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Applicant: Bayer S.A.
CNPJ: 18.459.628/0043-74
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Matter: Commercial release of genetically modified cotton.
Previous extract: 126/2004, published in the Federal Official Gazette no. 98, of 05.24.2004, Section 3, page 07.
Meeting: 115th Regular Meeting held on 08.21.2008.
Decision: GRANTED.

CTNBio, following examination of the application for commercial release of genetically modified cotton for resistance to glufosinate ammonium (LibertyLink Cotton Event LLCotton25), including all progenies resulting from LLCotton25 transformation event and their derivatives with non-transgenic cotton lineages and populations crossing with lineages carrying the LLCotton25 event, decided for GRANTING the application under this technical report.
Bayer S.A. requested CTNBio a Technical Opinion related to biosafety of genetically modified cotton (Gossypium hirsutum) tolerant to the herbicide glufosinate ammonium, styled LibertyLink cotton Event LLCotton25, for the purpose of its release to free registration, use in the environment, human and animal consumption, commerce and industrial use and any other use and activity related to this GMO, lineages and cultivars derived therefrom, including byproducts, under the remaining legislation and requirements applicable to any use of the cultivated species of Gossypium in effect within Brazil. Event LLCotton25, commercially known as LibertyLink is tolerant to glufosinate ammonium, a synthetic composite with herbicide properties, corresponding to the phosphinotricine produced by some microorganisms. Tolerance to glufosinate ammonium is granted by the bar gene that codes the synthesis of the phosphinotricine-N-acetyltransferase (PAT), that catalyzes the acetylation of glufosinate ammonium into N-acetyl-glufosinate or 4-methylphosphonico-butanoic (MPB) acid. The herbicide is registered with MInistério da Agricultura, Pecuária e Abastecimento (MAPA), the Brazilian Ministry of Agriculture and Supply, with Instituto Brasileiro do Meio Ambiente (IBAMA), the Brazilian Environment Institute and has a monograph approved by Ministério da Saúde, the Brazilian Ministry of Health, and is marketed in Brazil and several other countries. The commercial event LLCotton25 was obtained by transformation of cotton tissues of the region between the hypocotile and the radicle, collected three days after emergence and submitted to cultivation in a culture with Agrobacterium tumefaciens using the binary transformation system with plasmid Ti pGV3000 and the binary vector pGSV71. The crossing of information acquired with Southern Blot experiments and heritage of the inserted characteristics demonstrates that event LLCotton25 displays a single copy of the transgene that has been incorporated to the genome in a stable form. ELISA Enzyme Linked Immuno Sorbent Assay) show that the PAT protein is concentrated in the seed, and is also detected in fibers and linter. Amounts of proteins found in naked seeds of LLCotton25 plants were similar in specimens treated and not treated with glufosinate ammonium (127 and 118 ƒÝg/g), in linter (1.15 and 0.92 ƒÝg/g), and fiber (0.78 and 0.50 ƒÝg/g). In roots, stem, leaves, frozen and dried pollen, the amounts of PAT protein in plants with 2 to 4 leaves were 7.97ƒÝg/g (0.35% of total proteins), 36.8 ƒÝg/g (0.74% of total proteins), 52.9 ƒÝg/g (0.74% of total proteins), 8.23 ƒÝg/g (0.006% of total proteins) and 19.2ƒÝg/g (0.018% of total proteins) respectively. Biosafety tests support the conclusion that the PAT protein is highly specific and has no homologue sequence of any allergen and no characteristic associated with food toxins; has no N-glycosylation site; is rapidly degraded by gastric and bowel fluids; and failed to display adverse effect in mice receiving high doses of the protein, after intravenous administration. In practice, plants containing PAT protein are widely cultivated in the United States of America and Canada for almost a decade, without any report of adverse effect when used in human and animal feeding.
This protein action is well known and there is no evidence in the literature that it may have any biocide action against a non-target organism. The characteristic endowed by the bar gene – tolerance to herbicide – is acknowledged as unable to grant to receiving genotypes any adaptive advantage outside farming areas, since outside such areas potential receiving wild genotypes are not under selective pressure of the herbicide glufosinate ammonium and, therefore, any pollination of such genotypes would not result in genetic introgression. It is highly unlikely that the bar transgene of Event LLCoton25 shall be transferred to pest making them more invasive. Agronomic essays conducted, and enclosed to the proceedings, and the reports of planned releases to the environment failed to identify differences between LLCotton25 and its isoline in what concerns susceptibility to diseases and pests. The likelihood that the herbaceous LLCotton25 cotton may change into a pest is deemed negligible. The biochemical composition analysis of LibertyLink Cotton demonstrates that event LLCotton25 is substantially equivalent to non-genetically modified varieties, strongly suggesting that the event in study fails to display undesirable pleiotropic effects. None of the phenotypic characteristics of genetically modified cotton plants underwent changes as a result of insertion and expression of the bar gene in contrast to non-modified cultivars. Bibliographic data and results submitted confirm the transgenic variety level of risk as equivalent to non-transgenic varieties regarding soil microbiota, non-target vertebrate and invertebrate animals and other plants. The LibertyLink system facilitates implementation of the direct planting system, a practice that entails cuts in

production prices and reduces environmental impact, provides better water retention in soil, less soil compaction, less erosion, reduced loss of nutrients, less use of tractors and consequently cuts in fuel. Available information indicates that transgenic plants are no fundamentally different from the non-transformed cotton genotypes, except for the tolerance to glufosinate ammonium. Besides, there is no evidence of adverse reactions to the use of LibertyLink Cotton. For the above reasons, there are no restrictions to the use of this cotton or its derivatives, either for human or animal feeding. For the foregoing, the commercial release of LibertyLink Cotton is not potentially harmful to human and animal health, nor causes significant degradation to the environment. According to the provisions of 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." There are no indigenous varieties of cotton plants and the special cotton plant chains, both conventional and transgenic, had satisfactorily lived side-by-side, without known reports of coexistence problems. According to Annex I to Ruling Resolution no. 05, of March 12, 2008, applicant shall make appropriate amendments to its proposed post commercial release monitoring plan. In the context of competences envisaged by Article 14 of Law no. 11,105/05, CTNBio held that the request complies with the applicable rules and legislation aimed at securing the safety of the environment, agriculture, and human and animal health.

