THE BRAZILIAN FEDERAL SCIENCE AND TECHNOLOGY DEPARTMENT

EXECUTIVE SECRETARIAT

Brazilian National Biosafety Technical Commission - ctnBio

PREVIOUS CONCLUSIVE TECHNICAL OPINION

Nr. 513/2005

Proceedings number: 01200.001471/2003-01

Requesting Party: Monsanto do Brasil Ltda.

National Directory of Legal Entities ("CNPJ"): 64.858.525/0001-45

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Subject: Release for trading of cotton genetically modified resistant to the main plagues of the Order Lepidoptera

Previous Statement: 18/2003, published in the Federal Official Gazette ("D.O.U.") number 107, of June 5, 2003, section 3, pages 5 and 6

Meeting: 86th Annual Meeting of CTNBio, held on March 17, 2005

Decision: APPROVED

CTNBio, after appreciating the Previous Conclusive Technical Opinion for the trade release of cotton genetically modified resistant to insects, concluded for its APPROVAL, under this previous conclusive technical opinion.

Monsanto do Brasil Ltda. requested to CTNBio a conclusive technical opinion on the biosafety of the genetically modified organism ("GMO") designated "Algodão Bollgard Evento 531" [Bollgard Cotton Event 531], for purposes of trade release in Brazil. Said GMO resists to the main Lepidoptera Order plagues affecting cotton culture in Brazil, such as the cotton leafworm (*Alabama argillacea*), the pink bollworm (*Pectinophora gossypiella*), and the tobacco budworm (*Heliothis virescens*). The Bollgard cotton event 531 was genetically modified from the transformation of the commercial variety *Coker 312*, with the vector PV-GHBK04, through the system mediated by the *Agrobacterium tumefaciens*. The transformation inserted the genes *crylAc, nptII*, and *aad* in the genome of such a cotton variety. The protein CrylAc arises out of the *Bacillus thuringiensis*, a gram-positive soil bacterium capable of forming crystals containing endotoxins, proteins with insecticide action actuating before and during the sporulation phase of its lifecycle. Commercial formulation of *B. thuringiensis*containing such proteins have been used in Brazil and in other countries for controlling some agricultural plagues for more than 40 years. Once *B. thuringiensis* is a soil microorganism, the exposure of living organisms and the environment to such a bacterium or to any element extracted therefrom is an event occurring on an abundantly basis in the nature. The protein CrylAc has a quite specific action and actuates only by the ingestion in some species of Lepidoptera. The protein NPTII is produced by a number of prokaryotic microorganisms found in a ubiquitous manner in the environment, both in aquatic and terrestrial habitats, such as in human and animal intestinal microflora. The AAD protein is not an expressed one in tissues of the Bollgard cotton event 531. The genetically modified cotton event 531 did not show morphologic, phenological or plant architecture alteration. The gene insertion caused no effect on fibers' quality. Except for the tolerance to target insects during the crop, the Bollgard Cotton event 531 plants showed equivalence in all phenotypical and agronomical features as regards the standard shown by the non-transformed parental lineage and other varieties used in commercial production. The analysis of the documents submitted allows us to conclude that the cultivation of the Bollgard cotton event 531 shall not cause alteration in the soil and in its ecologic and functional relationships, different from those caused by conventional varieties. Due to the specificity of the CrylAc protein action on some Lepidopteran species, a direct negative effect on the third trophic level (natural enemies) is not expected. Studies conducted in other countries have demonstrated that there is no adverse effect by the CrylAc protein on natural enemies. Even after almost 10 years of use of the Bollgard cotton event 531 in other countries, up to now there are no reports on the evolution of resistance from any plague to the*B. thuringiensis* toxins in the field. The NPTII protein is quickly degraded, as the other proteins found in vegetal tissues, and it is not toxic for living beings. The resistance to the canamycin and neomycin, granted by the gene *nptII*, is a ubiquitous presence in microorganisms, and there are no evidences of gene transfer from the plant to the bacteria. Therefore, the horizontal gene transfer occurrence represents a minimum risk in cultivations of the Bollgard cotton event 531. Studies made in Brazilian Central-Western region reveal that the crossing rates obtained in the middle of big crops were always low, with averages varying from 3.9% to 4.3%. The crossing rates in plantations with 10 and 15m of alley were 0%, and the gene flow of the Bollgard cotton event 531 for conventional cotton was 0.85% in borders of cotton genetically modified. Studies in other countries showed that the likelihood of gene transfer from a field with Bollgard cotton event 531 to a field with conventional or sylvan cotton is a very low one, and trends to zero in distances higher than 15m. The reduction in the use of insecticides promoted by the use of plants genetically modified resistant to insects has positive repercussions in other aspects related to the obtainment, distribution, and use of agricultural defensives, significant reductions in the pollution caused by industrial wastes, reductions in the use of water to be used in spraying, and in corporate and environmental costs arising out of the transportation and storage of insecticides. Genetically modified plants resistant to insects collaborate for production reduction and the accumulation of agricultural toxic packages. Studies made do not show alterations in the main components and in natural anti-nutrients present in the cotton. The safety of feeding products from Bollgard cotton event 531 was determined by equivalence in the composition of macro and micronutrients in salubrity studies conducted with animals, and it was concluded that such product, as a component of animal ration, and proteins CrylAc and NPTII expressed in plant tissues, showed to be safe and with an equivalent nutrition value for human and animal consumption. The analysis of quality and composition of Bollgard cotton event 531 seed showed that the properties of the genetically modified cotton and its processed functions were comparable to those of the conventional cotton. It was concluded that despite of the absence of proteins in feeding products, the mode of action, specificity, and the history exposure, the lack of similarity with allergene and toxic proteins, the quick digestion in simulated gastric and intestinal fluids, as well as the lack of acute oral toxicity in animals, the Bollgard cotton event 531 expressing proteins CrylAc and NPTII shows safety for human and animal consumption equivalent to that of the conventional cotton. Moreover, it was demonstrated in other countries that the reduction of insecticide use caused a significant reduction in the number of intoxications of agriculturists. Because of the aforementioned, the Brazilian National Biosafety Technical Commission ("CTNBio"), after the analysis of Bollgard cotton event 531 biosafety, through proceedings 01200.001471/2003-01, hereby approves its release for commercial plantation and human and animal consumption under the following conditions: (i) Monsanto do Brasil Ltda., a company holding the Bollgard technology, shall provide the primers for detecting the specific event to the registration and inspection bodies; (ii) respect the exclusion zones as regards the plantation of the genetically modified cotton, as proposed by Barroso e Freire (2004), and determine and limit the crop time for the Bollgard cotton event 531 in the different cotton producing regions, mainly in locations with *safrinha* cotton crops; (iii) refuge areas with non-transgenic cultivars of cotton corresponding to 20% of the area to be cultivated with the Bollgard cotton event 531 shall be recommended, located at distances shorter than 800m; (iv) adopt conservationist handling practices of the cotton-plant culture, such as the destruction of the rootstock, the burn for disease control, crop rotation, the employment of trap cultures and the biologic control. The surveillance bodies shall be the one to ensure the compliance with the requirements contained in the Previous Conclusive Technical Opinion, mainly those relevant to refuge areas and exclusion zones. There are no restrictions to the GMO use in analysis and its byproducts, provided that the requirements contained in the Previous Conclusive Technical Opinion are complied with. So, CTNBio considers that such activity is not the potential causer of a significant degradation of the environment and human health.

