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

Applicant:
Name: Monsanto Japan Limited
Seiichiro Yamane, President
Address: 4-10-10, Ginza, Chuo-ku, Tokyo

Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Pima cotton tolerant to glyphosate herbicide (Modified *cp4 epsps*, *Gossypium barbadense* L.) (MON88913, OECD UI: MON-88913-8)
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) Taxonomy and distribution in nature

1) English name and scientific name

Scientific name: *Gossypium barbadense* L.
English name: Sea island cotton (Pima Cotton)

Scientific name: *Gossypium hirsutum* L.
English name: Upland cotton (Cotton)

2) Name of the variety or line of the recipient organism

G. barbadense (general name: Pima cotton) and *G. hirsutum* (general name: Cotton) are cultivated amphidiploid[1] species of the genus *Gossypium* that belong to the family Malvaceae. The varieties of Pima cotton that were used for crossing were W1377 and W2490.

G. barbadense is an amphidiploid (tetraploid: 2n = 4x = 52) which shares a common chromosome structure (AADD) with *G. hirsutum* (Percy *et al*., 1996; Robinson *et al*., 2001; Kulkarni, 1992; Pandey and Subrahmanyam, 1987; Khalifa *et al*., 1982).

3) The habitat in natural environment in Japan and overseas

G. barbadense is native to the tropical or subtropical regions such as Peru, which is its country of origin, and Ecuador, as well as Brazil, the countries of the Caribbean Basin, and the southern tip of the United States. It is not common that *G. barbadense* grows naturally in clusters. Instead, in many cases, it grows scattered in coastal areas and small islands (Lee, 1984).

It has not been reported that *Gossypium* plants, which are crossable with *G. barbadense* and *G. hirsutum*, grow naturally in Japan (*Wild Flowers of Japan-Trees II*, *Weed Flora of Japan: Illustrated by* Colour).

(2) History and present state of use

1) History of the Type 1 Use in Japan and overseas

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[1] A tetraploid which possesses two types of different genomes is called amphidiploid.

There are four (4) species of cultivated cotton plants, which include *G. herbaceum* and *G. arboreum*, which are diploids (n = 13) and collectively called “Asiatic cottons” of Old World, *G. hirsutum*, which is an amphidiploid (2n = 52) of New World and known as “Upland cotton, American cotton, and Mexican cotton”, and *G. barbadense*, which is known as “Pima cotton, Extra-long staple (ELS) cotton, Sea island cotton, Egyptian cotton, Creole cotton, and Indian cotton”. Each of these four species has been individually domesticated (Brubaker *et al*., 1999; Hiroshi Kurihara ed., 1981, *Industrial Crop*. Rural Culture Association).

G. barbadense is characterized by extra-long staple (ELS), and used as material for high-end textile. On the other hand, *G. hirsutum*, which is a recipient organism of genetically modified cotton, Type 1 Use of which has been approved, is called Upland cotton, and characterized by its medium length of staple. *G. hirsutum* is accounting for 90% of world cotton production, and used as material for textile (Jenkins, 2003).

Since *G. barbadense* is an amphidiploid (tetraploid: 2n = 4x = 52), which shares a common chromosome structure (AADD) with *G. hirsutum*, (Percy *et al*., 1996; Robinson *et al*., 2001; Kulkarni, 1992; Pandey and Subrahmanyam, 1987; Khalifa *et al*., 1982), there is no genetic barrier between *G. barbadense* and *G. hirsutum*. Therefore, it is easy to crossbreed these two species (Percival *et al*., 1999). In fact, cultivar improvement has often been performed by transferring useful trait of *G. barbadense* into *G. hirsutum* (Yuan *et al*., 2000), or transferring a trait of *G. hirsutum* into *G. barbadense* (Wang *et al*., 1995). In some countries, the hybrid between *G. barbadense* and *G. hirsutum* is commercially cultivated. For example, in India, the growing area of this type of hybrid is accounting for approximately 5% of total cultivation area of cotton plants.
(ikisan; http://www.ikisan.com/links/ap_cottonHybrid%20Cotton.shtml).

The species of cotton, which has a long history of cultivation in Japan, is *G. arboreum*, so-called Asiatic cotton. It is believed that the cotton was first introduced into Japan by Indian in 799 A.D., although this species appeared to become extinct soon after that. Later in Bunroku era (1592 to 1595), seeds of cotton plants were reintroduced into Kyushu and the cultivation of cotton plants had spread in the area south of the Kanto region. Around 15^{th} to 20^{th} year of Meiji era (1882 to 1887), the cotton plants were cultivated in the area of approximately 100,000 ha, with total production of approximately 24,000 tons. However, with the increase in the import of cotton from overseas, cultivation of cotton plants in Japan was gradually declined (Hiroshi Kurihara ed., 1981, *Industrial Crop*. Rural Culture Association). Currently, cotton plants are hardly cultivated on a commercial scale in Japan, and they are grown mainly for ornamental purposes.

2) Major cultivation areas, cultivation methods, distribution and use

There are four (4) species of cultivated cotton plants. They include *G. herbaceum*, which is a diploid (n = 13), collectively called "Asiatic cotton" of Old World and cultivated in dry land of Africa and Asia, as well as *G. arboreum*, which is also an diploid and mainly cultivated in India.

The most common cultivated species of cotton are amphidiploid (2n = 52) of New World, *G. hirsutum* and *G. barbadense*, which are cultivated in the major growing areas of the world, including the United States, Europe, China, Africa and Australia (Lee, 1984; Jenkins, 2003).

G. barbadense is mainly cultivated in Egypt, the United States and China, as well as in India, Turkmenistan, Peru, Israel, Sudan, Uzbekistan and Tajikistan. Within the United States, *G. hirsutum* is cultivated in so-called Cotton Belt region of southeastern and southern part of the United States (North Carolina, South Carolina, Georgia, Alabama, Mississippi, Louisiana, Arkansas, Tennessee and Texas), while *G. barbadense* is cultivated in the regions of Arizona, California, New Mexico and Texas, where the climate is drier than that in Cotton Belt region.