TECHNICAL OPINION

I. Identification of GMO

Characteristic introduction Method:

Name of GMO: LibertyLink Cotton – Event LLCotton25

Applicant: Bayer S.A.

Species: Gossypium hirsutum L.

Inserted Characteristics: Tolerance to the herbicide glufosinate ammonium

Method of insertion: Transformation mediated by Agrobacterium tumefaciens

Proposed use: Production of fibers for the textile industry and grains for human and animal consumption from the GMO and its derivatives

II. General Information

The herbaceous cotton (Gossypium hirsutum L.) belongs to the Malvaceae family, is an allotetraploid plant, native of Mexico and sexually compatible with all other allotetraploid species of the same genus. Out of cultivated plants, this is the most used by humankind(18) and, in Brazil, it is cultivated in small and large properties in regions with different economic features(31).

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 species, with a production nearing 90% of the total cotton fibers produced in the world, responsible for over 40% of world clothing(9).Cotton is held as a main agricultural product and is of great importance to Brazil, mainly for its complex production/industry and high use of manpower.

Two types of cotton plants are predominantly cultivated in Brazil: the conventional and the genetically modified one, the latter resistant to caterpillars. These cotton plants are responsible for practically all cotton produced in Brazil. In addition to the above ones, three other cotton plants with special genetic of ecologic characteristics are cultivated: the cotton featuring naturally colored fiber and the agro-ecological cotton. Colored cotton is almost exclusively concentrated in the State of Paraíba and the planted area in 2007 was about 300 hectares. Production of certified organic cotton takes place in the States of Paraná and Paraíba, and the area cultivated in 2007 reached 250 hectares (Alexandre Karkaly, et. al.). Agro-ecological cotton is cultivated by 235 farmers in the semi-arid biome of four States of the Brazilian Northeast region, with an output of 42t(51). Chains of special, conventional and transgenic cotton plants have lived together in a satisfactory way, without any known record of coexistence problems. The area occupied by cotton plants in the 2007/2008 crop was about one million and one hundred thousand hectares, of which over 85% in the Cerrado biome, especially in the States of Mato Grosso, Bahia, Goiás and Mato Grosso do Sul. The remaining cotton farming is present in other Brazilian States, particularly in the semi-arid part of the Northeast region and States of Paraná, Minas Gerais and São Paulo(43).

Besides the herbaceous cotton, other three cotton plants occur in Brazil, all allotetraploid and therefore compatible with the cultivars. None of such species is held as a pest in agricultural or natural environments.

The species G. barbadense has a domestication center in the Northern Peru and Southern Ecuador(12). It was introduced by pre-Colombian peoples and its fiber was used to make textile craftsmanship by some indigenous ethnic groups before the Portuguese arrival(54). Its use as a textile plant grew among the colonizers, but start decaying with the dissemination of the two exotic races of G. hirsutum. G. barbadense is not found in natural environments and is maintained basically as a backyard plant. It is widely distributed across most of the country and the in situ conservation is directly linked to the traditional maintenance of use as a medicine plant(8).

The only species indigenous in Brazil is G. mustelinium, with natural distribution restricted to the Northeastern semi-arid(33, 45). Known populations are restricted to the States of Bahia and Rio Grande do Norte, in municipalities that are not producers of herbaceous cotton. Two problems affect the in situ maintenance of G. mustelinium. The first, and most severe, is the destruction of gallery forest of rivers and intermittent rivulets, the habitat of the species. The second is the extensive cattle raising of the region, especially goats. These animals feed on sprouts, leaves, fruits, seed and stalk bark, affecting the

