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

Name: Syngenta Seeds K.K.
Hidero Ohtomo, President
Address: 401-2, Mukounodai, Takatsuhara,
Tako-machi, Katori-gun, Chiba Prefecture

Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Cotton resistant to Lepidoptera (Modified *cry1Ab*, *Gossypium hirsutum* L.) (COT67B, OECD UI: SYN-IR67B-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 development of cotton resistant to Lepidoptera (modified *cry1Ab*, *Gossypium hirsutum* L.) (COT67B, OECD UI: SYN-IR67B-1) (hereinafter referred to as "this recombinant cotton") are shown in Table 1 (pNOV4641) and Table 2 (pNOV1914).

Table 1 Composition of the donor nucleic acid pNOV4641 used for the development of this recombinant cotton and the origins and functions of component elements

Component elements	Size (bp)	Origin and function
Insect pest-resistant gene cassette		
Act2 promoter	1,408	Promoter region from the actin gene (actin-2 gene) of *Arabidopsis thaliana*, including the first exon and intron (Reference 20). Involved in the constant expression of the target gene (modified *cry1Ab* gene).
Modified *cry1Ab* gene	3,546	The modified Cry1Ab protein encoded by the modified *cry1Ab* gene is identical to the Cry1Ab protein encoded by the *cry1Ab* gene of *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 in all the amino acid sequences but the additional 26 amino acid sequence called "Geiser motif" in the C-terminal portion of the protein (Reference 21).
NOS terminator	253	Terminator sequence from the nopaline synthase gene of *Agrobacterium tumefaciens*. Its function is to terminate transcription of mRNA by polyadenylation (Reference 22).
Other regions (Plasmid backbone region)		
LB	25	Left border region of T-DNA from *A. tumefaciens* nopaline Ti−plasmid (Reference 23).
spec	789	Streptomycin adenylyltransferase gene (*aadA*) from *Escherichia coli* (*E. coli*) transposon Tn7 (Reference 24). Used as a vector selectable marker to confer resistance to erythromycin, streptomycin, and spectinomycin.
virG	726	A region involved in transfer of T-DNA, derived from *A. tumefaciens* plasmid pAD1289. To ensure the constant expression of traits from *virG*, the 54th amino acid of asparagine is substituted by aspartic acid (Reference 25).

repA	1,074	Replicon (a part of the minimal function replication unit to control the replication of DNA) region of plasmid pVS1 derived from *Pseudomonas* bacteria. Essential gene for maintenance of vector in *A. tumefaciens* (Reference 26).
VS1 ori	405	The replication origin consensus sequence derived from the plasmid pVS1 of *Pseudomonas* bacteria. Functions as the replication origin of plasmid in *A. tumefaciens* (Reference 27).
ColE1 ori	807	Origin of replication of plasmid in *E. coli* (Reference 28).
RB	25	Right border region of T-DNA from *A. tumefaciens* nopaline Ti-plasmid (Reference 29).

Table 2 Composition of the donor nucleic acid pNOV1914 used for the development of this recombinant cotton and the origins and functions of component elements

Component elements	Size (bp)	Origin and function
Selective marker gene cassette		
Ubq3 promoter	1,721	Promoter region including the first intron from the polyubiquitin gene (*ubi3*) of *A. thaliana* (Reference 30). Involved in the constant expression of selective marker gene (*aph4* gene).
aph4 gene	1.026	Phosphotransferase enzyme (hygromycin B phosphotransferase enzyme) gene derived from *E. coli*. Encodes APH4 protein. Catalyzes the phosphorylation of hygromycin and some related aminoglycosides (Reference 31), thereby conferring resistance to hygromycin. Serves as a selective marker for transformed cells for development of this recombinant cotton. This gene is not present in this recombinant cotton, since those individuals in which the *aph4* gene is segregated and only the modified *cry1Ab* gene is transferred have been selected in the inbred progenies.
NOS terminator	253	Terminator sequence from the nopaline synthase gene of *A. tumefaciens*. Its function is to terminate transcription of mRNA by polyadenylation (Reference 22).
Other regions (Plasmid backbone region)		
LB	25	Left border region of T-DNA from *A. tumefaciens* nopaline Ti-plasmid (Reference 23).
trfA	1,149	The gene that encodes the replication initiation protein essential for plasmid replication (Reference 32).
npt3	795	Aminoglycoside 3'-phosphotransferase enzyme Type III gene, conferring kanamycin resistance, derived from Actinomyces *Streptococcu faecalis* (Reference 33).
oRK2	711	Region covering the origin of replication *oriV* of plasmid RK2 (Reference 34)