According to the statistical information from the United States Department of Agriculture, total acreage of cotton cultivation[2] in the world in 2007/08 was 32,650,000 ha. Top countries include India (9,500,000 ha), China (6,200,000 ha), the United States (4,240,000 ha) and Pakistan (3,250,000 ha) (USDA, Oct 2008, World Agriculture Production).

G. barbadense accounts for approximately 5% of the world fiber production (Wu *et al.*, 2005) and approximately 3% of cotton cultivation acreage.

Although the details of the cultivation acreage of *G. barbadense* in each country have not been made clear, based on the production of 2006 (ICAC, 2009), it is estimated that the cultivation acreage of the top four (4) producers are 240,000 ha in Egypt, 140,000 ha in China, 133,000 ha in India and 131,000 ha in the United States.

The same cultivation method is used for *G. barbadense* and *G. hirsutum*. However, different method of harvesting and ginning are used for *G. barbadense*, in order to protect its characteristic extra-long staple.

Harvested seed cottons, which contain seeds, are transported to a cotton gin, where the lint and seeds are separated. The separated lint, which is called cotton or raw cotton, is used for milling to produce cotton yarn/fabric, as well as for guncotton and fillings. The remainders of the seed cottons after the lint is removed are seeds (cotton seeds). The seeds (cotton seeds) contain 17 to 23% of oil, which is extracted either by pressing or solvent extraction to produce seed oil (cotton seed oil). One (1) ton of seeds (cotton seeds) yield

[2] In the USDA statistics, *G. barbadense* is not distinguished from *G. hirsutum*.

approximately 130 kg of seed oil (cotton seed oil), which is used as cooking oil, as well as raw materials for margarine and soap. The seed meal (cotton seed meal) is used mostly for feed and fertilizer after refining (Hiroshi Kurihara ed., 1981, *Industrial Crop*. Rural Culture Association). Short fiber (average 3 to 5 mm in length), which is attached to the surface of the seeds of *G. hirsutum*, but not to *G. barbadense*, is called a linter. The linter is a byproduct of the process of oil extraction at the factory, and used as raw materials for synthetic fibers and guncotton. Some of the linters, which are relatively long, are used as raw material for thick thread as well.

G. barbadense is used as material for high-end textile due to its superior quality, such as extra-long staple and strength of fiber (Smith, 1999), and thus it is distinguished from *G. hirsutum*, which is characterized by its fiber of medium length (USDA, 2001). In 2007/2008, *G. barbadense* was traded with approximately 85% higher price than *G. hirsutum* in the United States (USDA-AMS, 2008). However, the seeds and its byproduct, cotton seed oil, are traded without any distinction (OECD, 2004; NCPA, 1993, 1997, 1999; USDA-FAS, 2005; O'Brian, 2002).

The amount of import of seed (cotton seed) in Japan in 2008 was 133,173 tons, among which approximately 75% came from the United States, approximately 14% from Australia and approximately 11% from Brazil (2009 Trade Statistics, Ministry of Finance, Japan). Approximately 27,000 tons of imported seeds were used to extract oil, and the rest were used for livestock feed (*Handbook of Feed*. Japan Scientific Feeds Association). Only the imported material is used to produce all seed oil (cotton seed oil) in Japan. A company in Osaka is the only producer of cotton seed oil in Japan.

(3) Physiological and ecological characteristics

G. barbadense possesses many characteristics that are in common with *G. hirsutum*, which belongs to the same genus *Gossypium* and is an amphidiploid. Table 1 shows main similarities and differences between *G. hirsutum* and *G. barbadense*.

Table 1 Main similarities and differences between *G. hirsutum* and *G. barbadense*[3]

Characteristics	*G. hirsutum*/*G. barbadense*	Reference
Similarity		
Number of chromosome	2n = 52 (AADD)	OECD, 2008
Main root length	Approximately 1-3 m	OECD, 2008
Phyllotaxy	Alternate phyllotaxis of 3/8	OECD, 2008; Wise *et al*., 2000
Number of flowers	6-8 flowers/fruiting branch	McGregor, 1976
Number of days from bud to flower	25-30 days	McGregor, 1976
Pollination method	Mostly self-pollination (Some entomophilous cross pollination)	McGregor, 1976; Llewellyn and Fitt, 1996; Galal *et al*., 1972; Reddy *et al*., 1992b
Form of gossypol	Free form and bound form	OGTR, 2008
Honey line location	Back of the leaf and involucral scale	OECD, 2008
Disease resistance	No difference was observed	Wang *et al*., 1995; Brubaker *et al*., 1999
Cold-tolerance	No difference was observed	Christiansen, 1963; Christiansen, 1967; Kittock *et al*., 1988
Pest resistance	No difference was observed	OGTR, 2008
Dormant period	2-3 months	Paiziev and Krakhmalev, 2006; OECD, 2008, OGTR, 2008
Photoperiodism	Neutral	Wang *et al*., 1995; Brubaker *et al*., 1999
Period of reproductive maturity	4-5 weeks	OGTR, 2008
Seed shape	Pointy oval, 8-12 mm in length	OGTR, 2008
Germination time	5-7 days after sowing	Smith, 1995
Flowering time	After 2nd and 3rd branching	OECD, 2008

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

Table 1 (continued) Main similarities and differences between *G. hirsutum* and *G. barbadense*