Under the Article 1 D of the Law 8974/95, CTNBio considered that the experimental protocol and the other biosafety measures proposed meet the relevant rules and laws intending to ensure the environment, agriculture, human and animal health biosafety.

CTNBio PREVIOUS CONCLUSIVE TECHNICAL OPINION

Technical Foundation

I. GMO Identification

GMO Designation: Bollgard Cotton Event 531

Requesting Party: Monsanto do Brasil Ltda.

Species: *Gossypium hirsutum* - cotton

Inserted Feature: resistance to the main plagues of the Lepidoptera Order (cotton leafworm [*Alabama argillacea*], pink bollworm [*Pectinophora gossypiella*], and tobacco budworm [*Heliothis virescens*])

Method for feature introduction: transformation measure by *Agrobacterium tumefaciens*

Proposed use: production of textile fibers, only of the cotton seed for animal ration, and oil of the cottonseed for human consumption.

II. General Information

Monsanto do Brasil Ltda. requests to CTNBio a conclusive technical opinion on the biosafety of the genetically modified organism ("GMO") designated "Bollgard Cotton Event 531", for purposes of trade release in Brazil. Said GMO resists to the main plagues of the Lepidoptera Order that affect the cotton culture in Brazil, such as the cotton leafworm (*Alabama argillacea*), pink bollworm (*Pectinophora gossypiella*), and tobacco budworm (*Heliothis virescens*).

