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Applicant: Monsanto do Brasil Ltda.
CNPJ: 64.858.525/0001-45
Address: Avenida Nações Unidas, 12901, Torre Norte, 7º e 8º Andares, 04578-000 São Paulo, SP.
Matter: Commercial release of insect-resistant Genetically Modified Cotton.
Previous extract: 1075/2007. Published on 06.28.2007.
Meeting: 123rd CTNBio Regular Meeting held on 05.21.2009.
Decision: GRANTED.

CTNBio, following examination of an application for commercial release of genetically modified insect-resistant cotton (Bollgard Cotton, event MON 15985), including all progenies generated from transformation event MON15985 and their derivatives from the crossing of non-transgenic cotton lineage and populations with lineages that included event MON 15985, was for the GRANTING of the application under the terms of this Technical Opinion.
Monsanto do Brasil Ltda. requested a CTNBio Technical Opinion related to biosafety of the insect-resistant genetically modified cotton (Gossypium hirsutum), namely Bollgard II Cotton, Event MON15895, for the purpose of free registration, use in the environment, human and animal consumption, marketing and industrial use and any other use and activity related to this GMO including derivative lineages and cultivars as well as byproducts, all under the remaining regulations and requirements applicable to any use of cultivated species of the genus Gossypium effective in Brazil. Bollgard II Cotton was produced by introducing, through biobalistics, genes cry2Ab2 and uidA in the Bollgard cotton genome, as approved by CTNBio in 2005. Plasmid PV-GHBK11 was used to insert genes cry2Ab2 and uidA to Bollgard cotton genome, generating MON 15895 15985 cotton. Therefore, Bollgard II cotton Event 15985 contains the exogenous genes

cry1Ac, cry2Ab2, nptII, aad and uidA, expresses proteins Cry1Ac, Cry2Ab2, NPTII and GUS, differing from its Bollgard parental in proteins Cry2Ab2 and GUS. Combination of proteins Cry2Ab2 and Cry1Ac represents an additional tool to fight plague resistance to protein Cry1Ac, since Cry2A is a class of proteins coming from Bacillus thuringiensis, different from Cry1Ac. The uidA gene, also known as gus or gusA gene, derived from the K12 strain of Escherichia coli, codifies the GUS enzyme, which was used as a selection mechanism of transformed cells. The cry2Ab2 gene that codifies the Cry2Ab2 protein is derived from bacterium B. thuringiensis, a gram-positive soil micro-organism. Commercial formulations of B. thuringiensis have been used in Brazil and other countries to control some agricultural plagues for over forty years. Cry2Ab2 and Cry1Ac are proteins that feature very specific action, showing toxic effect through ingestion only and acting in specific receptors located in the middle intestine of some insects of the Lepidoptera Order. Stability and segregation analyses, in ELISA essays for protein Cry2Ab2 in four generations, support the conclusion that event MON 15985 is a single copy event of stable insertion. Chi-square analysis indicates that the insert segregates according to Mendelian genetics, with a segregation pattern of a single gene, against detection of protein Cry2Ab2. Southern Blot analyses in generations R1, R2, R3 and R4 and two second generation retro-crossing lineages (BC2F3), digested with enzyme SphI and hybridized with a probe of the coding region of gene cry2Ab2, evidenced that the transgene is stable across different generations, since no difference was noticed in the band patterns obtained. From the molecular analyses showed, it becomes

evident that Bollgard II cotton event MON 15985 possesses a copy of genes cry2Ab2, uidA, cry1Ac, nptII and aad, in which the latter is not expressed in plants. As the vector sequences are not part of the insert, the real potential horizontal genetic transfer from the bacterium donor of the plasmid to the receiving cotton can be considered null. Agronomic characteristics of MON 15985 cotton are equally comparable to, or better than, those of conventional cotton. The control of A. Argillacea, H. virescens and P. gossypiella was efficient, especially under conditions of high infestation by the pests. In artificial infestations of Spodoptera frugiperda there was a significant reduction in the number of caterpillars and defoliation during Bollgard II treatment, yet efficacy in controlling

the pest was decreased when compared with other target pests. Apparently, insertion of segment PV-GHBI11L was not harmful for the plant development. Assessment of agronomic performance of MON 15985 lineages and cultivars against conventional cultivars in Brazilian agricultural conditions revealed normal variability between genetically modified and conventional plants regarding their agronomic features (plant height, cycle up to flowering, precocity of maturation, cycle up to harvest and boll weight) productivity and fiber quality. Though the combination Cry1Ac and Cry2Ab2 has higher efficacy than Bollgard, Bollgard II is currently susceptible to damages caused by Spodoptera ssp. and Helicoverpa zea in conditions of high infestation, especially flowering times. Practices of pest management associated to cotton Bt have caused a dramatic reduction in the use of insecticides, which leads to a significant increase in the population of beneficial insects and, consequently, contributes towards the natural control of some pests.
340/2009
5 49
Studies were conducted with non-target organisms, such as birds, fish and beneficial invertebrate species. The results evidenced that protein Cry2Ab2 in MON 15985 cotton fails to impose premature risks for non-target organisms. Adverse effects were not observed in concentrations significantly higher than the ones foreseen by exposure to the environment. In all cases, the concentration of the non-observed effect exceeds the top environmental concentration, indicating minimum risk of protein Cry2Ab2 to non-target organisms. Results of several studies indicated that protein Cry2Ab2 poses minimum risk for non-target beneficial organisms. Studies with populations of predator species, such as Geocoris spp., Orius Insidiosus, Nabis spp., Slenopsis invicta, spiders, coccinellidae, chrysopidae and hemerobiidae, evidenced that the populations were either equal or larger in treatments containing Bollgard and Bollgard II cotton contrasted with treatments with conventional cotton. In a sample of over 40 field experiments with cotton and maize expressing proteins Cry it became clear that, in general, non-target invertebrates are more abundant in cotton and Bt corn fields than in fields where conventional cultures were treated with insecticides. However, in insect-resistant genetically modified cotton and corn fields, when compared to fields with cultures that were not treated with pesticides, show a statistically significant reduction in the number of some non-target organisms. Other studies evidenced that, in general, there was no significant difference on populations of natural enemies between Bollgard cotton and conventional cotton. Whenever significant differences were apparent, natural enemies were more abundant in Bollgard cotton fields, probably resulting from the decreased
340/2009
6 49
employment of chemical pesticides. It was additionally observed that, when insect eggs or larvae were presented as preys, natural populations in Bollgard cotton fields exhibited predation rates significantly higher. In China, a monitoring of nontarget organisms was conducted in the northwestern region for Bt cotton fields and the results suggested an increase in natural predator populations such as ladybirds, earwigs, spiders and other non-target organisms, in addition to the reappearance of cotton aphis. In Brazil, it was shown that the Bt cotton is either harmless or brings positive effects to changes in life cycle, survival, fertility, and appearance of colonies of Aphys gossypii, under nursery conditions. Results obtained by the authors and data available in technical literature show the high specificity of the Bollgard technology in the control of target-organisms, without causing either positive or negative effects in non-target populations, such as Aphis gossypii. Regarding the risk of gene flow to wild populations and potential reduction of biodiversity, it is important to consider that for gene introgression, it is first necessary hybridization and later a series of retro-crossing to take place for permanent incorporation of a gene in a genome. Further, the potential of vertical genetic transfer from genetically modified corn to wild species in non cultivated ecosystems is low, due to the relatively isolated distribution of Gossypium species. In Brazil, there are not species sexually compatible with G. hirsutum that display characteristics of invading plants, and it is highly improbable that cry1AC and cry2AB2 be transferred to pests, making the latter more invasive. The likelihood that a Bollgard herbaceous cotton plant becomes a pest is negligible. The cry genes were isolated from a soil bacterium, B. thuringiensis and, therefore
340/2009
7 49
the exposure of living and environment organisms to this bacterium or to any element derived from it is an event that occurs abundantly in nature. It was

