35S promoter to the modified *pat* gene, though in the other regions, a difference was observed in the composition between the individual copies. It was confirmed that there exist the following three copies; [i) A copy containing relatively small deletion between the 5'-terminal of 35S terminator and the 5'-terminal of *bla* gene with the missing the 3'-region of *bla* gene], [ii) A copy containing relatively large deletion between the 5'-terminal of 35S terminator and the 5'-terminal of *bla* gene with the missing the 3'-region of *bla* gene], and [iii)A copy being intact without any gene deletion between the 5'-terminal of 35S terminator and the 5'-terminal of *bla* gene with any unknown sequence estimated to be inserted in the *bla* gene though the *bla* gene found inserted in the intact form].

In the copy of [iii] described above, regarding the unknown sequence inserted in the *bla* gene, the nucleotide sequence was identified by the sequencing (Annex 2). As a result, the 104bp sequence in the *bla* gene sequence was found tandem-repeated. It is considered that the *bla* gene has lost its function due to the nonsense mutation induced in the *bla* gene by the repetition.

In all of the three (3) copies, the *bla* gene sequence is incomplete and thus, it is considered it does not function in the plant body. Northern blotting analysis using the *bla* gene as a probe was conducted regarding RNA extracted from the leaves of the recombinant maize T14, though it was confirmed that the transcript of *bla* gene was not detected (Annex 3). In addition, also regarding activity of β-lactamase, the product of *bla* gene, an activity assay was conducted for decomposition of ^{14}C-labeled penicillin with the crude protein extracted from the leaves, roots and seeds and as a result, the activity of β-lactamase was below the limit of detection in all of the tissues assayed (Annex 3). Based on the above understanding, it was confirmed that the *bla* gene does not function in the recombinant maize T14.

In order to confirm the stability of inserted nucleic acid in multiple generations, Southern blotting analysis was conducted for the DNA obtained from the original transformant of the recombinant maize T14 and the BC5 generation after 5 times of back-crossing. In this analysis, the genome DNA was digested with EcoR I or BamH I , separated by agarose gel electrophoresis, and transferred to nylon membrane. Then the genome DNA was hybridized with ^{32}P-labeled modified *pat* gene (552bp Sal I fragment). The pattern of hybridization is found remaining unchanged in multiple generations and then, the stability of the inserted nucleic acid in multiple generations was confirmed (Annex 3).

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

Based on the Southern blotting analysis and PCR analysis, it was suggested that 3 copies of the inserted gene were introduced in the genome of the recombinant maize. Estimated positions of the introduced copies are shown in Annex 2, though detail position relationship is not clear. However, based on the findings from Southern blotting analysis that the original transformant of the recombinant maize T14 and the BC5 generation possess the same band pattern (Annex 3), it is estimated that the individual copies are at the positions from which they cannot separate easily.

4) The stability of the expression among individuals and generations under natural conditions with respect to the physiological or ecological characteristics that were accompanied by the expression of replication products of transferred nucleic acid

In an isolated greenhouse test at the National Agriculture Research Center for Hokkaido Region in 1997, glufosinate was sprayed to the seedlings of the recombinant maize T14 and the control variety of LH202×LH172 (hereafter referred to as "non-recombinant ") which were germinated from seeds sworn on a tray. As a result, the individuals of the non-recombinant control maize all died under the influence of the herbicide, whereas all the individuals of the recombinant maize T14 were confirmed to have survived (Annex 5).

Northern blotting analysis using the modified *pat* gene as a probe was conducted for all RNAs extracted from the leaves of the recombinant maize T14 and the control variety of LH82. As a result, the presence of the transcript of the modified *pat* gene was observed in the recombinant maize T14, though the transcript of the modified *pat* gene was not detected in the non-recombinant control maize. Consequently, it was confirmed that the modified *pat* gene is expressed in the recombinant maize T14 (Annex 3).

In addition, in safety studies conducted in the USA from 1992 onwards, enzyme activity of PAT protein in each part of the plant was measured using roots, leaves, stems, matured pollens and matured seeds of the recombinant maize T14. As a result, PAT protein’s activity was detected highest in leaves, low in seeds, and below the detection limit in pollens. Consequently, it was confirmed that the modified *pat* gene is expressed in the green tissues and the active PAT protein is produced (Annex 3). Based on the above understanding, it was suggested that the constitutive expression is induced in the tissues under the control of 35S promoter.