The cotton (*Gossypium* spp.) is one of the main cultures used for producing fibers in the world, it being one of the most important productive chains in Brazil. The main cotton producing regions of the country are the States of Mato Grosso, Goiás, Bahia, Mato Grosso do Sul, Ceará, São Paulo, Minas Gerais, and Paraná. It is a culture known for suffering serious damages because of the occurrence of plagues, weeds, and diseases.

Among the main cotton plagues in Brazil, we may name the cotton leafworm (*Alabama argillacea*), tobacco budworm (*Heliothis virescens*), pink bollworm (*Pectinophora gossypiella*), *fall armyworm* (*Spodoptera frugiperda*), melon aphid (*Aphis gossypii*), cotton plant bug (*Horcias nobilellus*), and cotton boll wevil (*Anthonomus grandis*). Such plagues' control has been primarily made through the use of insecticides. In Brazil, more than 10 tons of insecticides are consumed on a yearly basis only for the cotton culture, making production costs burdensome ones, around US$ 190 million. The excessive use of non-specific insecticides cause negative environmental impacts such as a serious reduction in the population of benefic organisms, and the potential appearing of plagues resistant to conventional insecticides.

The cotton species commercially cultivated in Brazil are the *Gossypium hirsutum*, and in a smaller area, the *G. barbadense*. *G. hirsutum* has a better adaptability, high productivity, and is predominant on a worldwide basis. Its fiber is used in the production of the textile fiber of other non-textile products, and is a source of industrial cellulose for a number of products. *G. barbadense* is important for its fiber quality and length, and it is used in the production of fine clothes.

The Bollgard Cotton event 531 was genetically modified from the transformation of the commercial variety Coker 213 with the PV-GHBK04 vector, through the system mediated by the *Agrobacterium tumefaciens*. The transformation inserted the genes *crylAc, nptII*, and *aad*in such a cotton variety genome.

The researches for evaluating the agronomic effectiveness and performance of the Bollgard Cotton in Brazil were made in crops 1997/1998 and 1999/2000, in the regions of Goiatuba and Edéia (State of Goiás), Ituverava and Santa Cruz das Palmeiras (State of São Paulo), Rondonópolis (State of Mato Grosso), and Capinópolis (State of Minas Gerais). Other studies for evaluating the effectiveness of the Bollgard Cotton against some plague species were made in green house. Studies confirmed the effectiveness of the Bollgard Cotton in the control of the cotton leafworm (*Alabama argillacea*), tobacco budworm (*Heliothis virescens*), and pink bollworm (*Pectinophora gossypiella*).

The Bollgard Cotton is an advanced technology, and of great interest for Brazil, where Lepidoptera plagues cause great production losses, and for its control enormous quantities of insecticides are applied. The adoption of such technology may reduce the use an approximately one million-liter of insecticides in the country every year, raise productivity and reduce production costs.

III. GMO Description

The genetically modified cotton developed by Monsanto do Brasil Ltda., designated Bollgard Cotton Event 531, was generated by the use of indirect transformation technique via*Agrobacterium tumefaciens*, consecrated technique and one of the most used in the obtainment of genetically modified plants. *A. tumefaciens* is a gram-positive bacterium found in soil, and causer of the plant tumor.

The mechanism for inserting the genes of *A. tumefaciens* in the hostess plant is almost all known. In wild *A. tumefaciens*, the existence of the plasmid Ti allows the bacterium to insert a portion of its genome (region T-DNA) in the plant genome. In disclosing such mechanism, the scientists, using molecular biology techniques, retired the genes inducting the tumor or the T-DNA region of plasmid Ti and substituted them for interest genes. The specificity of the mechanism in transferring only the sequences between the right and the left edge of the T-DNA region makes the transformation strategy quite safe, to the effect that other sequences of the bacterium mediating the process are not transferred.

Such process resulted in the stable introduction of three genes in the genome of a conventional variety of cotton, Coker 312, with the binary vector PV-GHBK04. The elements forming vector construction comprehend, further to the interest genes, sequences normally used in the construction of expression vectors by the specialized scientific community, such as genes of replication origin, genes promoting and terminating the transcription.