verified that adoption of Bt cotton in different countries caused significant reduction in the use of pesticides, with benefits to the environment and field workers. Available information suggests that transgenic plants are not fundamentally different from genotypes of non-transformed cotton, safe for resistance to some insects of the order Lepidoptera. There are no restrictions to the use of this cotton or derivatives, either for human or animal feeding. According to Article 1 of Law nº 11,460, of March 21, 2007, “research and cultivation of genetically modified organisms may not be conducted in indigenous lands and areas of conservation units”. The Bollgard technology proved to be useful under all agricultural practices commonly used in different regions and conditions, either for the availability of inputs, labor, among others, used in the cotton culture. There are no creole varieties of cotton plants and the chains of special, conventional and transgenic cottons have lived together in a satisfactory fashion, without records of coexistence problems. According to Annex I of Regulating Resolution no. 5, of March 12, 2008, the applicant shall have a term of thirty (30) days from the publication date of this Technical Opinion to adjust its proposal to the postcommercial release monitoring plan. Under Article 14 of Law no. 11,105/2005, CTNBio found that the request complies with the applicable rules and legislation securing the biosafety of environment, agriculture, human and animal health.

TECHNICAL OPINION

I. GMO Identification

GMO name: Bollgard II Cotton, Event MON 15985.
Species: Gossypium hirsutum
Inserted characteristics: Tolerance to certain pest insects
Method of insertion: Plant transformation by particle acceleration
Prospective use: Release into the environment, marketing, consumption and any other activities related to this GMO and its derivatives.

II. General Information

Herbaceous cotton (Gossypium hirsutum L.) of the Malvaceae family is a allelotetraploid plant, native of Mexico and sexually compatible with all the remaining allelotretraploid species of the same genus. It is one of the most cultivated plants used by humankind(12) and is cultivated in Brazil in small and large properties in regions featuring distinct ecological conditions(18).

Cotton plant is one of the main cultivated plants, represented by commercial species, such as G. hirsutum, G. barbadense, G. arboretum, and G. herbaceum. G. hirsutum is the main such plants, with a production of about 90% of the total cotton fibers produced worldwide, being such fibers responsible for 40% of

human clothes(7). Cotton is held to be one of the prime agricultural products and is very important in Brazil, for its complex production/industry process and high use of manpower.

Two types of cotton plants are predominantly cultivated in Brazil: conventional cotton and genetically modified caterpillar-resistant cotton. These plants are responsible for practically all the cotton produced in the country. In addition, other three types of cotton featuring special genetic or ecologic features are cultivated: the naturally colored fiber cotton and the agro-ecological cotton. Colored cotton is almost exclusively concentrated in the State of Paraíba, with a crop area in 2007 of about 300 hectares. Crops of agro-ecological were sown by 235 farmers in the semiarid bioma in four states of the Brazilian Northeastern region and produced 42 tons(37). Chains of special, conventional and transgenic cotton have satisfactorily lived together, without problems of coexistence being reported. The area planted with cotton in Brazil in the past 2007/2008 harvest reached about one million and one hundred thousand hectares, of which over 85% concentrated in the Cerrado bioma, especially in the states of Mato Grosso, Bahia, Goiás and Mato Grosso do Sul. Other cultures are present in other states of the country, mainly in the semiarid of the Northeastern region, Paraná, Minas Gerais and São Paulo(28).

Besides the herbaceous one, other three cotton plants grow in Brazil, all of them allelootetraploids and, therefore, sexually compatible with the cultivars. None of such species is considered to be a pest in agricultural or natural environments.

The species G. barbadense was domesticated mainly in the Northern Peru and

Southern Ecuador(9). It was introduced by pre-Columbian peoples and its fibers were used in the production of textile craftsmanship by some indigenous ethnic groups before the arrival of Portuguese colonizers(42). Its use as a textile plant was disseminated among colonizers but suffered a decline driven by the dissemination of two exotic races of G. hirsutum races. G. barbadense cannot be found in natural environments and is basically kept as a backyard plant. It is widely distributed, present in almost the whole country and the in situ conservation is directly linked to the maintenance of traditional use as a medicine plant(4).

The only species indigenous to Brazil is G. mustelinum, being its natural distribution restricted to the Northeastern semiarid(19,32). Populations are known only in the States of Bahia and Rio Grande do Norte, in places that do not produce herbaceous cotton. Two problems affect the in situ maintenance of G. mustelinum. The first and most severe is the destruction of non-perennial rivers and rivulets gallery forests, the natural habitat of the species. The second is the extensive cattle raising conducted in the region, especially caprines. The animals feed on buds, leaves, fruits, seeds and bark, harming the development and, in some cases, killing adult plants. Renewal of populations is also jeopardized, since the grazing on young individuals destroys part of the plants(10). The distance between known populations of G. mustelinum and cotton producing regions prevents the crossing between G. mustelinum and herbaceous cotton present in cultivars.