Characteristics	*G. hirsutum*/*G. barbadense*		Reference
Similarity			
Branch shape[4]	Vegetative branch and fruiting branch		OECD, 2008
Flower shape	Pentamerous perfect flower		McGregor, 1976; OECD, 2008
Difference			
Heat-tolerance	No difference at the early stage of the growth. *G. barbadense* shows higher sensitivity for heat during the reproductive period.		Wang *et al*., 1995; Brubaker *et al*., 1999; Reddy *et al*., 1993; Kittock *et al*., 1987.
Fiber length	Approximately 2.9 cm	Approximately 3.6 cm	SeedQuest
Fiber strength (g/tex)[5]	Approximately 30	Approximately 42	SeedQuest
Presence or absence of linter	Present	Absent	SeedQuest
Frostless period required for cultivation	180-200 days	200-250 days	Reddy *et al*., 2006; Unruh and Silvertooth, 1997; Duke, 1983
Optimal temperature for growth	30-35˚C	25-30˚C	Reddy *et al*., 1992a
Salt tolerance	Present	Present (stronger than *G. hirsutum*)	OGTR, 2008; Ashour and Abd-El'Hamid, 1970
Plant height	1-3 m	1-2 m	Fryxell, 1984; OECD, 2008
Number of leaf margin	3-7	3-5	Fryxell, 1984
Red patterns in petals	Absent	Present	Fryxell, 1984
Petal colour	Pale yellow	Yellow	BeeCulture
Number of segments of a boll	4-5	3	BeeCulture

4 Vegetative branch is, like a main stem, a monopodial branch, whose terminal bud extends to form a main axis, from which side branches are formed by elongation of side buds. Fruit bearing branch is a sympodial branch. With the elongation arrest or flower bud differentiation of the terminal bud, the side bud beneath the terminal bud starts to grow to form main axis by repeating this process.

5 g/tex (grams/tex) is calculated to express the strength of cotton fiber in terms of the strength required to cut 1,000 m of the fiber.

It is reported that the RFLP analysis of the genomes of 54 varieties of *G. barbadense*, which are cultivated in major cotton-growing countries, showed that the genome of *G. barbadense* contains average of 8 to 12% of transferred *G. hirsutum* genome (Wang *et al.*, 1995; Brubaker *et al*., 1999). It is considered that the outcome of this analysis indicates the result of frequent crossbreeding between *G. barbadense* and *G. hirsutum*, as stated in I-1-(2)-1) on p. 2.

In addition, regarding the genetic similarity between *G. barbadense* and *G. hirsutum*, Khan *et al.* reported the result of quantification of genetic similarity among 34 species (partially among the varieties) of cotton plants by use of RAPD method.[6] The results showed that in some cases, the homology between *G. barbadense* and *G. hirsutum* is higher than the homology between different varieties of the same species, with the homology between the different species being 0.788 (*G. barbadense* vs. *G. hirsutum*) and 0.828 (*G. barbadense* vs. *G. hirsutum* Yucatenanse), while the homology between the different varieties of the same species being 0.848 (*G. hirsutum* vs. *G. hirsutum* Yucatenanse) and 0.796 (*G. barbadense* vs. *G. barbadense* Mummy). Therefore, it is considered that *G. barbadense* and *G. hirsutum* are genetically highly similar to each other (Khan *et al.*, 2000).

1) Basic properties

G. hirsutum and *G. barbadense* are seed-propagated perennial crops that belong to the *Malvaceae* family. *G. hirsutum* and *G. barbadense* attain a plant height of 100 cm to 300 cm and 100 cm to 200 cm, respectively. There are 15 to 20 nodes on each plant. Each node bears a leaf and two (2) axillary buds, which give rise to either vegetative branches or fruiting branches. Under cultivation conditions, *G. hirsutum* and *G. barbadense* are grown as an annual shrub, and the plant height is controlled to range between 1 to 1.5 m by use of growth retardant.

2) Environmental conditions allowing inhabiting or growth

The optimal temperature for the growth of *G. barbadense* is in the range of 25 to 30˚C (Reddy *et al.*, 1992a), and at least 200 to 250 days per year of frostless period (average temperature of 21 to 22˚C) is required for the growth (Unruh and Silvertooth, 1997). It is reported that there is not significant difference in cold-tolerance between *G. barbadense* and *G. hirsutum* (Christiansen, 1963; Christiansen, 1967; Kittock *et al*., 1988). It is also reported that Pima cotton experiences cold stress under the temperature condition of 15˚C/10˚C (day/night), and unfavorable effect on the plant growth was observed under this condition (Reddy *et al.*, 1995; Reddy *et al.*, 1993). On the other hand, the optimal temperature for the growth of *G. hirsutum* is in the range of 30 to 35˚C (Reddy *et al*, 1992a), and at least 180 to 200 days per year of frostless period

[6] The homology was roughly calculated by comparing the number of the amplified bands of PCR analysis using 45 random primers (homology of 1.000 means that all the amplified bands are homologous).

(average temperature of 21 to 22˚C) is required for the growth (Duke, 1983).

The sensitivity to temperature of both *G. barbadense* and *G. hirsutum* at the early stage of growth are low (Reddy *et al*., 1993), while the heat sesitivity during reproductive period, when the fruiting branches develop, of *G. barbadense* is higher than that of *G. hirsutum* (Kittock *et al*., 1987). It is known that the number of seeds of *G. barbadense* decreases at the temperature of 35˚C, and when the temperature reaches to 40˚C, the plant stops producing seeds or retaining the cotton bolls. It is considered that these phenomena are attributable to the abscission of flower buds caused by high temperature (Reddy *et al*., 1992a; Reddy *et al*., 1992b; Reddy *et al*., 1993). In addition, it is considered that cotton plants are relatively salt-tolerant, and *G. barbadense* possesses higher salt tolerance than *G. hirsutum* (Ashour and Abd-El'Hamid, 1970).