The Bollgard cotton event 531 has three inserts originated during the transformation process: the gene *CrylAc*, which codifies the protein CrylAc of the *Bacillus thuringiensis* variety*kurstaki*; the gene *nptII*, which codifies the Phosphotransferase Neomycin type II ("NPTII"), which grants resistance to canamycin and neomycin antibiotics; and the gene *aad*, which codifies the protein 3"(9)-O-aminoglycoside adenililtransferase. The protein CryAl has a 99.4% homology with the *B. thuringiensis* protein, providing high specificity of action on some plague species of the Lepidoptera order. The genes *nptII* and *aad* were the selection markers for cells transformed with the gene *crylAc* in their *in vitro* phase. Only the genes *crylAc* and*nptII* are expressed on Bollgard Cotton event 531. The gene *aad* is controlled by a bacterial promoter, and the protein AAD is not detected in the GMO tissue.

Molecular analyses ("Southern Blot", Polymerase Chain Reaction ("PCR"), cosmid cloning, DNA sequencing, and "genome walking") showed that the DNA and the left and right edge of the vector PV-GHBK04 was inserted in two places of the cotton genome, separated by the plant genomic DNA. Only one insertion is functional for having the complete cassette for the*crylAc* gene expression. The second one corresponds to a small segment without functional activity, which provides independent segregation in conventional crossings. The retro-crossing results, including with tropical varieties, are consistent with the insertion of the functional *crylAc* gene in a sole *locus*, maintaining its entirety during several generations.

Once the Bollgard cotton event 531 is released for commercial use, the surveillance and registration bodies shall have access to information allowing the differentiation of event 531 among other events of other plants and raw materials not genetically modified. So, the information of DNA sequences for the genes inserted in Bollgard cotton event 531 and the primers for use in the PCR technique specifically identifying such event should be made available to the surveillance and registration agencies.

IV. Expressed Proteins

The protein CrylAc arises out of *Bacillus thuringiensis*, a gram-positive soil bacterium initially insulated in Japan by Ishiwata, and formally described by Berliner in 1915. Such microorganism forms crystals containing endotoxins, proteins with insecticide action actuating before and during the sporulation phase of its lifecycle. Commercial formulations of *B. thuringiensis* containing such proteins have been used in Brazil and in other countries for controlling some agricultural plagues for more than 40 years. Once *B. thuringiensis* is a soil microorganism, the exposure of living organisms and the environment to such a bacterium or to any element extracted therefrom is an event occurring on an abundantly basis in the nature.

The crystals from different lineages of *B. thuringiensis* may contain a series of different proteins that have insecticide action, toxic for different groups of insects. Among the toxins, those known as proteins Cry or d -endotoxins are emphasized. The protein CrylAc has a quite specific action, and actuates only through the ingestion of some species of Lepidoptera. Generally, the action mechanism for Cry proteins consists of three main steps: a) solubilizing and activation of the crystal in the insect medium intestine; b) linking of the activated toxin to specific receptors; and c) insertion of the activated toxin in the apical membrane of the medium intestine for creating ionic channels or pores.

The protein NPTII is produced by a number of prokaryotic microorganisms found in a ubiquitous manner in environment, both in aquatic and land habitats, as in human and animal intestinal microflora.

The *nptII* gene used for plant transformation is derived from the transposon Tn5 of the *Escherichia coli*, an enterobacterium present in man intestinal flora. The way of action of protein NPTII is well featured, and culminated with the inactivation of antibiotics such as the neomycin, the gentamicyn A, and the canamicyn A, B, and C. The protein NPTII is degraded in the gastrointestinal system of humans and animals.

The expression of proteins CrylAc and NPTII was determined in leaves and seeds of the Bollgard Cotton event 531, through the ELISA technique, in field experiments conducted during the crop 1999/2000 in Edéia (State of Goiás), Capinópolis (State of Minas Gerais), Rondonópolis (State of Paraná), and Ituverava (State of São Paulo). The average levels of protein CrylAc in leaves collected 20 and 130 days after the plantation of the lineage DP90B (derived from the Bollgard cotton event 531) were 2.93 µg and 3.02 µg of protein per gram of fresh tissue, respectively. In seeds, the average level in every location was 1.83 µg of protein per gram of fresh tissue. The average levels of protein NPTII in leaves collected 20 and 130 days after the plantation of lineage DP90B were 5.57 µg and 9.55 µg of protein per gram of fresh tissue, respectively. In seeds, the average in all locations was 6.88 µg of protein per gram of fresh tissue.