The third cotton plant is known as mocó cotton and belongs to a race different from the same species of herbaceous cotton (G. hirsutum var. marie galante (Watt) Hutch.). This cotton originated in the Antilles and its introduction to Brazil is uncertain. One conjectures that it may have been brought by the Dutch or Africans during colonial times(42). Mocó cotton was widely cultivated in the Northeastern semiarid until the end of the eighties, when a series of problems caused an abrupt interruption in planting(6). A small amount of arboreal cotton plants, mainly inter-racial hybrids of colored and white fiber cotton produced by the Embrapa improvement program are still cultivated. However, the planting of this material is in decline; 5,692 hectares were harvested during the 2004/2005 crop and just 1,326 hectares during the 2005/2006 crop(28). Tillage is cultivated with a minimum of external inputs and the most important one is the insecticide to control insect pests. Control of weed is almost exclusively conducted through manual clearing. Transient populations of this race with high biologic importance, derived from forsaken farming, may be found in the high ridges of some municipalities of the Seridó area in the States of Paraíba and Rio Grande do Norte(4). These populations are geographically isolated from herbaceous cotton farms and well represented in the Embrapa germplasm banks.

The cotton leafworm (Alabama argillacea), cotton budworm (Helotes virescens), pink bollworm (Pectinophora gossypiella), fall armyworm (Spodoptera frugiperda), cotton aphid (Aphis gossypii), cotton bug (Horcias nobilellus), and boll weevil (Anthonomus grandis) are the main cotton pests in Brazil. Control of such pests has mainly been conducted with the use of insecticides. In Brazil, over 10 tons of

insecticides are consumed each year in cotton fields only, causing a US$ 190 million increase in production costs. The excessive use of non-specific insecticides leads to negative environmental impacts, such as severe reduction of beneficial organisms and potential upsurge of pests resistant to conventional insecticides.

Bollgard II cotton (Event MON 15985) was developed from Bollgard cotton, Event 531, through introduction of the gene cry2Ab2 of Bacillus thuringiensis, of the variety kurstaki. Therefore, Bollgard II expresses d-endotoxins Cry1Ac and Cry2Ab2 that are highly specific and toxic to caterpillars and some Lepidoptera, including Spodoptera frugiperda (J.D. Smith) (Lepidoptera: Noctuidae) and other species of the Spodoptera genus(13, 39, 54, 53), in addition to Bollgard cotton target-pests, Alabama argillacea (Hüeb.) (Lepidoptera: Noctuidae), cotton budworm (Heliothis virescens), (Fabr.) (Lepidoptera: Noctuidae), and pink bollworm (Pectinophora gossypiella) (Saund.) (Lepidoptera: Gelechiidae). Expression of two toxic proteins gives large scope for action to control pest Lepidoptera and makes possible, in some cases, to delay the evolution of resistance.

Taking into account that Bollgard II cotton was developed from Bollgard cotton,

which has been approved for commercial use by CTNBio in 2005, the biosafety analysis in this technical opinion shall be focused in additional proteins expressed in Bollgard II: GUS and Cry2Ab2 and possible interactions.

After ten years from the first commercial release of a genetically modified organism, the genetically modified cotton – GM took 20% of all worldwide planted area in 2005, corresponding to 1/9 of the whole area sowed with GM plants in the world. However, China and USA were responsible for the most part of this increase in planted area, where GM crops exceeded 2/3 and 4/5, respectively. Other countries featuring high rates of transgenic cotton adoption in the world in 2005 were Australia and South Africa, both featuring about 4/5 of their respective cultivated areas planted with cotton. In 2008, out of the 15.8 million hectares covered with transgenic cultures in Brazil, 14 million are soybeans, 1.4 million corn, and 0.4 million cotton.
Bollgard II cotton Event MON 15985 is marketed in different countries, such as: United States of America (2002), Australia (2002), Japan (2002), South Africa (2003), Philippines (2003), Mexico (2003), Korea (2003), Canada (2003), European Union (2005), China (2006), India (2006), and Burkina Faso (2008)(2). Up to this moment, no severe damage to human and animal health and to the environment was recorded by such commercial use in the above countries. In Brazil, field experiments were conducted in different Brazilian states.
III. Description of GMO and Proteins Expressed
Bollgard II cotton (Event MON 15985) was developed from Bollgard cotton by introducing another gene cry1Ab2 from B. thuringiensis var. kurstaki. Therefore, Bollgard II expresses d-endotoxins Cry1Ac and Cry2Ab2, which are highly

specific and toxic to caterpillars of some Lepidoptera, including Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) and other species of the genus Spodoptera(13, 53), in addition to target-pests of Bollgard cotton, Alabama argillacea (Hüeb) (Lepidoptera: Noctuidae), Heliothis virescens (Fabr.) (Lepidoptera: Noctuidae) and Pectinophora gossypiella (Saund.) (Lepidoptera: Gelechiidae). Expression of two toxic proteins increases the scope of action for controlling pest Lepidoptera and makes possible, in some cases, delay the evolution of resistance. CTNBio approves the release of Bollgard cotton Event 531 in 2005.
Commercial event MON 15985 (Bollgard II) was obtained by genetic transformation of Bollgard cotton (variety CP50B) using the methodology of microparticle acceleration or biobalistics(36). Bollgard cotton, already approved for marketing in Brazil(14) contains genes cry1Ac, nptII, aad, introduced using the transformation technique via Agrobacterium tumefaciens, in the conventional variety Coker 312, by using plasmid PV-GHBK04. Despite the presence of gene aad, the Bollgard cotton expresses only proteins Cry1Ac and NPTII. The gene aad has no modifications for expression in plants, and is used only as a marker for selection in bacterial cells, transformed with the vector containing the genes of interest.
Bollgard II cotton, in turn, was generated through introducing, by biobalistics (which results in direct entry of the DNA of interest to the plant cell) of genes cry2Ab2 and uidA in the Bollgard cotton genome, which was approved by CTNBio