Moreover, in the USA, in a total of six (6) areas in 1993 and in a total of three (3) areas in 1994, the recombinant maize T14 was cultivated and harvested. Regarding the content of PAT protein in various feeds, forage, fodder, silage and grains, derived from the recombinant maize T14 harvested in the individual areas, measurement for the PAT protein content was conducted based on the ELISA method. The PAT protein content varied according to the cultivated area and the year, though it was confirmed that the individual feeds contained the PAT protein (Annex 3).

Overall, based on the results discussed above, it was confirmed that the PAT protein in the recombinant maize T14 is stably expressed among generations and individuals under natural condition.

5) Presence or absence, and if present, degree of transmission of nucleic acid transferred through virus infection and/or other routes to wild animals and wild plants

The vector used for production of the recombinant maize T14 contains no transferring factor and therefore, the vector never causes transmission ability.

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

Specific detection method for the recombinant maize T14 is available by PCR method using the nucleic acid of the plant genome of the inserted gene and its surroundings as 21mer primers. By using 50ng DNA, nearly 100% was able to be detected, so if the seeds and plant body of the recombinant maize T14 are very small in quantity, detection and identification is possible. The result of high reproducibility was obtained in replicated tests (Annex 6).

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

1) Specific contents of physiological or ecological characteristics that were accompanied by the expression of replication products of transferred nucleic acids
With the production of the PAT protein due to the expression of the transferred modified *pat* gene, tolerance to glufosinate herbicide is conferred to the recombinant maize T14.

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

In 1997, isolated field tests were conducted at National Agriculture Research Center for Hokkaido Region for comparison between the recombinant maize T14 and the non-recombinant plants (Annex 5).

(a) Morphological and growth characteristics

As a examination of the growth characteristics, for the imported seeds, comparison was made for the average values of time of germination and germination rate, time of tasseling, time of tassel opening, time of silking, date of harvesting, degree of maturity at harvest time, lodging, and incidence of sooty blotch/spot leaf blight. As a result, the germination rate of the imported seeds was found 97% for the non-recombinant and 79% for the recombinant maize T14, showing the recombinant maize T14 is inferior to the non-recombinant. In addition, it was also observed that the time of silking was slightly later in the recombinant compared to the non-recombinant, and that the progress of maturity was also relatively later in the recombinant. In the other characteristics examined, the recombinant and the non-recombinant were found equivalent to each other (Annex 5).

As an examination at the time of harvesting, comparison was made for the culm length, height of ear, culm diameter, total number of leaves, leaf length, leaf width, node number of bearing 1st ear, rate of sterile individuals, cob length, ear length, ear diameter, row number per ear, and grain number per row. As a result, regarding the average values, the recombinant and non-recombinant were observed equivalent to each other or the recombinant maize T14 had a tendency showing relatively lower values (Annex 5).

As a yield examination, comparison was made for the fresh yield [stems and leaves, ear, the entire body (kg/10a)], dry yield [stems and leaves, ear, fruit body, the entire body (kg/10a)], dry matter yield [stems and leaves, ear, the entire body (%)], dry ear weight ratio and 100-kernel weight. As a result, in all the characteristics examined, the recombinant maize T14 had the tendency showing lower values compared to the non-recombinant (Annex 5).

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

Cold-tolerance test at the early stage of growth of the plant body was not conducted. Instead, sensitivity to low temperatures of the seeds was examined for reference. The seeds of the recombinant maize T14 and the non-recombinant control maize were sown in the Wagner's pots, 150 seeds for each (50 seeds×3 repeats) under the highly humid soil condition, exposed to a low temperature of -0.2℃ for 82 days, and examined for the germination at 25℃ for 7 days. As a result, the germination rate was 0% for the recombinant maize T14 and 1.3% for the non-recombinant control maize, showing germination of smaller quantity of seeds of the non-recombinant, though the seeds of the both plants were observed very low tolerance to low temperatures (Annex 5).

(c) Wintering ability of the matured plant

This item is not applicable.

(d) Fertility and size of the pollen

The tolerance to glufosinate herbicide is the only characteristics conferred to the recombinant maize T14 and there exists no wild relative that can be crossed in Japan, therefore this item is not applicable.

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

For the characteristics referring to the production of seeds, dry yield (fruit body) and 100-kernel weight of the recombinant maize T14 and the non-recombinant were compared. As a result, for the average values of the both characteristics examined, the recombinant maize T14 indicated smaller values compared to the non-recombinant maize (Annex 5).

The corncob firmly holds the kernels of maize and the ears are entirely covered with bracts, and then individual seeds can never shed and scatter naturally (OECD, 2003). In addition, it was confirmed that the seeds of both recombinant maize T14 and non-recombinant are firmly attached to the corncob and covered with bracts and thus, the shedding habit was considered to be extremely low.