In North America, the cotton plant is cultivated within an area straddling from 37 to 39 degrees north latitude. In the northern hemisphere, the northern limit of the range of the cotton plant is 43 degrees north latitude in general, while in Europe and Central Asia, the cotton plants range up to 42 degrees north latitude and 44.3 degrees north latitude, respectively. In Japan, the cotton plants range up to the northern part of Fukushima prefecture (37.5 degrees north latitude).

3) Predacity or parasitism

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4) Mode of propagation or reproduction

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

The bolls of *G. hirsutum* are divided into 4 to 5 compartments, while the bolls of *G. barbadense* are divided into 3 compartments (BeeCulture, 2009). The cotton bolls burst open upon maturity; however, shedding habit is low because the seeds are covered with downy fibers (Llewellyn and Fitt, 1966). The dormancy period of the seed is 2 to 3 months (Paiziev and Krakhmalev, 2006; OECD, 2008; OGTR, 2008).

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

Both *G. hirsutum* and *G. barbadense* reproduce through seed production, not through vegetative propagation via tubers and rhizomes. There has been no report indicating the budding characteristics of the tissues and organs, from which a plant body can regrow in natural environment in either species.

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

The method of pollination of both *G. barbadense* and *G. hirsutum* is basically a self-pollination (Niles and Feaster, 1984). It is known that entomophilous cross pollination is also possible, with the pollination rate of 5 to 30% being reported (Kerkhoven and Mutsaers, 2003).

In Japan, there is no known closely related wild variety which can cross with *G. barbadense* and *G. hirsutum* (*Weed Flora of Japan: Illustrated by* Colour; *Wild Flowers of Japan-Trees II*).

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

The quantity of pollen production of cotton plant is approximately 45,000 per flower, and the difference in the quantity of pollen production between *G. hirsutum* and *G. barbadense* has not been reported (McGregor, 1976). A pollen of *G. barbadense* is 118 μm in diameter, with 4.9×10^{-3} prickles per μm^2, which is 15.4 μm in length. On the other hand, a pollen of *G. hirsutum* is 101 μm in diameter, with 8.3×10^{-3} prickles per μm^2, which is 12.1 μm in length (Kakani *et al*., 1999).

Since the pollens of *G. barbadense* are large in size, sticky and heavy, anemophilous cross-pollination hardly takes place. Instead, the pollens of *G. barbadense* are mediated by bumblebees (*Bombus* sp.) and honey bees (*Apis mellifera*) (McGregor, 1976).

Compared to the pollens of *G. hirsutum*, the pollens of *G. barbadense* are larger in size and have long thorn. However, it is considered that there is no difference in the distance the pollens are carried by the bees between these two species (Loper and Davis, 1985). The range of pollen dispersal by insect pollinators is generally limited. It is reported that when the distance between the plants of *G. barbadense* was 2 m or less, the pollination rate was approximately 8%, while the pollination rate decreased to 2% or less when the distance was 8 m, and no pollination was observed when the distance was 20 m (Galal *et al*., 1972).

When dispersion of the pollens of *G. hirsutum* to the surrounding flowers was examined by attaching fluorescent particles to the pollens, the pollen of the cotton plant was detected on approximately 1.6% of the flowers in the flower bed, which sits approximately 45 to 60 m away from the cotton field and has beehives arranged around the edge (McGregor, 1976). In addition, the crossability was 0.4% or less, when the distance from the cotton field was 1 m, and it decreased to as low as 0.03% or less, when the distance was 16 m (Llewellyn and Fitt, 1996). In the crossbreeding test by Umbeck *et al.*, in which the marker gene of *G. hirsutum* was used, the crossability was 5% in the place 1 m away from the cotton field (30×136 m), while the crossability decreased to 1% or less in the place 7 m away. However, the crossability of 1% or less was sporadically observed even in the farthest place, which was 25 m away from the cotton field (Umbeck *et al.*, 1991).

5) Pathogenicity

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6) Productivity of harmful substances

It is known that the cotton does not produce any harmful substances including allelochemicals which can affect the habiting or growth of wild animals and wild plants.

7) Other information

All cultivated cotton varieties contain terpenoid called gossypol in the secretory structures within the plant tissues including seed (Abou-Donia, 1976). It has been known that gossypol is a toxic substance which causes inflammation in the internal organs and lungs of mammals, as well as dyspnoea and paralysis in laboratory animals (*Seikagaku Jiten*, 1992, Tokyo Kagaku Dojin).

It is known that *G. barbadense* has higher gossypol content than *G. hirsutum*, and exhibits resistance to major pests of *G. barbadense* and *G. hirsutum*, leafhopper and spider mite, which belong to genus *Earias* (Reed, 1994; Matthews, 1994; Sengonca *et al.*, 1986; Matthews and Tunstall, 1994; Gannaway, 1994).

All cultivated cotton varieties also contain cyclopropene fatty acids (CPFA) such as dihydrosterculic acid, sterculic acid and malvalic acid, which account for approximately 0.5 to 1.0% of the total fatty acid content in the seeds (Schneider *et al.* 1968). In general, CPFAs content is higher in *G. hirsutum* than in *G. barbadense* (Frank, 1987). Although it is considered that CPFAs exert harmful effects such as discoloration of yolk and decrease in hatchability in chickens, these effects are decreased dramatically by deodorization treatment during the oil extraction process (OECD, 2004).

Gossypol and cyclopropene fatty acids are not the substances which affect the habitat or growth of wild animals and wild plants, such as allochenmicals. There has been no report that shows the example of seed (cotton seed) ingestion by wild mammals. In Japan, natural growth of *G. hirsutum* and *G. barbadense* from the spilled seeds during the transportation has not been reported.

It is also known that the fatty acid composition (Khattab *et al.*, 1977; Kerkhoven and Mutsaers, 2003; Khalifa *et al.*, 1982) and oil composition (Pandey and Thejappa, 1981) of the seed of *G. barbadense* and *G. hirsutum* are nearly identical.