in 2005. Plasmid PV-GHBK11 was used to insert genes cry2Ab2 and uidA in the Bollgard cotton genome to generate MON 15985 cotton. The plasmid was propagated in Escherichia coli, purified of bacterial suspensions and used for transformation. The exogenous DNA was introduced in cotton meristems using the method of particle acceleration and the DNA integration was detected by histochemical coloring for GUS (b-glucoronidase) in the vascular tissue. Selected plants were then tested for expression of the protein of interest Cry2Ab2. Therefore, cotton MON15985 contains exogenous genes cry1Ac, cry2Ab2, nptII, aad and uidA, and expresses proteins Cry1Ac, Cry2Ab2, NPTII and GUS, differing from its parental Bollgard in proteins Cry2Ab2 and GUS. The combination of proteins Cry2Ab2 and Cry1Ac represents one additional tool for resistance of pests to protein Cr1Ac, since Cry2A is a class of proteins coming from B. thuringiensis that is different from protein Cry1Ac.
Protein GUS is a product of expression of gene uidA, and was used as a selection mechanism for transformed cells (calorimetric selection marker). Gene

uidA, also known as gene gus and gusA, derived from E. coli strain K12, codifies enzyme b-D-glucoronidase (GUS).The enzyme catalyzes the hydrolysis of different b-glucoronides, among them the p-nitrophenyl-b-D-glucoronide, resulting in a chromogemic bluish compound. Bacterium E. coli is an inhabitant of the digestive system of vertebrates, including humans.
Gene cry2Ab2, which codifies protein Cry2Ab2, is derived from bacterium B. thuringiensis, a gram-positive soil microorganism, first isolated in Japan by

Ishiwata and formally described by Berliner in 1915. This pathogen displays the ability to form crystals containing endotoxins, which are proteins featuring insecticide action, during the sporulation phase of its development cycle. Among toxins, are the well known proteins Cry, or d-endotoxins. Commercial formulations of B. thuringiensis containing such proteins have been used in Brazil and other countries in controlling some farm pests for over forty years. Cry2Ab2 and Cry1Ac are proteins featuring very specific action, displaying toxic effects in case of ingestion and acting in specific receptors located at the middle intestine of some species of insects of the Order Lepidoptera.
Stability and segregation analyses, in ELISA essays for protein Cry2Ab2 in four generations, support the conclusion that event MON 15985 is a single copy event of stable insertion. Chi-square analysis showed that the insert segregates according to Mendelian genetics, with a single gene segregation pattern in relation to detection of protein Cry2Ab2. Southern Blot analysis of generations R1, R2, R3 and R4 and two lineages of the second retrocrossed generation (BC2F3), digested with enzyme SphI and hybridized with a probe of the codifying region of gene cry2Ab2, shows that the transgene is stable across different generations, since no difference was apparent in the pattern of bands obtained. The Mendelian segregation and stability of the transgene across tested generation of the MON 15985 cotton progeny was submitted by applicant.
As evidenced by molecular analyses shown, Bollgard II Event MON 15985 possesses a copy of genes cry2Ab2, uidA, cry1Ac, nptII and aad, the latter not

expressed in plants. Since the sequences of the vector (replication sequences or other elements of plasmid stability) are not part of the insert, any actual potential of horizontal genetic transfer from the bacterium donor of the plasmid to the receiving cotton is deemed null.
IV. Aspects Related to Human and Animal Health
Assessment and alimentary and nutritional safety studies for MON 15985 cotton were conducted based on the principle of Substantial Equivalence adopted by international organizations and regulatory bodies, such as WHO, FAO, OECD and ILSI. Under such approach, in case a new ration or a new food derived from a genetically modified culture is substantially equivalent to its conventional counterpart and the new proteins produced are held as safe, this genetically modified culture is held to be “as safe as” the conventional culture.
Protein GUS is an enzyme (b-glucoronidase), codified by gene uidA of E. coli and catalyzes the hydrolysis of b-d-glucoronides. By adding the artificial substract p-nitrophenyl-b-glucoronide, it is hydrolyzed imparting a bluish color that acts as a visible marker of the selection, being this the reason for its introduction in MON 15895 cotton. The protein is normally existent in the human organism due to the presence of E. coli and also for its presence in several foods derived from conventional plants such as potato, apple, oat, beet and others. Besides, it is degraded in the intestinal tract of humans and animals.
Gene uidA was not obtained from any clearly allergenic source, since bacterium

E. coli(31) is prevalent in the gastrointestinal tract of humans and animals. A data bank containing sequences of proteins associated to allergy and celiac disease was assembled from public domain data banks (GenBank, EMBL, PIR and SwissProt). A search for the sequence of enzyme GUS in such data banks shows that this protein has no similarity with allergenic sequences.
Protein Cry2Ab2, similarly to Cry1Ac, is a microbial d-endotoxin produced by B. thuringiensis (Bt.). The toxin acts in the intestine of larvae of different caterpillars of the Order Lepidoptera that have the related receptor. This bond causes the opening of pores for cations and prompts an osmotic imbalance between the digestive system and the hemolymph, causing the death of such insects. Humans and animals cannot be under the effects resulting from this bond

because they lack the related receptors.
According to the United States of America Environmental Protection Agency(58), no effects caused by this transgene were detected and, even with high doses, the Cry2Ab2 d-endotoxin was not considered to be toxic. Since the protein is promptly digested upon ingestion, effects of a chronic exposure to this protein are not expected.
For being proteins, the risks of allergenic effects were also assessed. Allergens originated from food are normally resistant to heat, acids and proteases, may be glycosylated and present in high concentrations. The proteins tested were promptly digested by gastric juices, are not glycosylated and their heating leads to