development and, in some cases, killing adult plants. Renewal of populations is also affected, since grazing on young plants causes their partial destruction(6). The distance among known populations and cotton producing regions prevents the cross of G. mustelinum with herbaceous cotton in the fields.
A third type of cotton plant is known as mocó cotton and belongs to a race different from the same species of the herbaceous cotton (G. hirsutum r. marie galante (Watt) Hutch.). Its origin is the Antilles and the history of its introduction to Brazil is uncertain, including hypotheses that it had been brought by Netherlanders or Africans during the colonial period(54). Mocó cotton plant was extensively cultivated in the Northeastern semi-arid up to the end of the 1980s, when different problems caused an abrupt interruption of its cultivation(8). A small amount of herbaceous cotton plants, mainly inter-racial hybrids of white and colored fiber cottons, produced by an Embrapa, the Brazilian Company for Agricultural Research, improvement program are still cultivated. However, cultivation of such materials is decreasing, and 5,692 ha were harvested in the 2004/2005 crop year and just 1,326 ha in the 32005/2006 crop year(43). The crops are cultivated with a minimum of external resources, being the insecticide for plague-insect control the most important one. Pest plants are controlled almost exclusively by hand weeding. Transient populations of high biological importance of this race, derived from abandoned farming, are found in higher parts of ridges in some municipalities of the Seridó region of the States of Paraíba and Rio Grande do Norte(6). There populations are geographically isolated from herbaceous cotton plant areas and well represented in Embrapa's germplasm banks.
Weed control is one of the main tillage made in cotton plant farming. Negative weed-cotton plant interactions, especially competition, allelopathy and interference in agricultural activities, cause reduced productivity and lower the market price of the cotton. Losses may be significant in case there is not appropriate and timely control(19). Main invading plants that affect cotton plant farming in Brazil are: the southern sunbur (Cenchrus equinatus), alexandergrass (Brachiaria plantaginea), Jamaican crabgrass (Digitaria horizontalis), Bermudagrass (Cynodon dactylon), Broom stick (Bidens pilosa), Bristly starbur (Acanthospermum hispidum) and morning glory (Ipomoea sp.). Weed management is conducted by cultivation, mechanical and chemical methods and control is achieved by application of herbicides, the main controlling method.
Event LLCotton25, commercially known as LibertyLink, is tolerant to glufosinate ammonium, a synthetic compound with herbicide property corresponding to phosphinothricin produced by some microorganisms. Tolerance to glufosinate ammonium is granted by the bar gene that codifies the synthesis of enzyme phosphinothricin-N-acetyltransferase (PAT), catalyzer of glufosinate ammonium acetylation to N-acetyl-glufosinate or 4-metylphosphonico-butanoic acid (MPB). These metabolites are not toxic to plants(59). The herbicide was registered with the Ministry of Agriculture and Supply (MAPA), Brazilian Institute for Environment (IBAMA), has a monograph approved by the Ministry of Health, and is marketed in Brazil and several other countries.
The LibertyLink cotton, event LLCotton25 is marketed in the United States of America since 2003, in Japan and Canada since 2004, and in Australia, Mexico and China since 2006(1). Until now, no severe damage to human or animal health or to the environment has been detected by the commercial use in the above countries. In Brazil, field experiments were conducted in different states.
III. Description of GMO and Expressed Proteins
LibertyLink Cotton has the bar gene in its composition, coming from Streptomyces hygroscopicus, strain ATCC-21705(55), a gram-positive actinobacterium common in the soil, non-pathogenic to plants, humans and animals(74, 58). This gen codifies the PAT – phosphinotricine-N-acetyltransferase, responsible for acetylation of phosphinotricine, also called glifosinate or glifosinate ammonium (GA). Inactivation of phosphotricine by PAT enables a selective use of herbicides that have glufosinate ammonium as active principle.
Commercial event LLCotton25 was obtained by transforming cotton tissues from the region between the hypocotile and the ridicule, collected at the third day after emergence and submitted to cultivation in Agrobacterium tumefaciens culture, using a binary transformation system with plasmids Ti pGV3000 and binary vector pGSV71. Explants were regenerated in an appropriate medium in the presence of Claforan 500 mg/L. The expression of the bar gene was used as a selection marker. Regenerated tissues formed the T0 plantelets, which were transferred for cultivation in the soil of a plant nursery up to flowering and production of seeds through self-impregnation.
LibertyLink Cotton Event LLCotton25 contains the following DNA sequences inserted in the cell genome:
(i) bar gene: derived from S. hygroscopicus, ATCC-21705 strain, this gene codifies the PAT-phosphinothricin-N- acetyltransferase enzyme responsible for phosphinothricine acetylation. The two first amino acid codifying codons of the beginning of the N-terminal region of the original sequence were replaced by ATG and GAC codons, in order to improve the beginning of the protein translation into plant cells. The recombinant protein has the same amino acid composition than the original protein derived from S. hygroscopicus and the bar gene final version has 551 pb, according to the full plasmid sequence pGSV71 used in the genetic transformation. Right and left border sequences of T-DNA of plasmid pTiB6S3, of A. tumefaciens and the synthetic sequences of endonuclease restriction sites are commonly used in molecular biology labs. According to available literature, there is no evidence that these sequences are expressed in plant cells(10).
(ii) CaMV 35S promoter: Sequence 1,324 pb used to direct the transcription of the bar gene.
(iii) nos terminator: 3'-nos sequence, of 260 pb, used as a terminating element of the bar transgene. The sequence comes from the nopaline-synthase gen (nos) derived from T-DNA of the pTiT37 plasmid