2 Information concerning preparation of living modified organisms

Pima cotton tolerant to glyphosate herbicide [modified *cp4 epsps*, *Gossypium barbadense* L. (MON88913)] (hereinafter referred to as "this recombinant Pima cotton") was developed by transferring the modified *cp4 epsps* gene, which was transferred into glyphosate herbicide-tolerant cotton [modified *cp4 epsps*, *Gossypium hirsutum* L. (MON88913)] [hereinafter referred to as "MON88913 (*G. hirsutum*)"], Type 1 Use of which had been approved on February 10 of 2006, into a cotton plant of different species, *G. barbadense*, by backcrossing.

MON 88913 (*G. hirsutum*) was developed as a line of glyphosate herbicide-tolerant cotton which expressed the modified CP4 EPSPS protein, by transferring the modified *cp4 epsps* gene, which was derived from *Agrobacterium* CP4 strain, into the cultivated cotton variety of Coker312, which belongs to *Gossypium hirsutum* species.

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

Composition and origin of component element of donor nucleic acid used for the development of MON88913 (*G. hirsutum*) are shown in Table 1 on p. 7 of Appendix 1.

2) Functions of component elements

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

Functions of component elements of donor nucleic acid used for the development of MON88913 (*G. hirsutum*) are shown in Table 1 on p.7 of Appendix 1.

(b) Functions of proteins produced by the expression of target gene and selective markers, and the fact, if applicable, that the produced any allergenicity

Functions of the CP4 EPSPS protein produced by the expression of the modified *cp4 epsps* gene transferred into this recombinant Pima cotton are shown from p. 4 to p. 6 of Appendix 1.

In order to investigate whether the modified CP4 EPSPS protein shares functionally important amino acid sequences with known allergens, the CP4 EPSPS protein was compared with allergens in the allergen database 10 (AD 2010[7]), using FASTA algorithm and ALLERGENSEARCH algorithm. The results showed that the CP4 EPSPS protein did not share structurally related sequences with any known allergens examined.

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

As shown from p. 4 to p. 6 of Appendix 1, it is considered highly unlikely that the modified CP4 EPSPS protein affects the metabolic system of the recipient organism.

(2) Information concerning vector

This recombinant Pima cotton was developed by transferring the modified *cp4 epsps* gene, which was transferred into MON88913 (*G. hirsutum*), Type 1 Use of which had been approved on February 10 of 2006, into a cotton plant of different species, *G. barbadense*, by backcrossing. Therefore, information concerning the vector PV-GHGT35 used for the development of MON88913 (*G. hirsutum*) is shown in the following 1) and 2).

1) Name and origin

See (2)-1) on p. 9 of Appendix 1

2) Properties

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

See (2)-2) on p. 9 of Appendix 1

[7] FARRP (Food Allergy Research and Resource Program): Database holding the sequences registered in the Allergen Online database (FARRP, 2010) as of January, 2010.

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

See (2)-2) on p. 9 of Appendix 1

(c) Presence or absence of infectivity of vector and, if present, the information concerning the host range

See (4)-4) on p. 13 of Appendix 1

(3) Method of preparing living modified organisms

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

The component elements of plasmid vector PV-GHGT35 used for the development of MON88913 (*G. hirsutum*) are shown in Table 1 on p.7 of Appendix 1. The position and direction of the donor nucleic acid and the restriction enzyme cleavage sites in the vector are shown in Figure 1 on p.10 of Appendix 1.

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

This recombinant Pima cotton was developed by transferring the modified *cp4 epsps* gene, which was transferred into MON88913 (*G. hirsutum*), into W1377 and W2490, which are the non-recombinant Pima cotton varieties of *G. barbadense* species, by backcrossing (Figure 1, p.15).

3) Processes of rearing living modified organisms

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

Since conventional breeding method was used, it is not applicable.

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

Since conventional breeding method was used, it is not applicable.

(c) Processes of rearing and pedigree trees of the following lines; cells to which the nucleic acid was transferred, the line in 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

This recombinant Pima cotton was developed by 4 successive backcrossing of F1 hybrid, which is the cross generation of MON88913 (*G. hirsutum*) and

W1377 (commercial variety of *G. barbadense*), with W1377, followed by 3 successive crossing of the product with W2490 (*G. barbadense* cultivar), followed by inbreeding for fixation (Figure1, p. 15). The existence and stability of expression of transferred gene in this recombinant Pima cotton, as well as the generation subjected to field tests in the United States are shown in Figure 1 (p. 15).

This recombinant Pima cotton in this assessment refers to all hybrid progenies derived from BC2F5 generation.

[Confidential: Not disclosed to unauthorized person]

Figure 1 Process of rearing this recombinant Pima cotton

(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

The existence of the transferred nucleic acid on the nuclear genome of the parent line of this recombinant Pima cotton, MON88913 (*G. hirsutum*), had been confirmed (p.13 of Appendix 1). Therefore, it was judged that transferred nucleic acid also existed in the nuclear genome of this recombinant Pima cotton, which was developed by conventional breeding using MON88913 (*G. hirsutum*) as a parent line.

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

This recombinant Pima cotton was developed by transferring the modified *cp4 epsps* gene, which existed in the genome of MON88913 (*G. hirsutum*), into *G. barbadense* by use of conventional breeding method. Based on this, it was considered that one copy of the T-DNA region containing the modified *cp4 epsps* gene, which existed in the genome of MON88913 (*G. hirsutum*), also existed in the genome of this recombinant Pima cotton. Southern blotting analysis was conducted to examine the existence of the transferred gene derived from MON88913 (*G. hirsutum*) in this recombinant Pima cotton.

It has been confirmed by the results of the Southern blotting analysis that the same transferred gene as that in MON88913 (*G. hirsutum*) existed in this recombinant Pima cotton (Figure 3 on p.17 of Appendix 2), and stably inherited in multiple generations (BC2F5 to BC2F7) (Figure 3 on p. 17 of Appendix 2).