loss of bioactivity. The amino acid sequences of this protein were searched against a data bank containing about 600 sequences of allergenic proteins and no similarity became apparent. Experiments conducted in animals failed to suggest any allergenic potential.
According to data submitted by applicant, oral acute toxicity of proteins Cry1Ac and Cry2Ab2 is low and are held to be non toxic to mammals. Besides, food products derived from cotton are highly processed, with in general degrades the proteins expressed by the Bolgard II cotton. However, in case the proteins are ingested, they will be immediately broken into their respective amino acids, which disable any chronic exposure. Protein Cry2Ab2 was searched against a data bank containing 4667 proteins held to be toxic and no similarity was found. A test with rats was also conducted with the supply of high doses, without evidence of any toxic effect.
In vitro digestion studies showed that when exposed to gastric juice, 98% of the protein was digested in just 15 seconds. In the intestinal fluid, it resisted for a quite longer period, but as almost all of the protein is digested in the stomach, the importance of intestinal digestion is low.
Results from 14 tests conducted in the United States evidenced that there was no bromatologic change in the composition of MON 15895 cotton against its conventional counterpart considering elements such as ashes, calories, carbohydrates, total fat, total fiber, fiber in acid detergent, fiber in neutral detergent and protein. An analysis of eighteen essential amino acids also failed to

find differences between the two varieties.
Though the composition of fat acids in MON 15895 cotton seeds is similar to that of conventional cotton, some acids were found in higher amounts in the new variety (myristic, stearic, linolenic, arachidonic and dehydro-sterculius). It is worth stressing that linolenic and arachidonic are essential fatty acids for man and animals. Despite the differences, the averages found were within the 95% confidence interval of conventional references. The composition of fatty acids in processed cottonseed oil was also similar between MON 15895 cotton and conventional cotton. Regarding minerals (Ca, Cu, Fe, Mg, P, K, Na and Zn), the contents were quite similar between the two varieties, with MON 15895 displaying lower values for copper, iron and phosphor, though all values were also within the expected variation range of conventional cotton. For gossypol, which is the toxic factor of the cotton kernel, the contents found were practically the same in the two varieties
Considering the analyses conducted in Brazil, MON 15895 cotton showed results similar to those of conventional cotton for ashes, carbohydrates, fat, protein and gossypol. The average of the latter was 12% lower in MON 15895 cotton. Nutritional composition also varied within the limits for conventional cotton adopted by ILSI(30). The conclusion was that Bollgard II Event MON 15895 has a composition similar to that of conventional cotton.
Cotton is primarily cultivated for the value of its fiber and, secondly, for the use of its kernel in the production of cottonseed oil and animal fodder(29). Cottonseed oil

and cellulose from processed fibers are the only products derived from cotton used in human food(45). Short fibers are the main source of cellulose used in the chemical and food industry (their cellulose content reaches 99%). Cotton kernel produces high quality oil that is used in a variety of foods as frying oil, salad and cooking oil, mayonnaise, salad dressing and margarine, among other uses. It is the oldest oil industrially produced and has been largely consumed in Brazil before the increased use of soybean oil. The high quality of refined cottonseed oil

is due to the presence of essential fatty acids (such as linoleic acid) and high content of E vitamin and a-tocopherol (a natural antioxidant) that increases its value for consumption compared with corn and soybean oils(16).
Kernel quality and composition analyses of Bollgard II event MON 15895 cotton showed that this genetically modified insect-resistant cotton and its processed fractions are comparable to those of conventional cotton, taking into consideration the natural variability between market cotton varieties. Studies performed with animas (dairy cows, catfish, quails and rats) assessed the nutritional quality of MON 15895 cotton and the effects of diets containing modified cotton kernels on the development of animals(26). MON 15895 cotton, as a component of animal fodder, and proteins Cry2Ab2, Cry1Ac, NPTII and GUS in plant tissues proved to be safe and had similar nutritional value for human and animal consumption.
Field experiments with MON 15895 cotton were conducted in three locations in Brazil (Santa Cruz das Palmeiras, SP; Santa Helena de Goiás, GO and Sorriso, MT) during the 2005/2006 harvest, with the purpose of generating samples to

quantify proteins CryAb2, Cry1Ac, NPTII and GUS in tissues of leaves and kernels. Samples were analyzed regarding the content of such proteins by the ELISA method. Average levels or proteins in leaves and kernels in the three locations for MON 15895 cotton were: Cry2Ab2, 660 and 250 mg/g of dry weight, respectively; Cry1Ac, 53 and 1.9 mg/g of dry weight, respectively; NPTII, 35 and 2.7 mg/g of dry weight, respectively; and GUS, 2600 and 140 mg/g of dry weight, respectively.
Proteins Cry2Ab2, Cry1Ac, NPTII and GUS have higher levels of expression in leaves, but they were also detected in samples from other tissues. After processing the fibers and kernels, such proteins are undetected. Since oil and processed fibers are the only products derived from cotton used in human food, consumption of exogenous bioactive proteins or any product of their degradation is not expected(33, 51, 52).
Nutritional equivalence of MON 15895 cotton with conventional varieties of cotton was assessed in dairy cows, catfish, quails and poultry, and the results showed that the MON 15895 cotton is as healthy and nutritious as conventional corn when used as fodder for those animals.
The studies in animals were conducted by comparing MON 15895 cotton with conventional cotton. A survey with dairy cows consuming an average of 2.250 kg of raw cotton kernel per day failed to detect any difference in milk production and composition. Among ruminants, a productive dairy cow is an animal very sensitive

to external factors: a change in temperature can affect milk production. Fodder may change not only milk production but mainly its composition, which did not happen with MON 15895. As an effect of ruminal microorganisms, gossypol is inactivated and about 60% of cotton meal protein is degraded, remaining 40% to be digested by the true stomach (abomasus), in addition to intestinal digestion. Studies were also conducted with catfish, quails and poultry (very sensitive to proteic quality), without any record of adverse effect. Given the presence of gossypol and the high content of fibers, cotton meal is either seldom used or used in small quantities (no more than 5%) as fodder for monogastric animals. As gossypol impairs the use of lysine (an amino acid of great importance to animals), its use for monogastric animals is not recommended, and it is only used to reduce the cost of industrial fodder.
Despite the absence of exogenous proteins in food products, the way of action, specificity and exposure history, the absence of similarity with allergenic and toxic proteins, the rapid digestion in simulated gastric and intestinal fluids and the lack of acute oral toxicity in mice demonstrate the safety of these proteins for human and animal consumption.
As the proteins are internal to cells, field workers are not exposed to them. Besides, fibers are mainly cellulose and practically devoid of proteins. Cotton plant is highly self-pollinating and the pollen is large and sticky, which makes dispersion by wind difficult, reducing human exposure.