In the isolated field tests in Japan, no tests were carried out on the germination rate and dormancy of seeds harvested from cultivated plant bodies and then, the results of tests conducted in 2 areas in the USA in 1994 were used for reference. The germination rates of the seeds harvested from the recombinant maize T14 and the non-recombinant maize were both very high between 92.7 % and 96.3% (Annex 4).

Dormancy test was not conducted, though it was reported in the field tests and cultivation experiences in the USA that the recombinant maize T14 does not exhibit higher dormancy. It is also reported that the level of seed dormancy of maize is extremely low and the seeds can germinate at temperatures of 10℃ or more even under considerable dry conditions (Encyclopedia of Crops Changing, Vol. 3, 2001). Moreover, it is generally known that the seeds of maize may be susceptible to delayed germination under low-temperature and high-humidity conditions and they are likely to become decayed and die in most cases before germination (Encyclopedia of Farming, 1981).

(f) Crossability

In Japan, the growth of wild relatives (teosinte) that can be crossed with maize in natural environment has not been reported. Therefore, this item is not applicable.

(g) Productivity of harmful substances

No test was conducted for this item. The possibility of growth of the recombinant maize T14 in Japan is considered to come up in cases when any seeds commingled in the imported seeds for cultivation are planted otherwise spilled during transportation and such spilled seeds germinate and grow. However, in actuality, 5 years or more have elapsed after cultivation of the recombinant maize T14 was discontinued in 1999 and there is no cultivation program in the future, therefore, it is considered that the possibility of commingling in imported cultivation seeds is extremely low. In addition, maize cannot survive without intervention of humans (OECD, 2003) and thus, it is considered that maize is unlikely to become volunteering even if any seeds are commingled in imported cultivation seeds and spilled during transportation. Moreover, also in the past field tests and commercial cultivation in the USA, there are no reports that the recombinant maize T14 has produced any harmful substances. Based on the above understanding, it was judged that there is no

possibility of causing any adverse effects due to the productivity of harmful substances.

2. 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.

Both at home in Japan and abroad, cultivation of the recombinant maize T14 will not be conducted in the future.

(2) The results of Use in laboratory or Use in similar environment to the environment in which Type 1 Use is intended

Isolated field tests were performed in the National Agriculture Research Center for Hokkaido Region in 1997 (Annex 5).

(3) Information obtained from Use abroad

Production of seeds of the recombinant maize T14 for commercial cultivation was started in 1996 in the USA and discontinued in 1997. The commercial cultivation of the recombinant maize T14 lasted for 3 years from 1997 to 1999, and in the winter of 1998 and spring of 1999, the seeds were bought back and the recovered seeds were all disposed of by incineration. The recombinant maize T14 was cultivated under contract and thus, it is considered that the seeds sold were recovered at a very high rate. In addition, based on the fact that production of seeds was discontinued in 1997, it is considered that if any farmers had been keeping the seeds after 1999, they never apply the seeds for commercial cultivation because the seeds became deteriorated in quality over time, and then there is no advantages for farmers who do not accede to the buy-back of seeds.

In 1999, the closing year of commercial cultivation, the planted area of the recombinant maize T14 accounted for 0.06% of the total acreage under maize cultivation in the USA. In addition, commercial cultivation of the recombinant maize T14 was performed only in the USA, and the grains harvested were all consumed in the USA without any export to overseas. Also, since 2000, planting of the recombinant maize T14 has not been done in any country and the seeds are all discarded and thus unavailable at present, so there is no possibility of planting in the future. Moreover, since 1998, production of seeds has been discontinued and therefore, commercial cultivation, selling and/or distribution of the recombinant maize T14 would not be implemented in any country.

The approvals received from organizations abroad (Table 2) and the total planted area and production of the recombinant maize T14 (Table 3) are provided. In Japan, the approval for the safety of use for feed was obtained on March 7, 1997 by the Ministry of Agriculture, Forestry and Fisheries, and the approval for the safety of use for food was obtained on May 26, 1997 by the Ministry of Health and Welfare (The Ministry of Health, Labor and Welfare, currently).