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

This item is not applicable because of one copy.

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

The amount of expression of the modified CP4 EPSPS protein in the leaves and seeds of this recombinant Pima cotton, which was cultivated in 3 replicates in 5 fields in the United States (California (2), Arizona, New Mexico and Texas), was analyzed by use of ELISA method.

The results showed that the expression level of the modified CP4 EPSPS protein in the leaves was within the range of 280 to 600 µg/g fwt, with the mean value of 410 µg/g fwt, while the expression level of the modified CP4 EPSPS protein in the seed was within the range of 190 to 420 µg/g fwt, with the mean value of 340 µg/g fwt (Table 1 on p. 17 of Appendix 3). The expression level of the modified CP4 EPSPS protein in the leaves and seeds of the non-recombinant control Pima cotton, W2490, were all under the limit of detection (leaf: LOD = 0.069 µg/g, seed: LOD = 1.7 µg/g).

Western blotting analysis was conducted on the leaves of 3 generations of this recombinant Pima cotton (BC2F5, BC2F6 and BC2F7) to examine inter-generational expression stability of the modified CP4 EPSPS protein in this recombinant Pima cotton. It was confirmed by the results of the analysis that the modified CP4 EPSPS protein (up to 43.8 kDa) was expressed in all the generations subjected to the test, while it was not expressed in the non-recombinant Pima cotton W2490 (Figure 1 on p. 16 of Appendix 4). The protein with a size of approximately 37 kDa, in addition to the full-length modified CP4 EPSPS protein (up to 43.8 kDa) which was detected in both this recombinant Pima cotton and the control (the modified CP4 EPSPS protein expressed by *E.coli*), was considered to be a fragment of the modified CP4 EPSPS protein.

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

See (4)-5) on p. 13 of Appendix 1

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

It was confirmed possible to detect and identify the MON 88913 event in recombinant Pima cotton (Appendix 6) by qualitative PCR method which was developed to detect MON88913 (*G. hirsutum*) (Appendix 5).

(6) Difference between the modified organism and 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

The modified *cp4 epsps* gene transferred into this recombinant Pima cotton confers glyphosate herbicide tolerance by expressing the modified CP4 EPSPS protein.

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[8]

(a) Morphological and growth characteristics

The following 6 items of morphological and growth characteristics were examined between this recombinant Pima cotton and the non-recombinant control Pima cotton W2490 at 3 fields in the United States (Arizona, California and New Mexico): number of established seedlings, plant's vigor at the early stage, main stem length, number of nodes to the lowest fruiting branch at flowering stage, number of nodes to the lowest fruiting branch and yield). As reference, 4 varieties of conventional commercial cotton of *G. barbadense* species were subjected to the test. The tests were conducted in 3 replicates (Table 1 on p. 5 of Appendix 7).

As a result, statistically significant differences were observed in the plant's vigor in the 8th week after sowing and the main stem length, as well as in the number of nodes to the lowest fruiting branch at flowering stage and number of nodes to the lowest fruiting branch; however, no significant difference was observed in the other items ($p > 0.05$) (Table 2 on p. 6-7 of Appendix 7).

Regarding the plant's vigor in the 8th week after sowing, statistically significant difference was observed in the field of California, with the value being 2.3 and 4.0 in this recombinant Pima cotton and the non-recombinant control Pima cotton, respectively. However, these values are within the range of the mean value in the conventional commercial cotton cultivars (1.0-3.3), subjected to the test as reference.

Regarding the main stem length in the 8th week after sowing, statistically significant differences were observed in the fields in Arizona and New Mexico. In the field in Arizona, the main stem length of this recombinant Pima cotton was 74.9 cm, while that of the non-recombinant control Pima cotton was 83.0 cm. However, the value of Arizona was within the range of the mean value in the conventional commercial cotton cultivars (74.5-84.0 cm), subjected to the test as reference.

In the field in New Mexico, the main stem length of this recombinant Pima cotton was 45.5 cm, while that of the non-recombinant control Pima cotton was 49.6 cm. This value was out of the range of the conventional commercial cotton cultivar (46.2-52.0 cm), subjected to the test as reference.

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[8] All the rights pertinent to the information in the following (a) to (g) and the responsibility for the content rest upoin Monsanto Japan Limited.

Regarding the number of nodes to the lowest fruiting branch at flowering stage, statistically significant difference was observed in the first observation in the field in Arizona, with the value being 10.6 and 11.9 in this recombinant Pima cotton and the non-recombinant control Pima cotton, respectively. This value was out of the range of the mean value in the conventional commercial cotton cultivar (10.9-11.3), subjected to the test as reference.

Regarding the number of nodes to the lowest fruiting branch, statistically significant difference was observed in the first observation in the field in New Mexico, with the value being 17.0 and 10.1 in this recombinant Pima cotton and the non-recombinant control Pima cotton, respectively. However, this value was within the range of the mean value in the conventional commercial cotton cultivars (11.5-19.3), subjected to the test as reference.

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

Cold-tolerance test at the early stage of growth was conducted by examining the plant's vigor, plant height, growth stage, fresh weight and dry weight, after the cultivation of this recombinant Pima cotton (21 days after sowing), the non-recombinant control Pima cotton W2490, and 6 varieties of conventional commercial cotton cultivars for 21 days in a climate chamber, in which the day/night temperatures were set at 15˚C/10˚C. Under the temperature condition of this test, Pima cotton experiences cold stress, and it is reported that harmful effects on the growth were actually observed under this temperature condition (Reddy *et al*., 1995; Reddy *et al.*, 1993).