For people who use short fiber products and cottonseed oil, besides the minimum(sometimes undetectable) level of protein in such products, thermal and chemical treatment generally inactivates or removes the residual protein, making the risk practically inexistent. As already said, in case of ingestion of some

amount of protein, which will be minimal, its digestion in the stomach is very rapid. Considering that protein Cry1Ac represents no more than 0.002% of cotton kernel total proteins, and protein Cry2Ab2, 0.02%, a possible effect to man is unlikely.

V. Environmental and Agronomic Aspects

Modern agriculture is an activity responsible for significant negative environmental impacts(3, 11, 57) and, therefore, the risk assessment of any GM event shall be conducted in relation to that impact inherent to conventional agriculture(5, 15, 43). Therefore, the analysis conducted by CTNBio intended to assess whether the impact caused by Bollgard II Cotton Event MON 15895 is significantly higher than the one caused by conventional cotton varieties considering the practices associated to each system.

All species of the Gossypium genus posses perfect flowers. Fecundation takes place promptly after anthesis, and either self-fecundation, crossed pollination or both are possible. The cotton plant pollen is relatively large, ranging from 81 to 143 micra, viscous (making the pollen grains to adhere to each other), spherical in format, covered by a large amount of spicules and in practice is not transported by wind(47). In the fields, its viability extends to late afternoon, but may last for up to 24 hours if stored at temperatures from 2ºC to 3ºC(10).

Cotton is often described as a partially crossed pollinating culture, though a large number of improvers treat the plant as fully auto-fertile and self-pollinating, except for crossed pollination through pollinating insects. Freire (2000) argues that the cotton plant has a reproductive system intermediating between that of allogamic and autogamic plants, with crossed pollinating rates between 5% and 95%(19). Self-pollination is the preferred form of hybridization in cotton culture, though natural crossing may also occur(46). Seeds are produced at a rate of 20 to 30 per fruit when crossing and self-pollination are well performed(21). The cotton plant flowering time may vary according to environmental conditions and cotton variety, but in general starts about 50 days after emergence and lasts 120 or more days, with the peak of the curve situated around 70 or 80 days. Self-pollinating and crossing procedures shall take place at the most opportune time, 30 to 40 days from flowering.

Genetic improvement requires controlled pollination and maintenance of purity by physical barriers or isolation by distance. Cotton pollen grains are heavy and viscous, which makes dispersion unlikely. Pollen transfer is made by insects, especially by wild bees, bumble bees (Bombus sp.) and honeybees (Apis mellifera) that reach semi-open flowers. In Brazil, cotton genetic improvement programs focus on gathering the most desirable features, according to the region of culture, taking into consideration production components and agricultural adequacy, fiber and thread quality, as well as characteristics of the product for special purposes(21).

Cotton commonly does not propagates by vegetation, but through apples or seeds(19). Natural crossing may occur thorough pollinating insects, since there is not pollen dispersion by wind. However, pollen reach tend to be limited to very close cotton flowers, surrounded by bee colonies. Pollen movement is small, just 1.6% of flowers receive material from other plants. Pollinating insects are used as a tool in improvement programs in order to obtain fresh cotton varieties. One of the most important effects of crossing, known as heterosis or hybrid vigor, may result from interspecific, intraspecific and intervarietal crossing. The use of hybrid vigor in cotton became interesting after it was noticed that excessive introgression (self-pollinating) causes detrimental effects(50). The natural crossing rate detected in Brazil has ranged from 1% to 100% in the Northeastern region, and from 0% to 71% in the Central-Western region. Different crossing rates in boundary regions are explained by the presence of native forests and pollinating insects, mainly honeybees. It shall be emphasized that crossing rates in the Cerrado crops have always been low, about 6%. However, in Cerrado regions with significant occurrence of native vegetation, rates range from 19% to 42% and, in areas cultivated by small farmers, rates are even higher (45% to 69%), because of preserved forests and high population of bees(20).

The application for commercial release of Bollgard II cotton is based on three field experiments conducted in the 2005/2006 crop by the Monsanto do Brasil Ltda. Experimental Stations, located in Santa Cruz da Palmeira, SP; Sorriso, MT and Santa Helena de Goiás, GO. According to data submitted, the agronomic features of MON 15895 cotton (including phenotype, fiber quality, productivity) are

comparable or better than those of conventional cotton (DP50). According to the results, control of A. argillacea was excellent in the three experimental areas, mainly in conditions of high infestation of the pest in Sorriso, MT and Santa Helena de Goiás, GO. The high efficacy of Bollgard II cotton was also observed against H. virescens in these two locations. Apparently, there was not infestation by H. virescens in Santa Cruz das Palmeiras, SP. Infestation by P. gossypiella took place only in Sorriso, MT, and the performance of MON 15895 cotton was also excellent in controlling such species. Efficacy of Bollgard II cotton in controlling H. virescens and P. gossypiella was already recognized from studies conducted in other countries, especially the United States. Due to the low infestation by S. frugiperda it was not possible to assess efficacy of Bollgard II in natural infestations in the three experimental areas. Therefore, the results shown were based in artificial infestations by the pest, conducted only in Santa Helena de Goiás, GO. Leaves and flower buds were infested with two caterpillars by structure, comprising ten blocks (10 repetitions), each with five structures. The structures were separately infested with large and small caterpillars. Each repetition was screen protected to avoid any interference from the environment and escaping by the caterpillars. Assessments were conducted three days after infestations. Significant reduction was recorded in the number of caterpillars and defoliation in the treatment with Bolgard II, however the efficacy in controlling S. frugiperda was lower when compared with other target pests (A. argillacea, H. virescens and P. gossypiella).
Regarding agronomic characteristics, insertion of the segment PV-GHBK11L

apparently has not harmed the plant development. Since 1998, assessments of field essays have been conducted in the United States, Porto Rico, Argentina, South Africa, Costa Rica and Australia.
Comparison studies between event MON 15895 and conventional corn DP50, conducted in the United States, show that features such as yield, morphology and fiber maturity and quality are within a normal range of variability, with significant variation. Assessment of agronomic performance of MON 15895 cotton lineages and cultivars against conventional cotton cultivars under Brazilian conditions also show normal variability between genetically modified plants and conventional ones regarding agronomic characteristics (plant height, cycle up to flowering, precocity of maturation, cycle up to harvest and boll weight), productivity and fiber quality.
Results obtained abroad evidenced an additional and independent activity of Cry1Ac and Cry2Ab2, due to the absence of crossed resistance between the proteins(22, 34, 38), enabling greater biologic activity and enhancing the scope for action against species of genus Spodoptera(54). However, though the combination of Cry1Ac and Cry2Ab2 has proved to be more efficient than Bollgard, Bolgard II is still susceptible to damages caused by Spodoptera ssp. and H. zea under conditions of high infestation, especially when flowering(1, 13, 54). Under such conditions, insecticides are still needed to control the pests. These fields observations are in line with toxicological data reported by Sivasupramanian et al. (2008) verifying the greater tolerance of S. frugiperda (CL50=82ppm) to protein