traJ	372	The gene that encodes the relaxosome protein for plasmid replication (Reference 35).
oriT	40	Origin of plasmid conjugal transfer initiation essential for conjugal transfer of plasmid in bacteria derived from *E. coli* (Reference 35, Reference 36).
virG	726	A region involved in transfer of T-DNA, derived from *A. tumefaciens* plasmid pAD1289. To ensure the constant expression of traits from *virG*, the 54th amino acid of asparagine is substituted by aspartic acid (Reference 25).
tetR	651	Tetracycline resistant repressor gene from *Klebsiella aerogenes* (Reference 37). Controls the expression of tetracycline resistant gene (*tetA*).
RB	25	Right border region of T-DNA from *A. tumefaciens* nopaline Ti−plasmid (Reference 29).

2) Function of component elements

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

Functions of component elements of donor nucleic acids used for the development of this recombinant cotton are listed in Table 1 and Table 2**.**

(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 (excluding allergenicity as food)

Modified Cry1Ab protein

The Cry1Ab protein family, to which the modified Cry1Ab protein expressed in this recombinant cotton due to the modified *cry1Ab* gene belongs, was found to provide the proteins exhibiting the insecticidal activity against the insects of order Lepidoptera produced during the spore forming period of *Bacillus thuringiensis*. The Cry protein is known to become an active polypeptide (core protein) through specific digestion of protein when fed and digested by sensitive species of insects, which specifically binds to the specific receptors on the surface of midgut of insects, causing formation of ion channels and leading to destructed digestive tracts and death of the insects (Reference 37). This mechanism of action is also attained similarly in the Cry1Ab protein derived from *Bacillus thuringiensis* subsp. *kurstaki*.

The modified Cry1Ab protein is composed of 1,181 amino acids. The modified Cry1Ab protein contains an additional 26 amino acid sequence called “Geiser motif” in the C-terminal region. The Geiser motif sequence was added to enhance the production efficiency of the modified Cry1Ab protein at the time of incubation

of *Bacillus thuringiensis* (Reference 21), though it does not function in plants.

All the Cry1 proteins but the Cry1Ab protein possess the sequences equivalent to or very highly homologous with those of Geiser motif by nature, and the Geiser motif sequence is contained also in the Cry1Aa protein, the Cry1Ac protein, the Cry1Ca protein and the Cry1Da protein which have been already applied as biological agrochemicals. Absence of Geiser motif in the unmodified Cry1Ab proteins is considered to result from deletion of Geiser motif from the *cry1Ab* gene (Reference 21). The Geiser motif sequence added to the modified Cry1Ab protein is derived from the Cry1Ac protein.

The amino acid sequence of core protein exhibiting the insecticidal activity in the modified Cry1Ab protein is maintained and found identical to that of the Cry1Ab protein and then, it is considered that the modified Cry1Ab protein and the wild-type Cry1Ab protein are equivalent to each other in the insecticidal activity. For the insecticidal activity of the Cry1Ab protein, the results of study are detailed in the Canadian Government Database (Reference 39), and the Cry1Ab protein exhibits the insecticidal activity against Lepidoptera including Cotton bollworm (*Helicoverpa Zea*), Tobacco budworm (*Heliothis virescens*) and Pink bollworm (*Pectinophora gossypiella*), which are the major pest insects of order Lepidoptera that damage cotton cultivation. In addition, the similar results have been confirmed in the tests using this recombinant cotton. For the Cry1Ab protein and the recombinant plants expressing this protein, possible toxic effects on the non-target living organisms including honeybees and other flower-visiting insects have been evaluated. Based on the evaluation, it was confirmed that the Cry1Ab protein offers a very low toxicity against honeybees and has no toxic effect on the larvae and adults (Reference 15, Reference 40). In addition, it has been also suggested that the recombinant plants expressing the Cry1Ab protein have no adverse effect on the other non-target living organisms (Reference 15, Reference 40).