No statistically significant difference was observed in the items statistically analyzed (plant's vigor, plant height, fresh weight and dry weight) between this recombinant Pima cotton and the non-recombinant control Pima cotton ($p > 0.05$) (Table 3 on p. 6 of Appendix 8). In addition, no difference was observed in the item not statistically analyzed (growth stage) between this recombinant Pima cotton and the non-recombinant control Pima cotton (Table 3 on p. 6 of Appendix 8).

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

Whereas *G. hirsutum* and *G. barbadense*are are classified as perennial plants, the cotton plants are cultivated as annual plants in the United States and many other cotton-growing countries in the world. After the raw cotton and seeds are harvested following fruition, the cotton plants wither and die in winter. In several fields in the United States, this recombinant Pima cotton plants were completely defoliated and became brown in winter, in the same way as the non-recombinant control Pima cotton. There was no difference in withering between this recombinant Pima cotton and the non-recombinant control Pima cotton.

(d) Fertility and size of the pollen

Pollens were collected from this recombinant Pima cotton, the non-recombinant control Pima cotton W2490, and 4 varieties of conventional commercial cultivars that were cultivated in the fields in the United States, and their fertility and size were examined. The fertility of this recombinant Pima cotton was 93.6%, which was not statistically significantly different from the fertility of the non-recombinant control Pima cotton of 94.9% (Table 1 on p. 4 of Appendix 9).

Regarding the size of the pollen, there was a statistically significant difference between this recombinant Pima cotton and the non-recombinant control Pima cotton ($p < 0.05$). The mean of the size of the pollen in this recombinant Pima cotton and the non-recombinant control Pima cotton were 105.5 µm and 102.6 µm, respectively. This value was out of the range of the mean in the conventional commercial cultivar (103.3-105.0 µm), subjected to the test as reference. This value was also smaller than the range reported in the test using conventional commercial variety of *G. barbadense* species (non-recombinant Pima cotton) (115-117.9 µm; Saad, 1960; Kakani *et al.*, 1999). No difference in the morphology of the pollen was observed by visual examination (Figure 1 on p.5 of Appendix 9).

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

As mentioned earlier in I-2-(6)-2)-(a) on p.18, no statistically significant difference in the quantity of seed production was observed between this recombinant Pima cotton and the non-recombinant control Pima cotton.

Regarding the shedding habit, it was considered that shedding habit of both this recombinant Pima cotton and the non-recombinant Pima cotton W2490 are similarly low, since the seeds of both plants are covered with lint and difficult to be isolated.

Some species of cotton plants are known to possess 2 to 3 months of dormancy period; however, in the process of cultivation, the plants with shorter dormancy period have been selected (Paiziev and Krakhmalev, 2006; OECD, 2008; OGTR, 2008).

The germination rate of this recombinant Pima cotton, the non-recombinant control Pima cotton W2490, and 4 varieties of commercial Pima cotton cultivars was examined, in 4 replications using 100 seeds of each variety sown in a greenhouse, under the dark condition with the repeating cycle of temperature condition of 16 hours at 20˚C and 8 hours at 30˚C. The germinated seeds were separately examined for normal germination rate and abnormal germination rate, while the seeds that did not germinate were separately examined for the percentages of viable hard seeds, withered seeds, and viable firm-swollen seeds (Appendix 10). As a result, no statistically significant difference was observed in all items examined (Table 1 on p. 4 of Appendix 10).

(f) Crossability

It has not been reported that *Gossypium* plants, which are closely related to and crossable with cultivated amphidiploid cotton of *G. barbadense* species, which include this recombinant Pima cotton, grow naturally in Japan (*Wild Flowers of Japan-Trees II*). Therefore, the evaluation of crossability was not conducted.

(g) Productivity of harmful substances

This recombinant Pima cotton produces substances such as gossypol and cyclopropane fatty acid, which are also contained in the non-recombinant Pima cotton. Gossypol and cyclopropene fatty acids are not the substances which affect the habitat or growth of wild animals and wild plants, such as allochenmicals. There has been no report that shows the example of cotton seed ingestion by wild mammals.

The productivity of harmful substances of this recombinant Pima cotton and the non-recombinant control Pima cotton W2490 was examined by conducting plow-in test, succeeding crop test and soil microflora test.

[Plow-in tests]

To examine whether the forage of this recombinant Pima cotton produced any harmful substances that pose a threat of affecting the surrounding flora, the forages of this recombinant Pima cotton, the non-recombinant control Pima cotton W2490, and 6 varieties of conventional commercial Pima cotton cultivars were subjected to the plow-in test in a greenhouse in the United States.

None of the tests indicated a statistically significant difference in the number of germination, growth stage, plant height, fresh weight and dry weight of the test plant, lettuce, which was sown in the soil plowed with the forage of Pima cotton ($p > 0.05$) (Table 1 on p. 4 of Appendix 11).

[Succeeding crop tests]

To examine whether subterranean part of this recombinant Pima cotton secreted any harmful substances that pose a threat of affecting the surrounding flora, the soil collected at the harvesting time of this recombinant Pima cotton, the non-recombinant control Pima cotton W2490, and 6 varieties of conventional commercial Pima cotton cultivars were subjected to the succeeding crop test in a greenhouse in the United States.

None of the tests indicated a statistically significant difference in the number of germination, growth stage, plant height, fresh weight and dry weight of the test plant, lettuce, which was sown in the soil collected at the harvesting time of Pima cotton ($p > 0.05$) (Table 1 on p. 4 of Appendix 11).

[Soil microflora tests]

To examine whether this recombinant Pima cotton secreted any harmful substances that pose a threat of affecting soil microflora, the soil microflora test was conducted in a greenhouse in the United States.

The number of bacteria, filamentous fungi, and actinomyces were examined in the soil used to cultivate this recombinant Pima cotton, the non-recombinant control Pima cotton W2490, and 6 varieties of conventional commercial Pima cotton cultivars. Although a statistically significant difference was observed in the number of bacteria and filamentous fungi between this recombinant Pima cotton and the non-recombinant control Pima cotton (Table 2, and Table 2 on p. 23 of Appendix 12), the numbers were within the range of the mean value of conventional commercial variety (Table 2 on p. 23 of Appendix 12). Moreover, no statistically significant difference was observed in the number of actinomyces (Table 2 on p. 23 of Appendix 12).