Cry2Ab2 regarding H. virescens (CL50=0.549 ppm) and P. gossypiella (CL50=0.036 ppm)(53).
With the worldwide increase in area cultivated with insect-resistant genetically modified cultures, concerns about the impact of the technology in non-target organisms, including important ones in biologic control, have been frequently raised. However, management practices of pests associated with cotton Bt have resulted in dramatic reduction in the use of insecticides, leading to a significant increase in populations of beneficial insects and, consequently, contributing to natural control of some pests(59).
Studies were conducted with non-target indicator organisms such as birds, fish and beneficial invertebrate species. Non-target organisms were exposed to leaves or seeds of MON 15895 cotton or to purified protein Cry2Ab2 incorporated in a diet during five to eight weeks, depending of the study. Doses were chosen in so to exceed envisaged environmental exposure, therefore increasing the safety margin of conclusions generated by the studies. Results showed that protein Cry2Ab2 in MON 15895 cotton does not impose previous risks to non-target

organisms. Adverse effects were not recorded in concentrations significantly higher than the ones foreseen for exposure to the environment. In all cases, the no observable effect concentration (NOEC) largely exceeds the maximum environmental concentration, indicating minimum risk posed by protein Cry2Ab2 to non-target organisms. Besides, results obtained in different international research centers showed that the populations of A. mellifera (honeybee),

Folsomia candida (collembolan), Chrysomera carnea (green lacewing), Hippodamia convergens (ladybug), Nasonia vitripennis (jewel wasp), Eisenia foetida (redworm) fail do display any significant adverse effect in concentrations exceeding the one forecasted by exposure in the environment. Study results indicate that protein Cry2Ab2 poses minimum risk to such non-target beneficial organisms. Adverse effects were not recorded at the maximum concentration foreseen in the environment to which such organisms may be exposed.
Additionally, Hagerty et al. (2005) conducted studies with populations of predator species, such as Geocoris ssp., Orius insidiosus, Nabis ssp., Slenopsis invicta, spiders, coccinellidae, chrysopidae and hemerobiidae, evidenced that the populations were either equal or larger in treatments containing Bollgard cotton and Bolgard II cotton contrasted with treatments with conventional cotton(23).
Marvier et al. (2007) analyzed over forty field experiments with cotton and corn expressing proteins Cry, including Cry1Ac, and found that, in general, non-target invertebrates are more abundant in fields of Bt cotton and corn than in conventional ones treated with insecticides(40). On the other hand, fields of insectresistant genetically modified cotton and corn, when compared to fields of cultures untreated with pesticides, display a reduction statistically significant in the number of some non-target organisms(41, 49). Such differences are expected: in general, insecticides are little selective, which explains the fact that fields with Bt plants (and, consequently, with reduced applications of insecticides) show more non-target organisms; however, tillage of conventional plants without pest control (and, consequently, without application of insecticides) will not display reduction

in the population of pests and non-target organisms.
Head et al. (2005) conducted field studies comparing populations of natural enemies in fields of Bollgard and conventional cotton, in the period from 2000 to 2002 in the United States. Results show that, in general, there were no significant differences in populations of natural enemies between Bollgard and conventional cotton. Whenever significant differences were recorded, there was greater abundance of natural enemies in the fields of Bollgard cotton, probably due to the lower use of chemical pesticides. The study also observed that, when insect eggs or larvae were offered as preys, populations of natural enemies in the field of Bollgard cotton exhibited predation rates significantly higher(27).
In China, a monitoring or non-target organisms was conducted in the northeast of the country in Bt cotton fields(60). The results indicate an increase in populations of natural predators, such as ladybug (Coccinella septempunctata), lacewing (Chrysopa sinica), spider and other non-target organisms, in addition to the reappearance of cotton aphis.
In Brazil, Sujii et al. (2008) verified that the Bt cotton plant, the expressing of protein Cry1Ac has no harmful action and fails to positively favor changes in life cycle, survival, fecundity and colony formation of Aphis gossypii in nursery conditions. Results obtained by the authors and data available in the scientific literature show the high specificity of Bollgard technology for controlling target organisms, without positive or negative effects to non-target populations, such as cotton aphis(56).

Regarding the risk of gene flow to wild populations and potential reduction of biodiversity, it is worth stressing that in order for gene introgression to occur, first, hybridization and then a series or retro-crossing are necessary for a gene to be permanently incorporated into the genome(24,25). Additionally, the potential of vertical gene transfer from genetically modified cotton to wild species in noncultivated ecosystems is low, due to the relatively isolated distribution of the species of Gossypium. Some conditions are necessary for vertical gene transfer and gene introgression: physical proximity of the plants (less than 30 meters), simultaneous fecundity times, sexual compatibility of parents, production of viable seeds, generation of fertile progeny ecologically adapted to the environment and