Moreover, it has been also confirmed based on the homology search using the publicly available protein database (SWISS-PROT, FARRP, etc.) that the amino acid sequence in the modified Cry1Ab protein does not have any homology with known allergens and toxins examined.

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

The modified Cry1Ab protein expressed due to the modified *cry1Ab* gene is considered unlikely to possess any enzyme activity and thus, it is considered to function independently from the metabolic system of recipient organism. Therefore, it is considered extremely low that the modified Cry1Ab protein could affect the metabolic system of the recipient organism.

For the *aph4* gene, two traits (hygromycin resistance and Lepidoptera resistance) were separated, and those individuals were selected that do not contain the *aph4* gene encoding the APH4 protein for use as mother plants for crossing with inbred lines and commercial varieties. Therefore, this recombinant cotton is free from any effect of the APH4 protein on the metabolism of recipient organism.

Based on the above understanding, it is considered unlikely that the transferred genes could affect the metabolic system of recipient organism.

(2) Information concerning vectors

1) Name and origin

For the development of this recombinant cotton, the vectors pNOV4641 and pNOV1914 were used. The pNOV4641 was constructed based on the pBluescript II SK(+) derived from *E. coli*, and the pNOV1914 was constructed based on the pBR322 derived from *E. coli*.

2) Properties

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

The total number of base pairs of the vector pNOV4641 is 10,995 bp, and the nucleotide sequences have been disclosed.

The total number of base pairs of the vector pNOV1914 is 11,727 bp, and the nucleotide sequences have been disclosed.

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

The vector pNOV4641 contains the *spec* gene which expresses the resistance to streptomycin, erythromycin, and spectinomycin as a selective marker for growth of vector in microorganisms, though the gene is not transferred in this recombinant cotton. The vector pNOV1914 contains the *npt3* gene which expresses the resistance to kanamycin as a selective marker for growth of vector in microorganisms, though the gene is not transferred in this recombinant cotton. The *aph4* gene, which is contained in the vector pNOV1914 and expresses the resistance to hygromycin, was used as a selective marker for recombinant plants.

In the generation obtained by self-fertilization, two traits (hygromycin resistance and Lepidoptera resistance) were separated and then, the presence or absence of the modified *cry1Ab* gene and the *aph4* gene was identified based on the TaqMan PCR. As a result, those individuals were selected as parents for line breeding that contain the modified *cry1Ab* gene and do not contain any aph*4* gene. Therefore, the *aph4* gene is not contained in this recombinant cotton.

(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 vectors contain no sequence that exhibits the infectivity.

(3) Method of preparing living modified organisms

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

To the recipient organism, two gene expression cassettes (insect pest-resistant gene cassette and selective marker gene cassette) between RB and LB of T-DNA region of the vector pNOV4641 and the vector pNOV1914 used for the development of this recombinant cotton were transferred.

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

Agrobacterium method was used to transfer the T-DNA region of the vector pNOV4641 and pNOV1419 to the petiole tissues of cotton.

3) Processes of rearing of living modified organisms

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

Transferred genes were cultured on the medium containing hygromycin to select the cells which express the APH4 protein, and the selected cells were regenerated to plant bodies. In addition, those individuals that contain the modified *cry1Ab* gene were identified based on the TaqMan PCR to select only the plant body into which the genes derived from two transformant vectors pNOV4641 and pNOV1914 were transferred.

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

The antibiotic Cefotaxime was added to the medium for selective culture of transformed cells to remove any residual *Agrobacterium* used for transformation. Then, transformed cells were cultured on the medium containing no Cefotaxime and it was confirmed that there is no remaining *Agrobacterium*.

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

From the cells selected after transferring of genes, plant body was regenerated and conditioned then cultivated in a greenhouse. Then, two traits (hygromycin resistance and Lepidoptera resistance) were separated. Based on the TaqMan PCR, presence or absence of the modified *cry1Ab* gene and the *aph4* gene was identified, and those individuals were selected that contain the modified *cry1Ab* gene and do not contain the *aph4* gene.