Table 2 Soil microflora tests[9]

	Mean value (CFU/g dwt soil)		The range of the mean value of conventional commercial variety
	This recombinant Pima cotton	Non-recombinant control Pima cotton	
Bacteria	*2.84×10^{8}	1.37×10^{8}	1.15×10^{7}-3.74×10^{8}
Filamentous fungi	*2.29×10^{6}	1.46×10^{6}	1.10×10^{6}-3.03×10^{6}
Actinomyces	1.93×10^{7}	2.36×10^{7}	2.19×10^{7}-3.28×10^{7}

* Significant difference was observed compared to the non-recombinant control Pima cotton W2490 ($\alpha = 0.05$).

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

Assessment Result by the Committee for Review on the Biological Diversity Risk Assessment

1. Name of the Type of Living Modified Organism: Pima cotton tolerant to glyphosate herbicide (modified *cp4 epsps*, *Gossypium barbadense* L.) (MON88913, OECD UI : MON-88913-8)
   Content of Type 1 Use: Provision as food, provision as feed, processing, storage, transportation, disposal and acts incidental to them
   Applicant: Monsanto Japan Ltd.

(1) **Item-by-item assessment of Adverse Effect on Biological Diversity**

This recombinant Pima cotton was developed by crossing genetically modified cotton (MON88913), whose Type 1 Use Regulation based on the “Low Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms” (Law No. 97, 2003, hereinafter referred to as “law for the Cartagena Protocol on Biosafety”) was approved (February 10, 2006), with the non-recombinant Pima cotton variety by conventional breeding method (such as backcrossing). Since cotton and Pima cotton are plants of different species, the application for approval of Type 1 Use Regulation, based on the law for the Cartagena Protocol on Biosafety, of this recombinant Pima cotton was also submitted, in accordance with “The application for approval of Type 1 Use Regulation concerning the genetically modified plants whose production and distribution are under the jurisdiction of the Minister of Agriculture, Forestry and Fisheries” (December 10, 2007, 19 Consumer Product Safety Act No. 8999, No. 071210001, Notice of the Ministry of the Environment, Natural Environment Bureau, Chief of Wildlife Div. to the chief of relevant bureau).

Cotton and Pima cotton share a common genome structure, and genetically resemble to each other. Since no significant difference in physiological and ecological characteristics, which exert the effect on biological diversity in Japan, was observed between these two plants, it was judged to be possible to evaluate the effect of this genetically modified plant on biological diversity without examining the characteristics of this genetically modified plant in the isolated field in Japan.

1) Competitiveness

Currently, commercial cultivation of cotton plants including Pima cotton is hardly seen in Japan, and cotton plants are grown for mainly ornamental purposes. There has been no report indicating that the seeds of the cotton imported to Japan for oil and feed production spilled during the transportation and grew to become self-seeding in the natural environment.

Although glyphosate herbicide tolerance, which is attributable to the expression of the modified CP4 EPSPS protein, is conferred to this recombinant Pima cotton, it is unlikely that this glyphosate-tolerant trait enhances the competitiveness of this recombinant Pima cotton in the natural environment, where it is unlikely that glyphosate is used.

The results of the examination of the characteristics that are related to the competitiveness of this recombinant Pima cotton and the non-recombinant control Pima cotton in 3 fields in the United States indicated that there were statistically significant differences in the plant's vigor and main stem length in the 8th week after sowing, the number of nodes to the lowest fruiting branch at flowering stage, the number of nodes to the lowest fruiting branch and the size of pollen between this recombinant Pima cotton and the non-recombinant control Pima cotton. However, compared to the conventional Pima cotton varieties subjected to the test as reference, these differences were fairly small, and it was considered that they were not beyond of the range of Pima cotton species.

Based on the above understanding, it was judged that the following conclusion made by the applicant was valid: There are no specific wild plants or wild animals that are possibly affected by this recombinant Pima cotton, and the use of such cotton poses no risk of adverse effect on biological diversity that is attributable to competitiveness.

2) Productivity of harmful substances

There has been no known report indicating that Pima cotton and cotton plants produce substances which are harmful on the habitat or growth of wild animals and wild plants, such as allochenmicals.

This recombinant Pima cotton possesses a trait of producing the modified CP4 EPSPS protein, which confers glyphosate herbicide tolerance. However, there has been no report indicating that this protein is a harmful substance. It has been also confirmed by homology search of amino acid sequence that modified CP4 EPSPS protein does not contain sequence which is structurally analogous to known allergens.

The modified CP4 EPSPS protein is an enzyme of the shikimic acid pathway for aromatic amino acid biosynthesis. Since CP4 EPSPS is not a rate limiting enzyme of shikimic acid pathway, it was considered that increase in EPSPS activity would not increase the concentration of the final product of the pathway, aromatic amino acid.

In a greenhouse in the United States, the presence or absence of the productivity of harmful substances (the substances secreted from the roots which can affect other plants and microorganisms, the substances existing in the plant body which can affect other plants after dying) of this recombinant Pima cotton was examined by conducting a plow-in test, succeeded crop test and soil microflora test. As a result, statistically significant differences were observed in the number of bacteria and filamentous fungi in the soil microflora test. However, these values were within the variation range of conventional Pima cotton varieties subjected to the test as references.

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, if cannot be specified and that the use of this recombinant Pima 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 plants which can cross with Pima cotton. Therefore, it was judged that there are no specific wild plants that are possibly affected by this recombinant Pima 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 Pima cotton in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity in Japan. It was judged that the conclusion above made by the applicant is reasonable.