of A. tumefaciens.
Both elements regulating the transcription, CAMV 35S and 3'-nos, have their functions widely described in the scientific literature(49, 35, 53).
A good number of Southern Blot hybridizations, using different probes covering the full T-DNA extension, were submitted to demonstrate the integration of the exogenous DNA fragment to the plant genome, the number of genic copies and the presence or absence of other DNA elements. The results concur with the applicant's representation that only a single transgenic copy was integrated to the genome of the kindred plant and, from that one, transferred to the progenies in hemizygosis, initially, and in homozygosis in the final version of kindred lineages. No other DNA sequence present in the binary plasmid and not included in the T-DNA was detected in the genome of transgenic plants.
The genic cassette "CaMV 35S-bar-nos" therefore enables the synthesis of the PAT recombinant protein, able to chemically modify herbicides derived from glufosinate ammonium, making resistant the transformed cells and plants derived thereof. The chemical change of glufosinate ammonium prevents inhibition of the endogenous glutamine-synthase, important for the synthesis of the glutamine amino acid, fundamental in protein synthesis. Inhibition of this enzyme by the herbicide leads to toxic accumulation of ammonia in plant cells and consequent death of the plant. The PAT enzyme has a described and well known activity(32,71,77). In plant cells, there is no known substratum for such enzyme, except in cases where phosphinotricin-derived herbicides were applied to the plants.
Southern Blot analyses conducted to check the presence of pGSV71 plasmid fragments revealed that just one T-DNA fragment is present in the event. Therefore, the transformation process resulted in insertion of a functional copy of T-DNA. The absence of other sequences in the binary vector used to obtain the LLCotton25 event was confirmed by Southern Blot, to the detection limit of the methodology used.
Studies to determine stability of the insert were conducted from molecular characterization essays using the Southern Blot technique and by analyzing the transgene segregation in progenies derived from the LLCotton25 event. Southern Blot analyses show that one single copy of the transgene with a definite pattern is unaltered in the progenies, inherited according to a Mendelian pattern for several generations. Analyses of the T-DNA presence in T4, T5 and RC3F3 generations (with six different recurrent genitors) indicate that the transgene has been transmitted to such generations. Analysis of the expression of tolerance to glufosinate ammonium (phenotypic analysis) in progenies T1 and generations T2, F1, RC1 and F2 indicate that the inheritance of the transgene is of the dominant and monogenic type. Therefore, crossing the information on experiments of Southern Blot and inheritance of the inserted characteristics show that event LLCotton25 displays one single copy of the transgene that was incorporated to the genome in a stable form.
Nucleotide sequences of DNA of the cotton plants bordering the insert were included in the process, primer sequences that amplify DNA fragments containing part of the insert sequence and part of the cotton plants sequence. Using this information it is possible to unambiguously identify event LLCotton25 from other plants possessing similar inserts. The use of bordering sequences 5' and 3' in the BLAST algorithm failed to identify any similarity with either the regulatory or coding sequences(57). This is an indication that there shouldn't have been interruptions in regulatory or coding sequences of other cotton plant genes.
Studies of genetic stability and integrity for several generations used Mendelian segregation analysis (RFLP – Restriction Fragment Length Polymorphism) and Southern Blot were submitted and demonstrate existence of a single locus of insertion of the construct containing the bar gene in event LLCotton25 and derived lineages. The absence of other sequences in the binary vector (gene aadA resistance to Sm/Sp, streptomycin and spectinomycin; pVS1ori = replication origin) in event LLCotton25 was confirmed by Southern Blot. Four probes were used covering the remainder of plasmid pGSVB71.
ELISA (Enzyme Linked Immuno Sorbent Assay) analyses show that the PAT protein concentrates in the seeds, and is also detected in fibers and linter. Amounts of protein found in naked seeds of LLCotton25 Cotton plants were similar to the amounts found in plants treated and untreated with glufosinate ammonium (127 and 118 ƒÝg/g), in linter (1.15 and 0.92 ƒÝg/g) and in fiber (0.78 and 0.50ƒÝg/g). In roots, stems, frozen pollen and dried pollen, the amounts of PAT protein present in plants with 2 to 4 leaves were 7.97 ƒÝg/g (0.35% of total proteins), 36.8 ƒÝg/g (0.74% of total proteins), 52.9 ƒÝg/g (0.74% of total proteins), 8.23 ƒÝg/g (0.006% of total proteins) and 19.2 ƒÝg/g (0.018% of total proteins), respectively. PAT concentration in samples of fresh pollen displayed the higher variation interval and in some plants examined, the concentration of ƒÝg of PAT by g of fresh weight reached very high values. However, when compared with the amount of PAT relatively to the protein content, the average observed value was 20 to 40 times less than that found in roots, stems and leaves.
IV. Aspects Related to Human and Animal Health
PAT enzyme belongs to a common class of biologic catalyzers, the acetyltransferases, present in microorganisms, plants and animals. Studies of enzyme kinetics show that the PAT enzyme has no activity on other amino acids, showing its substrate-specific activity(75), i.e., it acts solely on the glufosinate ammonium composite.
PAT protein characterization studies, involving homology of its nucleotide sequence with other recognizedly allergenic proteins, have shown that there is no homology between PAT and any other known allergen. The PAT molecule also fails to display any glycosylation sites, a common characteristic of allergenic proteins(40,41).