Regarding this recombinant cotton, Type I Use (Cultivation, storage, transportation, disposal and acts incidental to them in isolated fields) in accordance with the “Law concerning the conservation and sustainable use of biological diversity through regulations on the use of living modified organisms” was approved in May 2007 by

the Ministry of Agriculture, Forestry and Fisheries and the Ministry of the Environment. In addition, application for approval of safety of use for food and application for approval of safety of use for feed will be made sequentially to the Ministry of Health, Labour, and Welfare and the Ministry of Agriculture, Forestry and Fisheries, respectively.

(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 (on the chromosome, in the cell organelle, or in the protoplasm)

Based on the findings that the transferred genes in this recombinant cotton are stably inherited across multiple generations in accordance with the law of Mendelian inheritance, the transferred nucleic acid is considered to exist on the chromosome.

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

Southern blotting analysis was conducted using the T-DNA region and the backbone region of the vector pNOV4641 as probes by breaking the genome DNA extracted from multiple generations of this recombinant cotton through the restriction enzyme treatment. As a result, when the T-DNA region of the vector pNOV4641 including the modified *cry1Ab* gene cassette was used as a probe, the identical band was detected, suggesting that one copy of T-DNA is transferred in multiple generations. Consequently, it was indicated that one copy of the modified *cry1Ab* gene cassette is stably inherited across multiple generations, and it was confirmed that the transferred genes in individual generations are identical to each other. When the backbone region of the vector pNOV4641 was used as a probe, no band was detected in all generations and thus, it was confirmed that the backbone region of the vector pNOV4641 does not exist in this recombinant cotton.

In addition, when the Ubq3 promoter of the vector pNOV1914 or the *aph4* gene region and the backbone region were used as probes, no band was detected in individual generations. Consequently, it was confirmed that the Ubq3 of the vector pNOV1914 or the *aph4* region and the backbone region do not exist in this recombinant cotton.

Based on the above results, it was confirmed that one copy of T-DNA region of the vector pNOV4641 is transferred in the genome in this recombinant cotton at one site and it is stably inherited in offspring.

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

In 2004, in the fields in the US at 4 sites, this recombinant cotton was cultivated, and individual tissue samples were taken at individual growth stages to determine the level of expression of the modified Cry1Ab protein based on the ELISA method. As a result, it was considered that the modified Cry1Ab protein in this recombinant cotton was stably expressed across the individuals and through the generations.

In the selection of this recombinant cotton, the individuals that do not contain the *aph4* gene were selected for use as the parent for inbred lines and backcross lines; therefore, the expression level of the APH4 protein has not been determined.

4) 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 plant

The transferred nucleic acid does not contain any sequence allowing transmission. Therefore, it is considered unlikely that the nucleic acid transferred could be transmitted to any other wild animals and wild plants.

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

Existence of the target genes in this recombinant cotton can be confirmed based on the results of Southern blotting analysis using the modified *cry1Ab* gene as a probe after breaking the genome DNA by the restriction enzyme. In addition, a method for specific detection of this recombinant cotton was developed based on the nucleotide sequence of transferred genes and the nucleotide sequence of the neighboring genome.

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

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

This recombinant cotton is given the trait to be resistant to Lepidoptera due to the modified Cry1Ab protein that is expressed by the modified *cry1Ab* gene. This recombinant cotton, which expresses the modified Cry1Ab protein, exhibits the Lepidoptera resistance against Cotton bollworm (*Helicoverpa zea*), Tobacco budworm (*Heliothis virescens*), Pink bollworm (*Pectinophora gossypiella*) and other pest insects of the order Lepidoptera, which are produced in the cotton cultivation in the US (Reference 38).

2) With respect to the physiological or ecological characteristics listed below, presence or absence of difference between genetically modified agricultural products and the taxonomic species to which the recipient organism belongs, and the degree of difference, if present

In 2007, isolated field tests were carried out at the Kanza Site of Central Research Station, Research Department, Syngenta Japan K.K. using this recombinant cotton and the non-recombinant control cotton.