V. Agronomic Features

The field evaluations with the Bollgard Cotton event 531 conducted in cotton-producing regions in Brazil showed that there are no significant differences in morphologic features and in agronomic performance in relation to the Coker 312 non-transformed parental lineage. The following parameters were evaluated: effectiveness in the control of target-insects during the crop; plant growing and development morphologic features as germination, plant vigor, flowering, number and size of cotton bolls; susceptibility to plagues and diseases; yielding; fiber quality such as length, *micronaire* (fiber fineness and weaving capability), yarn resistance and elongation capability; and grain composition, where proteins, fats, fibers, carbohydrates, aminoacids, mineral residues, caloric contents, lipids, fatty acids, a -tocopherol, gossypol, and aflatoxins were evaluated.

The results of researches made in Brazil are similar to those observed in other countries. The levels of insect-laying plagues *A. argillacea/P. gossypiella* , and *H. virescens* in the Bollgard cotton event 531 were similar to those observed in the non-transformed parental lineage. The protein CrylAc expressed by plants of genetically modified cotton controlled and maintained the plague *P. gossypiella* within population satisfactory levels, even in high population pressure situations. The plagues *A. argillacea* and *H. virescens* had not a significant presence along the culture cycle of the Bollgard Cotton event 531 in evaluated experiments. The parasitism levels observed in eggs of *A. argillacea* and *H. virescens* species in the genetically modified lineage showed that parasitoids were not affected by the insecticide effect of protein CrylAc, compared with the non-transformed parental lineage.

Fiber qualities were also evaluated, and did not show any effect of the gene insertion. Data on the length, length uniformity, *micronaire* index, resistance and elongation capability of the yarn obtained from the Bollgard Cotton event 531 in commercial production in other countries showed equivalence or higher quality in relation to fibers produced by the Coker 312 parental variety, and by the traded conventional varieties.

The composition of genetically modified cotton grains showed to be equivalent in the composition standard of parental variety grains and commercial varieties.

The studies conducted showed that a reduction in the use of wide spectrum insecticides in cotton areas in countries where Bollgard technology is already available is a possible one.

Except for the tolerance to target-insects during the crop, the Bollgard Cotton event 531 plants showed to be equivalent in all phenotypic and agronomic features in connection with the standard shown by the non-transformed parental lineage, and other varieties used in commercial production. Thus, and within the Integrated Plague Handling (IPH) in the cotton culture, Bollgard technology may be introduced in the Brazilian market with a relative facility.

VI. Environmental Aspects - Environmental Safety

The environmental safety evaluation is based on the expression of functional genes and protein level in the plant, the mode of action and specificity of express proteins, its abundance and behavior in the environment and in the history of exposure and safe use of proteins produced by the bacterium *B. thuringiensis*, toxic for Lepidoptera plagues of the cotton-plant.

As formerly reported, the protein CrlAc is an express one in plant tissues, with concentration lower than 4 mg/g of fresh tissue in young leaves, and less than 2 mg/g in fresh seeds. The concentration of protein CrylAc in the whole plant is to the order of millionth parts of the total protein of the cotton-plant residues. Estimates point to an amount of *B. thuringiensis*, variant*kurstaki* HD73, incorporated to the soil upon the harvest lower than 2g/acre, which would correspond to 0.5 mg/g of soil in the plowing layer.

Studies made in Escola Superior de Agricultura "Luiz de Queiroz" [Luiz de Queiroz Graduate Agriculture School] of Universidade de São Paulo [The São Paulo State University] show that proteins above 70 Kda are detected in vegetal rests at fields, and suffer quick proteolytic action upon the maturation of fruits and in soil by the microbiota. Those results corroborate another study in samples of soils of transgenic cotton fields, in which tests employing the ELISA, an effective and sensible method, and biological trials of activity show the absence of protein accumulation in biologically significant levels. A number of other studies show that the Cry proteins are quickly dissipated in soil, and that even though there is an accumulation, such proteins are practically atoxic for non-target organisms, as disclosed by a number of *in vitro* studies, or with the addition of GM plant wastes to the soil. Tests with protein concentrations of up to 50 mg/g of soil did not show effects on the increase of caterpillars. Similarly, populations of detritus eaters as *collembolans* and earthworms, and microbiota components as protozoon, nematodes, fungus, bacteria, and actinomycetes are practically insensible to the protein CrylAc as to the reproductive and growing aspects. Other studies with more refined experimental techniques show the absence of change in the microbial density of a number of specialized soil groups, such as diastrophic antibiotic-resistant bacteria. It was observed, in studies employing Ecoplates and the molecular analysis ARDRA, that the effects of the Bollgard cotton event 531 on soil prokaryote metabolic and genetic diversity are similar to those of the conventional cotton. The analysis of the documents submitted allow us to conclude that the cultivation of the Bollgard cotton event 531 shall not cause alterations on the soil and its ecologic and functional relationships are not different from those caused by the conventional varieties.