Table 2 Approvals for the recombinant maize T14 received from organizations abroad

Country	Approval organization	Time of approval	Content of approval
USA	US Department of Agriculture (USDA)	June 1995	Safety confirmation for environment
	Food and Drug Administration (FDA)	December 1995	Safety of use for food and feed
Canada	Canada Department of Agriculture and Agri-Food	May 1996	Safety confirmation for environment
		March 1997	Safety of use for feed
	Health Canada	April 1997	Safety of use for food

Table 3 Total planted area and production of the recombinant maize T14 in the USA

Year	Total planted area of maize (ha) [1)]	Estimated planted area of T14 (ha) [2)]	Total production of maize (ton) [1)]	Estimated production of T14 (ton) [3)]
1997	29,409,000	88,227	233,867,008	745,518
1998	29,376,000	[4)]117,504	247,882,000	992,909
1999	28,525,000	16,187	239,548,992	136,780
Subtotal (1997-1999)	87,310,000	221,918	721,298,000	1,875,207
2000	29,316,000		251,854,000	
2001	27,845,910		241,484,864	
2002	28,050,280		228,805,088	
2003	28,789,240		256,904,560	
Sum total (1997-2003)	201,311,430	221,918	1,700,346,512	1,875,207

1) FAOSTAT (http://apps.fao.org/faostat)

2) In units of hectare obtained by the conversion of estimated planted area in acreage on the assumption that planted maize are all harvested.

3) Values calculated by multiplying the yield per hectare (8.45 ton/ha) converted from the sum of total planted areas of maize and the sum of production of maize from 1997 to 2003 by the estimated planted area of T14.

4) No data available for the planted area in the year concerned, though the listed value was estimated based on the information that T14 was planted in 0.4% of total acreage under maize cultivation at a maximum.

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

Regarding the StarLink, the recombinant maize, which has been approved for safety of use for feed in USA though not approved for safety of use for food, noticed about

detection from food in Japan in 2000 and a recall was done in 2000 in the USA . In addition, monitoring was started with the import in April 2000 by the Fertilizer and Feed Inspection Station of the Ministry of Agriculture, Forestry and Fisheries [The Incorporated Administrative Agency Fertilizer and Feed Inspection Services (FFIS), currently] for possible commingling of StarLink into maize for feeds. As a result, in the second half of 2003, 3 years from the start of monitoring, the samples examined were all found free from any commingling (Annex 7).

The planted area of StarLink in 2000 in the USA was 137,960 ha (accounting for 0.43% of the total planted area of maize in the USA) [Annex 7: Response on StarLink Corn (for use as feed)]. On the other hand, the planted area of the recombinant maize T14 was 133,691 ha (accounting for 0.47% of the total acreage under maize cultivation in the USA in 1999) even in the sum in 1998 and 1999, which is equivalent to the planted area of StarLink in 2000. In addition, the recombinant maize T14 was recalled one year earlier than the StarLink. Based on the above understanding, the commingling rate of the recombinant maize T14 in Japan is estimated lower than that of the StarLink and it is considered to be further decreased in the future.

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 applied 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

Regarding the maize (*Zea mays* subsp. *mays* (L.) Iltis.), to which the recipient organism belongs, cultivation has long been conducted in Japan, though there is no report that it has grown voluntarily in Japan.

This recombinant maize is given a trait to be tolerant to glufosinate herbicide by the transferred modified *pat* gene, though it is generally considered that the glufosinate does not exert selective pressure under a natural environment.

Based on the above understanding, it is judged that 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

For the maize, the biological species to which the recipient organism belongs, there is no report that it possesses productivity of any harmful substances that could affect wild animals and wild plants.

The recombinant maize produces the phosphinothricin acetyltransferase (PAT protein) that inactivates the glufosinate, though there is no report that the protein affectssome adverse effects towild animals and wild plants. In addition, based on the findings that the PAT protein does not transfer any acetyl group to the amino acids which is similar to the glufosinate in structure and that, even in the presence of excessive amount of various amino acids, it never inhibits the acetyl group transfer reaction to glufosinate, and also based on the suggestion that it possesses high substrate specificity, it is considered that the PAT protein does not affect the

metabolic system of the recipient organism.

Moreover, production of seeds and cultivation of this recombinant maize was performed only in the USA, though it has been discontinued since 2000. In addition, the seeds were recovered and disposed of by incineration and therefore, there is no possibility of cultivation in the future. Consequently, the use in Japan is limited to a case when the recombinant plant is commingled in the maize grains imported for use as food or feed.

Based on the above understanding, no wild animals and wild plants are specified to be possibly affected, and it was judged that 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 Japan, the native growth of wild relatives (teosinte) that can be crossed with maize in natural environment has not been reported.

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.

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

Based on the above understanding, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this recombinant maize in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion above made by the applicant is valid.

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