(a) Morphological and growth characteristics

For the morphological and growth characteristics, examination was conducted regarding the progress of germination, germination rate, flowering date, flower shape and petal color, leaf length and width, boll length and width, boll opening

time (boll opening date of the first boll), the color of fiber, harvest time (the day on which the plant lost the vigor and leaf drop was observed), plant height, the number of nodes, the number of flower buds, the total number of branches, the number of bolls harvested per plant, the total number of bolls per plant, the number of segments of a boll, the number of seeds per boll, seed color and shape, fresh weight of a boll, and the weights of above- and under-ground parts at the harvest time. For the germination rate, leaf length and leaf width, boll length and width, plant height, the number of nodes, the number of flower buds, the total number of branches, the number of bolls harvested per plant, the total number of bolls per plant, the number of segments of a boll, the number of seeds per boll, fresh weight of a boll, and the weights of above- and under-ground parts at the harvest time, statistical treatment was conducted. As a result, in all the items examined but boll width, flowering time and boll opening time, no significant difference or difference was observed between this recombinant cotton and the non-recombinant control cotton. The width of a boll, in which a significant difference was observed, was found 34.8 mm for this recombinant cotton and 33.5 mm for the non-recombinant control cotton. In addition, the flowering time was found September 15 for this recombinant cotton and September 17 for the non-recombinant control cotton. The boll opening time was December 4 for this recombinant cotton and November 28 for the non-recombinant control cotton.

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

The first leaf stage seedlings of this recombinant cotton and the non-recombinant control cotton were cultivated in the low-temperature conditions representing the winter season (10℃ with a 12-hour lighting and 2℃ with a 12-hour darkness) to compare the severity of damage due to the low temperature stress. As a result, this recombinant cotton and the non-recombinant control cotton both died completely, and there was no difference observed in the rate of death between the both plants.

(c) Wintering ability of the matured plant

The plant bodies of this recombinant cotton and the non-recombinant control cotton cultivated in the isolated field tests shed the leaves and died due to the low temperatures and frosting in the winter season. Based on the above findings, it was judged that there would be no difference between this recombinant cotton and the non-recombinant control cotton regarding the wintering ability of the matured plant.

(d) Fertility and size of the pollen

Pollens were collected from this recombinant cotton and the non-recombinant control cotton, and observed under a microscope for comparison of fertility, shape and size of pollens. As a result, no significant difference was observed between this recombinant cotton and the non-recombinant control cotton regarding the fertility of pollen stained with Acetocarmine solution. In addition, the shape of pollen was found circular for the both plants, and no significant difference was observed in the diameter of pollen between the both plants.

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

Regarding seed production, comparison was made for the total number of bolls per plant and the number of seeds per boll and as a result, no significant difference was observed between this recombinant cotton and the non-recombinant control cotton.

Regarding shedding habit of the seed, examination was made for the presence or absence of seeds shed naturally from open bolls at the harvest time and the number of seeds shed. As a result, no seed shed was identified in both this recombinant cotton and the non-recombinant control cotton, showing no difference in the shedding habit between the both plants.

The germination rate was found 95% or more for the seeds from both this recombinant cotton and the non-recombinant control cotton, showing no significant difference between the both plants.

It is generally known that the level of seed dormancy of cotton is low (Reference 12) and then, the test concerning dormancy was not conducted.

(f) Crossability

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

(g) Productivity of harmful substances

Regarding the productivity of harmful substances of this recombinant cotton, the following evaluation tests were carried out in the isolated fields.

Plow-in test:
From each plant body, above-ground parts (leaves and stems) were harvested, and dried and powdered then mixed with soils, to which the seeds of radish were sown as test plant. On the 7th day of sowing, the germination rate was examined, and on the 21st day of sowing, plant bodies were harvested and the dry weight was determined. As a result, no significant difference was observed between this recombinant cotton and the non-recombinant control cotton regarding the germination rate and the dry weight of radish.