Due to the specificity of the action of the protein Cryl Ac on some Lepidoptera species, a direct negative effect on the third trophic level (natural enemies) is not an expected one. As the population of natural enemies depends on the plague density, if the population of a certain plague is controlled with the Bollgard cotton event 531, it is expected that the population of its respective natural enemies, mainly that formed by specialists, trends to be reduced. In general, studies conducted in other countries have shown that there is no adverse effect by the CrylAc on natural enemies. Some studies showed that there was even an increase in biodiversity with the use of the Bollgard cotton event 531. It occurs mainly because of the reduction in the use of wide spectrum insecticides.

The toxicity evaluation of the pollen arising out of the Bollgard cotton event 531 in bees *Apis mellifera* conducted by Escola Superior de Agricultura "Luiz de Queiroz" of Universidade de São Paulo did not disclose significant adverse effects.

Due to the continued expression of insecticide toxins, the insect-resistant plants exercise a high selection pressure on plague insect populations under control. Even after almost 10 years of use of the Bollgard cotton event 531 in other countries, up to this moment there are no reports on the evolution of resistance of any plague to toxins from *B. thuringiensis*in the field, from the exposure to genetically modified insect-resistant plants. In different countries, the results of resistance handling strategies may be given in data collected through the susceptibility monitoring of plague insect populations to insecticide proteins Bt. So, the sustainability of Bt cultures depends on the adoption of proper release and handling programs for such plants in the environment, in order to retard, to a maximum extent, the evolution of insect resistance. Among the immediate consequences of insects' resistance to insecticide proteins expressed by Bt plants, we have the loss of such technology in the IPH. Moreover, there is the possibility of restriction to use of bio-pesticides formulated based on Bt, and the increase in the use of conventional insecticides.

The main strategy of handling adopted in other countries, having as grounds the philosophy of the Integrated Plague Handling IPH), was that of "high dose of toxin associated to the refuge area". The main assumptions of such strategy are the low initial frequency of the resistant alleles; the standard of recessive inheritance for resistance; the dose of protein enough for causing the high mortality of susceptible individuals and heterozygotes; and the production of susceptible individuals in refuge areas located at a certain distance, for making random crossings possible. Based on the Caprio mathematic pattern for simulating resistance evolution, the company proposal for the refuge area (plantation of conventional cotton) is 20% of the total area cultivated with Bollgard cotton event 531, for a technology durability of no less than 10 years. The maximum recommended distance between the refuge area and the Bollgard cotton area event 531 is 800 to 1,500m, depending on the occurrence or not of *P. gossypiella* in the area. Further to the preservation of the plague susceptibility source to*crylAc* in refuge areas, such areas may also serve for preserving from special natural enemies of the plagues.

The protein NPTII is expressed in Bollgard cotton event 531 leaves in higher concentration that the CrylAc. Such protein is quickly degraded, as the others found in vegetal tissues, and is not toxic for living beings. Its eventual expression in other organisms shall not imply behavioral alterations, excess in the capacity of tolerating aminoglycoside antibiotics, such as the canamycin and neomycin.

The transfer of the plant *nptII* gene to microorganisms is possible from the biologic point of view, but it happens with a very low frequency in optimized conditions, and there are no evidences of such a phenomenon in real field conditions. In prokaryotes, the transfer of such gene is made by mobile elements (plasmids and conjugative transposon), which ensure wide distribution of the gene among the species, or within the same prokaryote species. That is why the resistance to canamycin and neomycin is a ubiquitous presence in microorganisms, there is no evidence of transfer from the plant to the bacteria. So, the occurrence of horizontal gene transfer represents a minimum risk in the cultivations of the Bollgard cotton event 531.