The PAT protein has no characteristics of a toxin. In vitro essays show that the molecule is easily inactivated and denatured in acid pH, especially with digestive enzymes from the stomach and bowels. Hematologic, biochemical and urine tests, conducted in mice for a period of fourteen days, failed to show significant changes or a trend to be considered a significant toxicological parameter. Still, according to the data, the enzyme has no adverse effect to mammals, even when administered intravenously as pure protein and in high doses (1 and 10 mg/kg of live weight). Apart from the dose, the animals (mice) did not display and sign of toxicity, in contrast with the results obtained with the administration of melitin, which was 100% lethal for mice after ten minutes of treatment(46).
Stability analyses showed that the PAT enzyme is not stable when submitted to temperatures higher than 400ºC (for 15 minutes), nor when submitted to acid environments, such as the digestive system of animals and humans, and is easily destroyed when it goes through the gastrointestinal tract of animals. The data show that the introduction of the expression cassette containing the bar gene, as well as other genic elements described above, fail to change the substantial equivalence of the LibertyLink Cotton compared to the quality and quantity standards of metabolites, such as macronutrients, proteins, lipids and carbohydrates, minerals and E vitamin. Anti-nutritional factors in seeds (gossypol, and phytic and fatty acids) showed that in both genotypes (genetically modified and conventional) the detected quantities of the above compounds were identical.
According to the data, the PAT protein allergenic and toxic potential is practically null. One may assume that cotton plants of event LLCotton25 and its progenies may be considered and substantially equivalent to the non genetically modified cotton plants.
Studies in mice showed that the levels of PAT protein100 to 1,000 times higher than the ones found in plants did not cause any evident damage to animal health when the protein was added to their diet. Intravenous injection of the protein was also assessed, without any noticeable difference as against the control group, injected with recognizedly atoxic proteins.
Besides these tests, Wehrmann and collaborators(75) describe that when the PAT protein was tested in conditions that simulated gastric juice, it was degraded within seconds. Studies conducted by Esdaile (2002)(27, 28) show that the protein was digested in 30 seconds when incubated in the presence of gastric juice. Other studies performed by the European Commission (1996)(29) accounted for inactivation of the enzyme within one minute in gastric juice conditions. These studies corroborate the results attained in studies carried out by the inspection agency of Canada(14).
Studies conducted by ILSI (International Life Science Institute) showed that similar genes exist in nature without causing any adverse effect to man and that the DNA molecule is a natural component of food, with no evidence that it may have adverse effect to man when ingested in food in acceptable quantities (no direct toxic effect)(25). FAO/WHO (2000)(30) and ILSI(25) reports describe that there is no evidence that intact genes of plants may be transferred and functionally integrated to the genome of humans or other mammals exposed to the DNA or food produced with such elements.
In an extensive review of scientific literature on transgenic plants (including glufosinate-resistant plants), Aumaitre (2004) concluded that there is a host of data involving in vitro tests and tests in animals verifying alimentary safety of products obtained from genetically modified plants(5). In practice, plants containing PAT protein are widely cultivated in the United States of America and Canada for almost a decade without reports of adverse effect when used in human and animal feeding(42).
Biosafety tests support the conclusion that PAT protein is highly specific and does not have a homologue sequence with any allergen nor has it any characteristic associated to food toxins; has no N-glycosylation site; is rapidly degraded by gastric and intestinal fluids; and failed to show any adverse effect in mice receiving high doses of the protein, after endovenous administration. Therefore, it was evidenced that inclusion of the PAT protein in human and animal feeding does not produce any harm(42).
V. Environmental and Agronomic Aspects
Modern agriculture is an activity responsible for significant negative environmental impacts(3, 17, 37, 66, 73) and, therefore, risk assessment of any MG event shall be conducted in relation to the impact that is inherent to conventional agriculture(7, 20, 56). Thus the CTNBio analysis aimed at assessing whether the environmental impact caused by the LibertyLink system is significantly higher than the one caused by conventional varieties, considering the agricultural practices associated to each system.
All species of the Gossypium genus have perfect flowers. Fecundation takes place immediately after anthesis, and self-impregnation or cross-pollination may take place. The cotton plant pollen is relatively large, measuring from 81 to 143 microns, viscous (making the grains adherent to each other), spherical in format, covered by large amount of spicules, and practically not wind-transported(60). In the field, its viability extends up to dusk, though it may last for up to 24 hours if stored in temperatures from 2ºC to 3ºC(13). Cross-pollination requires the presence of pollinating insects, mainly of the order Hymenoptera(15, 62, 63, 68). Cross-pollination rates observed in cotton plant tillage is relatively low, showing values that enable classifying G. hirsutum as a partially autogamous species or a species featuring a mixed reproduction system.
Some authors suggest that the genic flow of MG plant to wild genotypes may cause reduced biodiversity. However, reduction in genetic variability is the result of a genetic introgression phenomenon, a process far more complex than simple hybridization(20, 24, 38, 70). In order for introgression to occur, hybridization is required and, following, a series of retro-crossings so that one gene is permanently incorporated to a genome(38, 39). Introgression of a transgene to wild cotton plants could only take place if it was to grant strong selective advantage, higher than the disadvantages granted by the alleles that are genetically

linked to the transgene(34, 44, 70). However, the feature granted by the bar gene – tolerance to herbicide – is recognized as unable to endow the receiving genotypes any adaptive advantage outside farming areas(22, 70), since in non-farming areas potential receiving wild genotypes are not exposed to the selective pressure of the glufosinate ammonium herbicide and, therefore, a pollination of such genotypes would not result in genic introgression. Therefore, the transfer of the tolerance to herbicide feature to non-farming areas is extremely unlikely(22, 70).

A question raised about genetically modified plants tolerant to herbicides (TH) is the likelihood of crossing of such plants with weeds and consequent invasion of better adapted TH plants(26, 34). However, in order to be formed, such "super weeds" would need hybridization of the genetically modified plant with an invading species and a selective pressure (application of herbicide) in the same physical area where the hybrid is located(70). Without all such pre-conditions, appearance of herbicide tolerant weed is negligible(21, 36, 48, 65). Indeed, available experimental data surveyed in large-scale herbicide tolerant GMO farming regions confirm that the development of resistance to herbicide in weeds is unrelated to the genetic modification, yet related to the management of cultures and herbicides used by farmers(16, 67). Moreover, there are not in Brazil species sexually compatible with G. hirsutum displaying characteristics of invading plants. Thus, one reaches the conclusion that it is extremely unlikely that the bar transgene of Event LLCotton 25 is transferred to weeds, making them more invasive. As explained above, customary care with the management of cultures and herbicides, such as rotation of crops and herbicides with different action mechanisms, shall be the focus to mitigate the appearance of weeds tolerant to herbicides.

Agronomic essays conducted and enclosed to the proceedings in addition to the reports of planned releases to the environment failed to identify differences between the LLCotton25 cotton plant and its isoline in what regards susceptibility to diseases and plagues. The same was ascertained regarding phenology in different farming conditions and stress caused by low temperatures. The behavior of LLCotton25 was not assessed outside the agricultural environment. Despite this, an increase in dispersion and survival ability associated to the inserted feature is expected in the presence of the selective agent glufosinate ammonium. Analyses conducted in agricultural conditions of competition with bush vegetations evidence that the ability of competition in other environments where this cotton plant may occur shall be similar to the conventional cotton plant. Therefore, the likelihood that the herbaceous cotton LLCotton25 may change into a pest was deemed negligible.