Succeeding crop test:
To each soil after cultivating the plant body, the seeds of radish were sown and the seedlings were cultivated in a greenhouse. On the 7th day of sowing, the germination rate was examined, and on the 21st day of sowing, plant bodies were harvested and the dry weight was determined. As a result, no significant difference was observed between this recombinant cotton and the non-recombinant control cotton regarding the germination rate and the dry weight of radishes.

Soil microflora test:
At the time of harvesting of this recombinant cotton and the non-recombinant control cotton, soil was sampled from the cultivation field to measure the number of

colonies of filamentous fungi, bacteria and Actinomyces for the microorganisms in soil based on the dilution plate technique. As a result, no significant difference was observed between this recombinant cotton and the non-recombinant control cotton.

Bibliography

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

Cotton (*Gossypium hirsutum* L) has been long used in Japan, though there is no report that it has become self-seeding in Japan.

This recombinant cotton is given the trait to be resistant to insects of the order Lepidoptera due to the expression of the modified Cry1Ab protein. In addition, as a result of isolated field tests in Japan, regarding the width of bolls among the characteristics relating to competitiveness, a statistically significant difference has been observed between this recombinant cotton and the non-recombinant control cotton, and regarding the flowering period and boll opening time, a difference has been observed between this recombinant cotton and the non-recombinant control cotton. However, it is considered that the insect damage by Lepidoptera is not the major factor restricting the growth of cotton under the natural environment in Japan. Moreover, it is considered that the differences observed in the width of boll, flowering period and boll opening time would not increase the abilities to grow and survive under a natural environment and that this recombinant cotton is unlikely to grow or become self-seeding in Japan.

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

(2) Productivity of harmful substances

For the cotton, the biological species to which the recipient organism belongs, there is no report that it produces any harmful substances such as allelochemicals that could affect wild animals and wild plants.

This recombinant cotton produces the modified Cry1Ab protein which exhibits resistance to pest insects of the order Lepidoptera, though it has been confirmed that there is no amino acid sequence homology with any known allergen and toxin. The modified Cry1Ab protein is considered unlikely to have any enzyme activity and thus it is considered not to affect the metabolic pathway of recipient organism and produce any harmful substances.

There is a concern about possible impacts of pollens of this recombinant cotton on the non-target species of insects of order Lepidoptera, though the pollens of cotton are

relatively heavy and viscous and then, they are considered unlikely to disperse. Even if the pollens of cotton disperse, the extent of dispersing is extremely limited. Then, it is considered low that the non-target species of insects of the order Lepidoptera, which do not feed cotton, would be exposed to the pollens of this recombinant cotton.

In addition, as a result of succeeding crop tests, plow-in tests and soil microflora tests carried out in the isolated field to examine the production of harmful substances of this recombinant cotton (the substances excreted from the roots which can affect other plants, the substances existing in the plant body which affect other plants after dying, and the substances excreted from the roots which can affect microorganisms in soil), no statistically significant difference from the recipient organism was observed in the productivity of all possible harmful substances.

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

(3) Crossability

In the Japanese natural environment, there are no wild species which can cross with cotton. Therefore, it was judged that there are no specific wild plants or wild animals that are possibly affected by this recombinant cotton, and that the use of such cotton poses no risk of Adverse Effect on Biological Diversity that is attributable to crossability. It was judged that the conclusion above made by applicant 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 cotton in accordance with Type ~~1~~ 1 Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion above made by the applicant is reasonable.

Cotton resistant to Lepidoptera
(Modified *cry1Ab*, *Gossypium hirsutum* L.)(COT67B, OECD UI: SYN-IR67B-1)

Outline of the Biological Diversity Risk Assessment Report

Annex List

Annex 1 Comparison of insecticidal activity using the genetically modified cotton that expresses the Cry1Ab protein

Annex 2 Nucleotide sequences of the vector pNOV4641 and the vector pNOV1914

Annex 3 COT67B: Evaluation on the stability of transferred genes based on the segregation ratio

Annex 4 COT67B: The number of copies and the stability of transferred genes in multiple generations

Annex 5 COT67B: Determination of protein expression levels based on the ELISA analysis

Annex 6 COT67B: Event specific detection method

Annex 7 COT67B: Report on the isolated field test results

Syngenta Seeds K.K.