The vertical gene transfer of the genetically modified cotton for conventional cultivars and sylvan species sexually compatible is a possible one, mainly through pollen grains, generally transported by pollinators. Studies made by Embrapa Algodão showed that a relatively small number of conventional cotton rows used as borderline for the genetically modified cotton (10 lines) was enough for containing the pollen of the quota inner portion with transgenic plants, even in places where the crossing rates were elevated.

Evaluation studies of the pollen dispersion distance and the frequency of natural crossing using plants marked with methylene blue and molecular markers showed that in Brazilian Central-Western Region the crossing rates obtained in the environment of big crops were always low, with averages varying between 3.9% and 4.3%. The crossing rates in plantations with 10 and 15m of road were 0%, and the gene flow of Bollgard cotton event 531 to the conventional cotton was 0.85% in borderlines of genetically modified cotton.

Studies conducted by Embrapa Algodão address the geographic distribution of such plant in Brazil, suggesting exclusion zones for cultivations of genetically modified cotton, and provide the actions taken by other countries where there are cotton sylvan species for avoiding the escape of transgenic for sylvan populations. The data submitted did not show aspects impacting the transgenic cotton environment. Data on the gene transfer potential between the Bollgard cotton event 531 and the close relatives and the conventional cotton in Australia, United States, India, and Israel, showed that the features of crossings were similar in different geographies and environments and that the gene transfer likelihood of a filed with Bollgard cotton event 531 for a field with conventional or sylvan cotton is a very low one, and trends to zero in distances greater than 15m.

The other possibility of gene flow is through seeds, in which the anthropic action performs an important role as the dispersion of cotton seeds hardly occurs from field seeds, as they are big, covered with fibers, and are rarely transported by animals. In Brazil, dispersion generally occurs through the undue use of seeds (seeds destined for animal feeding or oil manufacturing) as propagative material, and during the cotton transportation in plumes, kernels, and seeds, further to mixtures in cotton-plants of the Brazilian Northeastern Region, which, in general, seed more than one cultivar per month.

The reduction in the use of insecticides caused by the use of insect-resistant genetically modified plants provides positives repercussions in other aspects related to the obtainment, distribution, and use of those agricultural defensives. In China, the Bt cotton has been cultivated since 1997, with a reduction of 78,000 tons in the amount of insecticides used in 2001. A reduction in the exploitation rate of raw materials used in insecticide manufacturing, and, as a consequence, significant reductions in pollution caused by industrial wastes, were observed. We may also mention reductions in the water to be used in spraying and in corporate and environmental costs arising out of the transportation and storage of insecticides. Finally, insect-resistant genetically modified plants collaborate for the reduction in production and accumulation of agricultural toxic packages, which many times have not a final safe destiny in environment.

VII. Aspects related to the Human and Animal Health

The safety of the proteins CrylAc and NPTII is based on the knowledge of the biology of organisms containing the genes that codify such proteins, in their abundant occurrence in environment, in the function, and in the mode of action of proteins, and in the safe use history.

The exposure of living organisms to Cry proteins produced by *B. thuringiensis* is an event that occurs on an abundantly basis on the nature, and the mode of action of such protein is already well-known. The United States Environmental Protection Agency (EPA), in 1998, concluded, from the big volume of toxicology data submitted, that the subspecies of *B. thuringienis* are not toxic or pathogenic for mammals, including human beings. Recently, the World Health Organization (WHO) revised the extensive safety data banks, and concluded that the Bt does not cause adverse effects to human health when present in fresh water or foods.

The exposure of living organisms to the protein NPTII also occurs on an abundantly basis in the nature, due to the prevalence of bacteria producing such a protein. The safety of the NPTII was evaluated by the United States Food and Drug Administration (FDA) in 1994. It was concluded that such a protein is safe for use in foods. For WHO, there are no reasons for concerns as regards the safety of NPTII. Such protein has not a pesticide or insecticide activity, and is produced in the plant with the only purpose of causing an effective transformer selection system.

The safety of a genetically modified plant considers the new express proteins and the anticipation of use intention and consumption of products arising out of the relevant plant by human beings and animals. For determining the feeding safety as regards genetically modified plants, the Substantial Equivalence Principle is used as a guide for evaluations. Such principle sets forth that the genetically modified plant should be as safe as its conventional counterpart. Comparisons focus substances that are outstanding from the toxicological, nutritional and salubrious point of view.