The bar gene product grants tolerance to glufosinate ammonium by acetylation of the compound, making it to loose its herbicide action(23,50,76). Alimentary safety tests demonstrate that the PAT enzyme has no toxic effect, such safety already ascertained in rice, corn, soy and canola plants(58). Thus, the action of the genic product is well known and there is no evidence in literature that it has biocide action against non-target organisms.

Although structural rearrangements have occurred during the evolution of allotetraploid Gossypium species and there are self-incompatibility mechanisms in certain genitor combinations, the existing sexual barriers are partial and unable to prevent that the vertical genic flow occurs among species of this genus. Therefore, the transgene may be transferred by crossing to other types of cotton plants in the country. In addition to sexual compatibility, for the genic flow to occur, certain factors must be present as, for instance, the ability of hybrids to produce fertile descendants; coincidence of flowering; the same pollinating agent; absence of pre- and post-sexual barriers; sympatry between donor and receiving populations; and that reproductive systems allow crossing. Part of such factors is well possible to occur between genetically modified cotton plants and wild species of the genus Gossypium, while others are not applicable or only partially applicable. In case the genic flow occurs, the main likely negative consequences are the loss of in situ diversity and a positive adaptive effect to cause an increase of the receptor species aggressiveness in farm or natural environments, making it a pest. The only species of Gossypium able to survive in a sustained mode in natural environments is G. mustelinum. There is not a farmed form of the species nor any use mentioned in the literature or by inhabitants of the regions where it occurs. Populations of this species are known only in the Caatinga Biome of the States of Bahia and Rio Grande do Norte. It inhabits a very specific Caatinga niche, gallery forests of rivulets, rivers and intermittent ponds. The species does not occur in farm environments and, in case it did, would not be classified as an invading plant, since it displays features such as limited dispersion, long cycle and low reappearance ability when young. It is not, in addition, a pest in natural environments, coexisting in harmony with other native species. Part of known populations occurs in a strictly natural environment, non-managed by herbicides. Other populations occur in areas where the natural vegetation has been degraded of partially degraded for cattle raising, yet the management or pastures does not include the use of herbicides. Considering that the management in areas where G. mustelinum occurs does not include the use of herbicides, the absence of a selection agent makes the gene to have a neutral adaptive effect. Therefore, the likelihood of G. mustelinum changing into a pest in farm and natural environments is deemed negligible in case an introgression of the transgene occurs. G. mustelinum populations are located in regions that currently are not producers of herbaceous cotton. The distance between populations and tillage is large enough (at least some tens of kilometers) for the pollen of the tillage not to reach natural populations. Therefore, the likelihood of direct transfer of pollen from tillage to populations of G. mustelinum is negligible, being unlikely that the population structures be affected due to a genic flow with herbaceous cotton plants carrying Event LLCotton25. Unintentional dispersion by seeds may occur mainly during seed and whole cottonseed transportation, though it may also occur by

feces of animals fed with cottonseeds and other animals. Scattered seeds may fall in anthropized locations, such as roadsides, germinate and flower. The plants may serve as a bridge to introduce transportation of the transgene to G. mustelinum populations and mocó cotton. Though the frequency of spontaneous plants is low and in general they do not occur near the populations, the likelihood that the transfer of spontaneous plants may mediate the transgene transfer is low. This assumed low-frequency transfer will have null adaptive effect, as discussed above.

Absence of sympatry with biologically important populations of mocó cotton plants and lack of employment of glufosinate ammonium in locations where the mocó variety occur make the same considerations already made for G. mustelinium valid for the mocó cotton (G. hirsutum var. marie galante).

G. barbadense is a domesticated species introduced by indigenous people in the country thousands of years ago. There are no reports of stable G. barbadense populations in natural environments, being them restricted to locations with strong anthropic action. G. barbadense persists as a cultivated plant for the production of textile craftsmanship in a few indigenous and rural communities, and the majority of plants are located in backyards in all Brazilian States. Its low competitive ability, probably due to the domestication process, prevents fertile populations to establish. As it was for the two species discussed above, G. barbadense is not cultivated in places where herbicides are used. Therefore, one expects a null adaptive effect in case a genic flow takes place.

Due to the larger area where G. barbadense occurs, some tillage and backyard plants coexist in a distance in which pollen transfer may, theoretically, occur. Some facts contribute for a small amount of inter-specific hybridization. Noticeably, there is preferential pollination in oospheres of G. barbadense with male gametes of G. barbadense(61). Indeed, the way in which G. barbadense is kept makes the in situ plants to reproduce, basically, through self-impregnation(2, 64), and endogamy coefficients estimated from assessments with microsatellite markers are equal to one or near one in samples collected in the States of Pará, Amapá, Pernambuco and Tocantins. Besides, the majority of plants are not near the tillage, being sympatry an exception. Considering the wide distribution of the species, low expected hybridization frequency, and that the variability contained in places where sympatry occurs is duly represented elsewhere, the likelihood of loss of diversity is negligible.

Although the location of the genome insert of a transformed plant may influence directly the level of expression of one or more feature (47,52), in risk analysis molecular characterization shall be considered taking also into account studies related to compositional, agronomic and physiological characterization of the event analyzed.

A series of field studies conducted by the applicant company enabled analyzing comparatively phenotypic characteristics such as density of foliage, staple retention, flower morphology, pilosity, reaction to plagues and diseases, finding no significant difference from conventional varieties. Similarly, the agronomic performance is equivalent, as well as the characteristics regulating survival, reproduction and adaptability of the species. There are no reports on changes in agronomic performance that were observed following commercial cultivation of the event in other countries. Besides, biochemical composition analysis shows that event LLCotton25 displays substantial equivalence to non genetically modified varieties. The results strongly suggest that the event fails to display undesirable pleiotropic effects.