occurrence of gene transfer in the following generation, at least(55).
There are not in Brazil species sexually compatible with G. hirsutum displaying characteristics of invading plants, and it is extremely unlikely that genes cry1Ac and cry2Ab2 be transferred to pests making them more invasive. The cotton plant has not any characteristic associated to potential invasiveness, such as seed dormancy, persistence in soil, germination under adverse environmental conditions, rapid vegetative growth, short life cycle, high production of seeds and dispersion of seeds at long distance. Therefore, it is deemed unlikely that herbaceous Bollgard II cotton may change into a pest plant.
It is worth noticing that genes cry were isolated from a soil bacterium B. thuringiensis. Therefore, exposure of living organisms and environment to this bacterium or to any element thereof is an event that occurs abundantly in nature.
340/2009
33 49
The fear of adverse effects to the environment and concerns of alimentary safety were not justified during the first decade of adoption of the Bollgard technology. On the contrary, data suggest that water and soil quality improved, due to the less use of pesticides in GM cotton cultivars. King (2003) concluded that there is no evidence that GM plants are harmful to the environment, human and animal health(35).
An important characteristics regarding adoption of Bt cotton in different countries is that the use of pesticides has been significantly reduced(44). An ensuing benefit favors the environment and field workers due to the reduced use of pesticides.
According to FAO – Food and Agriculture Organization of the United Nations, the use of Bt cotton has caused a strong positive environmental impact, resulting in significant reduction in contamination of water sources and less impact to beneficial insects(17).
VI. Restrictions to the use of GMO and GMO derivatives
Technical opinions related to agronomic performance concluded that there is equivalence between transgenic and conventional plants. Therefore, the data suggest that transgenic cotton plants are not fundamentally different from the genotypes of non-transformed cotton plants, except for the resistance to certain insects of the order Lepidoptera. In addition, there is no evidence of adverse reactions to the use of Bollgard II cotton. For the foregoing, there are no restrictions to the use of this cotton or its derivatives, either as human or animal food.
340/2009
34 49
As established by Article 11 of Law no. 11,460, of March 21, 2007 “research and cultivation of genetically modified organisms may not be conducted in indigenous lands and areas of conservation units.”
VII. Considerations on particulars of different regions of the country (contribution to supervision agencies)
Bollgard technology was shown to be usable under all agricultural practices commonly used in different regions in different conditions, considering availability of inputs and labor, among other inputs used in the culture of cotton.
There are not creole varieties of cotton plants and the chains of special cotton plants, both conventional and transgenic, have lived together in a satisfactory fashion, without any record of coexistence problems.
VIII. Conclusion
Long experience with traditional plant improvement techniques, over three decades of experience in research and over one decade of marketing transgenic varieties over the world, in addition to knowledge advancements in the structure and dynamics of genomes, indicating whether a certain gene or characteristic is safe, indicate that the genetic engineering process, by its own, leaves little room for appearance of unexpected consequences that would not be identified or eliminated during the process of development of commercial genetically modified varieties(8).
340/2009
35 49
Considering that Bollgard II cotton belongs to a well characterized species (Gossypium hirsutum) with a solid background of safety for human use and that the cry1Ac and cry2Ab2 genes introduced in this variety do not codify any toxic protein, and is harmless to humans;
Considering that Bollgard II cotton was developed from Bollgard cotton, which was approved for commercial use by CTNBio in 2005 and, up to this moment, there is no evidence of risk to human and animal health and to the environment;

Considering that Event MON 15895 is a single-copy event of stable insertion and that Chi-square analysis evidenced that the insert segregates according to Mendelian genetics, with a segregation pattern of a single gene regarding detection of protein Cry2Ab2;
Considering that composition data failed to point significant differences between the genetically modified and conventional varieties, suggesting an equivalence between such varieties; and
Whereas:
1. Cotton plant is one of the most used plants among those cultivated by the human being;
2. Proteins Cry1Ac and Cry2Ab2 are promptly digested after ingestion and that effects of chronic exposure to such proteins are not expected;
3. Acute oral toxicity of proteins Cr1Ac and Cr1Ac is low, and they are

considered to be non toxic for mammals;
4. The DNA molecule is a natural component of food and there is no evidence that it may have any adverse effect to humans when ingested in food within acceptable amounts (no direct toxic effect);
5. There is no evidence that intact genes of plants may be transferred and functionally integrated to the human or other mammals genome exposed to the DNA or to food produced with such elements.
6. The likelihood that the herbaceous Bollgard II cotton plant becomes a pest plant is negligible;
7. Exposure of living organisms and environment to B. thuringiensis or to any element extracted from this bacterium is an event that occurs abundantly in nature;
8. Insertion of segment PV-GHBK11L apparently failed to harm the development of the plant regarding agronomic characteristics;
9. There are no reports in change of agronomic performance observed in the commercial cultivation of this event in other countries;
10. Analysis of biochemical composition showed that event MON 15895 displays substantial equivalence with non-genetically modified varieties, a robust suggestion that such event has no undesirable pleiotropic effects;

11. Literature and field experiment data suggest that event MON 15895 has no impact against non-target organisms except those already inherent to the culture of cotton;
12. Field data indicate that water and soil quality improved due to the less use of pesticides in cultivars of GM cotton;
13. Adoption of Bt cotton in different countries has significantly reduced applications of pesticides;
Summarizing, considering the criteria internationally accepted in the process of analyzing the risk of genetically modified raw materials, it is possible to reach a conclusion that Bollgard II Cotton Event MON 15895 is as safe as its conventional equivalent.
For the foregoing, commercial release of Bollgard Cotton Event MON 15895 is not potentially harmful to human and animal health and does not cause significant environment degradation.
The CTNBio analysis considered the opinions issued by the Commission members; ad hoc consultants; documents delivered by the applicant to the CTNBio Executive Secretariat; results of planned releases into the environment; lectures, texts and discussions in a public hearing held on 08.17.2007. Independent third party scientific studies and publications submitted by the applicant were also considered.

According to Annex I of Ruling Resolution 5, of March 12, 2008, applicant shall make adjustments to its proposed post-commercial release monitoring plan within thirty (30) days from publication of this Technical Opinion.
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Walter Colli
President of CTNBio

Dissenting Vote:
Rapporteur, Doctor José Maria Gusman Ferraz (Permanent Sector

Subcommission for the Environment) voted against approval of this product on grounds that the data for commercial release of Bollgard II cotton event MON 15895 are not sufficient to insure its biosafety, suggesting a biorisk, since it affects the environment as a whole and does not guarantee a more sustainable production. In addition, Doctor Ferraz considered that the reported data on control efficiency of pests are inconsistent, that environmental impacts are not sufficiently known, and that recent findings on negative impacts of Cry protein on arthropods acting on natural biological control and on the soil biota, as well as its permanence in the soil suggest the need for more studies in Brazilian conditions to assess possible advantages of its commercial release, vis-à-vis its environmental, social and economic impacts. CTNBio Members Doctors Leonardo Melgarejo, Paulo Kageyama, Rodrigo Roubach and Graziela Almeida da Silva followed the above Rapporteur opinion.