In the case of the relevant product, the studies conducted have not shown alterations in the main components and natural antinutrients present in the cotton. The safety of feeding products from the Bollgard cotton event 531 was determined by the equivalence in macro and micronutrient composition in salubrity studies made with animals, and it was concluded that such product, as a component of the animal ration and CrylAc and NPTII proteins expressed in the plant tissues, showed to be safe and with an equivalent nutrition value for human and animal consumption. After fiber and seed processing, the proteins expressed by the plant are not detected. As the oil and processed fibers are the only cotton products used in human feeding and for clothing, respectively, the protein consumption is not expected.

The analyses of the seed quality and composition of the Bollgard cotton event 531 showed that the properties of the genetically modified cotton and its processed fractions were comparable to those of the conventional cotton. Studies made with animals (milk cows, catfishes, quails, and rats) showed that the nutritional quality of the relevant product seed was the same than that obtained in the conventional cotton, and that the development of animals was not altered by the ingestion of either material. So, notwithstanding the lack of proteins in feeding products, the mode of action, specificity, and exposure history, the lack of similarity with allergenic and toxic proteins, the quick digestion in simulated gastric and intestinal fluids, and the lack of acute oral toxicity in animals, the Bollgard cotton event 531 expressing the proteins CrylAc and NPTII shows to be safe for human and animal consumption to the same extent of the conventional cotton.

In addition to that, with the reduction of the insecticide use in China, in 2001, a reduction of up to 75% in the cases of rural producers' intoxication caused by insecticides was registered. Similarly, the cotton Bt has assisted the South African agriculture in crops' MIP, resulting in the reduction of insecticide use, as well as in the ratio of workers' intoxication and culture production cost.

VIII. Conclusion

Thus, the Brazilian National Biosafety Technical Commission ("CTNBio"), after the biosafety analysis for the Bollgard cotton event 531, proceedings 01200.001471/2003-01, resolves for its release for commercial plantation and human and animal consumption pursuant to the below-mentioned conditions.

Monsanto do Brasil Ltda., a company holding the Bollgard technology, shall provide the DNA sequences of the genes inserted in the Bollgard cotton event 531, and the primers to be used in the PCR technique, which specifically identify such event before the surveillance and registration bodies.

Further to respect the exclusion zones as regards the plantation of the genetically modified cotton for containing the gene flow, as proposed by Barroso e Freire (2004), the crop time for the Bollgard cotton event 531 in the different cotton producing regions, mainly in locations with *safrinha* cotton crops, should also be determined and limited, in order that the period of plagues' exposure to CrylAc is the shortest possible.

Refuge areas with non-transgenic cultivars of cotton corresponding to 20% of the area to be cultivated with the Bollgard cotton event 531, located at distances shorter than 800m, shall be recommended. However, it may be required to review the refuge area when the total area of cultures Bt (cotton and maize, or only cotton in the event that the maize Bt is not released for commercial use) reach 50% of the cultivated area in a certain region. That is due to the fact that despite of the *S. frugiperda* not to be deemed a target-plague of control as regards the Bollgard cotton event 531, there are reports in the literature of the low toxic activity of the CrylAc against such species, and the answer to the selection pressure for a greater tolerance to CrylAc. In additional to that, there are reports in the literature on the genetic similarity between populations of *S. frugiperda,* arising out of cotton and maize cultures. If the genetically modified maize expressing the protein CrylAb be released for trading, the selection pressure for resistance shall be even greater in a certain region, as there are reports on the crossed resistance between the CylAc and the CrylAb (similarity of action). That could be avoided with the increase of the refuge area.

Further to the specific aspects approached, it is important to consider the different practices recommended in the plan of management of the cotton-plant culture. Further to those already mentioned exclusion zones and refuge areas, the destruction of the rootstock, the burning for disease control, the crop rotation, the employment of trap cultures, and the biologic control.

The relevant surveillance bodies shall be responsible for ensuring the compliance with the requirements contained in this opinion, mainly those relevant to refuge areas and exclusion zones.

There are no restraints to the use of the GMO in analysis, and its byproducts, provided that the requirements contained in this previous conclusive technical opinion are complied with. So, CTNBio considers that such activity is not the potential causer of a significant degradation of the environment and human health.

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Title: Chairman of CTNBio