Results of agronomic behavior in genetically modified cotton plants, both in quantity and quality, were equivalent to the ones observed in conventional cotton plants either in the absence or presence of herbicides derived from glufosinate ammonium. Experiments were conducted mainly from 2001 to 2003 (two crop years) and performed by technicians from Bayer CropScience Ltd. and researchers of Universidade Federal de Uberlândia, the Federal University of Uberlândia and Universidade de São Paulo, the São Paulo University in regions of the States of São Paulo and Minas Gerais. Features assessed include: height of plants; production of cotton staple; flowering pattern along the plant development; glandulation of plants and presence of foliar nectarines; size and form of leaves; pilosity of stems and leaves; color of flowers, stems and pollen grains; density of plants; number of vegetative and reproductive branches; retention of staple; and form and pattern of fruit (apples) development. Analyses of this series of genotype descriptors used for recording and protecting cultivars (among others) and behavior of genetically modified and non genetically modified plants in response to environmental factors, including culture plagues, lead to the conclusion that none of the phenotypic characteristics of genetically modified cotton plants underwent any change as a result of insertion and expression of the bar gene vis-à-vis non genetically modified cultivars. No difference was observed by authors even when the plants were submitted to different invading plant controlling methods or absence of use of herbicides. Data submitted for different phenotypic, morphologic and phonologic descriptors, agronomic performances and reactions to environmental factors were consistent, well grounded and supported by equivalent results developed in the USA.

In the analyses conducted, possible changes were taken into account resulting from the transgenic event on adaptability of genetically modified plants to agro-systems, including higher adaptation and survival potential, growth rate, development of vegetative (aerial parts and radicular system) and reproductive organs, fertilization, germination and initial vigor of plants, greater resistance to plagues and diseases, greater catchment of solar light and absorption of water and mineral salts, among others. The results obtained stress that the LibertyLink Event LLCotton 25 has competitiveness and adaptability equivalent to isogenic plants not genetically modified. Conclusions of studies developed in Brazil and the United States of America show that the crossing with other cotton species, either cultivated or native, and the transfer of genes are eminently identical to non genetically modified species. The requirement of isolation of tillage of

genetically and non-genetically modified seeds to secure purity of germinative material may comply with the criteria already defined by the Brazilian Ministry of Agriculture and Supply.
Bibliographic data and results exhibited confirm the risk level of the transgenic variety as being equivalent to the non transgenic one regarding the microbiota of soil, vertebrate and invertebrate animals as well as other plants. Summarizing, the environmental safety of LibertyLink Event LLCotton25 is based on the nature of the transgene and other sequences of exogenous DNA introduced in the plant, on the behavior of the plant itself, the cotton plant, and the plant proliferation environment that is restricted to tillage areas of small, middle and large extensions.
Besides, the applicant conducted sample surveys for two years of cultivation where interaction with several pathogens was observed, such as Ramulosis, Fusarium Wilt, Angular Wilt, Alternaria Wilt, Blue Mold and Common Mosaic, and no difference was noticed when compared to non genetically modified plants. This way, both literature data and field experiments suggest that Event LLCotton25 does not cause impact against non-target organisms in addition to those already inherent to the cotton culture.
Glufosinate ammonium is a synthetic compound with herbicide properties and corresponds to the phosphinothricin produced by some microorganisms. Glufosinate ammonium belongs to the replaced Homoalanine chemical group and Herbicide Class and growth regulator(4). The product is used as post-emergent, unspecific and of wide spectrum herbicide. It inhibits the glutamine synthase that promotes the incorporation of ammonia to glutamic acid to produce glutamine. As a result, a deficit of glutamine becomes takes place together with accumulation of ammonia in toxic levels, causing the plants to die.
Tolerance to glufosinate ammonium is granted by the bar gene that codifies the phosphinothricine-N-acetyltransferase (PAT), which catalyzes acetylation of glufosinate ammonium to N-acetyl-glufosinate, or 4-methylphosphinico-butanoic acid. These metabolites are not toxic to plants(59). Glufosinate ammonium is registered with the Brazilian Ministry of Agriculture and Supply (MAPA) , Institute of Environment (IBAMA) and has a monograph approved by the Ministry of Health, being marketed in Brazil and other countries. It is considered persistent and mobile in the soil and, in sandy soils up to 80% may be lixiviated.
Depending on management and edaphoclimatic conditions, microbian activity and other factors, glufosinate ammonium has a mean-life in soil from 12 to 70 days. However, residues persisting in the soil for up to 100 days have been reported(69). Therefore, if used outside recommendations, the glufosinate ammonium has the potential to contaminate watercourses, and ground waters such as any other herbicide used in genetically modified cultures or otherwise(72).
Thus, theoretical assessments and studies based on scientific literature taking into account the plant, the bar gene, the PAT recombinant protein, the LibertyLink technology and the glufosinate ammonium herbicide enable ranking the risk levels to the environment as the lowest possible. The LibertyLink system facilitates implementation of direct planting since it enables controlling the post-emergence invading plants. In this system, straw and other plant residues of previous cultivations are kept at the soil surface, securing coverage and protection against harmful processes, such as erosion. Direct planting entails several benefits that reduce production costs and environmental impact, with better retention of water in the soil, less soil compaction, less erosion, lower loss of nutrients, less use of tractors and, consequently, less fuel. This way, direct planting and cultivation of Event LLCotton25 make possible to extend this system's environmental benefits to new farming areas.
VI. Restrictions to the use of GMO and its derivatives
Technical reports related to agronomic performance concluded that there is equivalence between transgenic and conventional plants. Thus, the information indicates that transgenic plants are not fundamentally different from the genotypes of non modified cotton, except for the tolerance to glufosinate ammonium. Additionally, there are no records of adverse reactions to the use of LibertyLink Cotton. Therefore, there are no restrictions to the use of this cotton of its derivatives in human and animal food.
According to Article 1 of Law no. 11,460, of March 21, 2007, "research and cultivation of genetically modified organisms are forbidden in indigenous and conservation unit areas."
VII. Considerations on particulars of different Brazilian regions (assistance to monitoring bodies)
LibertyLink technology proved possible to be used according to all farming practices commonly used in different regions and under different conditions, regarding availability of inputs, manpower, among others, used in cotton farming. Additionally, the technology may grant improved success to direct planting.
Studies reached a conclusion that the use of genetically modified varieties to the selective use of glufosinate ammonium does not restrict any procedure of cotton farming. There are not local cotton varieties and special cotton chains, either conventional or transgenic, have satisfactorily lived along each other, without any record of coexistence issues.
VIII. Conclusion
The long experience with traditional plant improvement methods, the experience amassed over three decades of research and over a decade of marketing transgenic varieties all over the world, in addition to the advanced knowledge on the structure and dynamics of genomes, indicating whether a certain gene or characteristic is safe or not, are an indication that the genetic engineering process on its own displays little potential for the appearance of unexpected consequences that would not be identified or eliminated during the process of development of genetically modified commercial varieties(11).
Whereas LibertyLink Cotton belongs to a well known species (Gossypium hirsutum) that has a solid history of safety for human use, and that the gene introduced in the variety does not code a toxic protein and is innocuous to humans;

Whereas the genic construct used to insert this gene in cotton resulted in a stable insertion of a functional copy of pat, that granted the plants tolerance to glufosinate ammonium;
Whereas the composition data fail to display significant differences between genetically modified and conventional varieties, suggesting equivalence between them;
Whereas
1. Cotton is one of cultivated plants most used by humankind;
2. The PAT protein is highly specific and does not have a homologue sequence with any allergen or N-glycolisation site associated with food toxin;
3. The PAT protein is rapidly degraded by gastric and bowel fluids and did not cause adverse effect in mice receiving high doses of the protein, after intravenous administration;
4. The DNA molecule is a natural component of food, showing no evidence that it may have an adverse effect to man when ingested in food in acceptable quantities (no direct toxic effect);
5. There is no evidence that intact plant genes may be transferred and be functionally integrated to the human genome or the genome of other mammals exposed to the DNA or to food made with such elements;
6. The applicant answered to all questions under CNBio Ruling Instruction no. 20 (effective at the time the application was submitted) and that none of the answers indicated that this cotton may display adverse effects to human or animal feeding;
7. The possibility that the herbaceous LLCotton25 plant to change into a weed is negligible;
8. Streptomyces hygroscopicus is a gram positive actinobacterium ubiquitous in soil, non pathogenic to plants and animals;
9. The characteristics granted by the bar gene – tolerance to herbicide – is recognized as unable to grant the receiving genotypes any adaptive advantage outside farming areas, since outside such areas the potential of receiving wild genotypes do not undergo selective pressure from the herbicide glufosinate ammonium and, therefore, pollination of these genotypes would not result in genic introgression;
10. It is highly unlikely that the bar transgene of Event LLCotton25 shall be transferred to weeds making them more invasive;
11. The action of the PAT protein is well known and there is no evidence in the literature of any biocide activity of such protein against non-target organisms;
12. The supposed low frequency genic transfer between different cotton species will have practically null adaptive effect;
13. There are no reports of changes in agronomic performance recorded from commercial cultivation of the event in other countries;
14. Analysis of biochemical composition showed that event LLCotton25 displays substantial equivalence to non genetically modified varieties, strongly suggesting that such event fails to display any undesirable pleiotropic effect;
15. Literature data and field experiments suggest that event LLCotton25 has no impact against non-target organisms in addition to those already inherent to the cotton culture;
16. Glufosinate ammonium is registered with the Brazilian Ministry of Agriculture and Supply (MAPA), Brazilian Institute of Environment (IBAMA), with a monograph approved by the Ministry of Health, and is marketed in Brazil and several other countries;
17. The LibertyLink system facilitates implementation of direct planting, a practice bringing different benefits such as reduced production costs and environmental impact, increased water retention in soil, less soil contamination, less erosion, reduced loss of nutrients, fuel savings because of less tractor use.
Finally, taking into account the criteria internationally accepted in the process of analyzing genetically modified raw materials, one may reach a conclusion that LibertyLink Cotton Event LLCotton25 is as safe as its conventional equivalent.
For the foregoing, commercial release of LibertyLink Cotton is not potentially harmful to human and animal health nor significant for environment degradation.
CTNBio analysis took into consideration previous opinions by the Commission members; ad hoc consultants; documents delivered by applicant to the CTNBio Executive Secretariat; results from planned releases to the environment; and lectures, texts and discussions of the public hearing held on 08.17.2007. Independent scientific publications of applicant, conducted by third parties, were additionally considered and consulted.
Under Annex I of Ruling Resolution no. 5, of March 12, 2008, applicant shall have a term of thirty (30) days from the publication of this Technical Opinion to change accordingly its proposal for the post-commercial release monitoring plan.
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Walter Colli
President of CTNBio

Dissenting vote:
Author Dr. Paulo Yoshio Kageyama (Permanent Environment Sector Sub-Commission) had a dissident opinion on the approval of this product, arguing that the questions posed by CTNBio during the analysis of the LibertyLink Cotton commercial release were not adequately answered by the applicant and that there are uncertainties related to pollination of cotton in several Brazilian biomes, emphasizing that biosafety shall be fundamentally pursued, mainly in what regards genic flow. Dr. Paulo Black and Dr. Leonardo Melgarejo followed this dissenting vote.