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This is to certify that I, Marco Antônio Rochadel, Official Public Translator, designated and installed in Office according to The Official Gazette of June 23, 1982, page 5428, have received and translated, to the best of my knowledge and belief, a document with the following contents:

Ministério da Ciência e Tecnologia

GOVERNO FEDERAL BRASIL PAÍS RICO É PAÍS SEM POBREZA

Ministry of Science and Technology – MCT

National Biosafety Technical Commission – CTNBio

comissão técnica nacional CTNBio de biossegurança

Office of the Executive Secretary

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Technical Opinion no. 2955/2011

Proceedings:	**01200.003895/2010-21**
Applicant:	**Du Pont do Brasil S.A. – Divisão Pioneer Sementes**.
CNPJ:	**61.064.929/0043-28**
Address:	**SGAS 902, Lt 74, Conjunto B, Bloco A, salas 221 a 224, Ed. Athenas, Asa Sul, 70390-020 Brasília, Distrito Federal**
Previous Extract:	**2537/2010, of 09.24.2010**
Meeting:	**143rd Regular Meeting held on 06.16.2011**
Decision:	**GRANTED**.

CTNBio, following examination of an application for a Technical Opinion on commercial release of TC1507 x MON810 x NK603 corn, featuring resistance to insects and tolerance to herbicides, as well as all progenies therefrom, reached a **FAVORABLE** conclusion, in the terms of this technical opinion.

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Du Pont do Brasil S.A. – Divisão Pioneer Sementes, holder of *Certificado de Qualidade em Biossegurança – CQB* (Biosafety Quality Certificate) no. 013/97, requested CTNBio an opinion on biosafety of TC1507 x MON810 x NK603 corn resulting from crossing, by classical genetic improvement, of genetically modified corn events for resistance to insects attack and tolerance to herbicides, TC1507, MON810 and NK603 for the purposes of cultivation, human and animal consumption, manipulation, transportation, disposing of, import and export, as well as any other activities related to such corn and progenies therefrom. The event, obtained by TC1507 x MON810 x NK603 gene stacking, inherited from its parental TC1507 genes *cry1F* and *pat*, from parental MON810 gene *cry1Ab* and from parental NK603 gene *cp4 epsps*. Stability of inserts in crossings containing the three events was confirmed by Southern blot analyses. Gene *cry1F* was isolated from *Bacillus thuringiensis var. aizawai* and gene *cry1Ab* from *Bacillus thuringiensis* subsp. *Kurstaki*, and proteins coded by them, Cry1F and Cry1Ab grant the plants protection against certain corn pest insects of the Lepidoptera order. The action of the proteins is mediated by specific receptors located at the intestinal cells of susceptible insects (mammals, fish, birds and other non-target insects fail to have such receptors). Gene *pat* inherited from parental TC1507, was isolated from *Streptomyces viridochromogenes* strain Tu494 and codifies for enzyme phosphinothricin N-acetyltransferase (PAT), capable of chemically inactivating herbicides derived from phosphinothricin, such as ammonium glyphosate, making cells and plants containing it to acquire resistance. Protein CP4 EPSPS (CP4 5-Enol-Pyruvylshikimate-3-Phosphate Synthase) coded by gene *cp4 epsps* (isolated from *Agrobacterium tumefaciens* strain CP4) grants tolerance to the glyphosate herbicide. The action of glyphosate is due to its ability of blocking the activity of the target enzyme, EPSPS, therefore interrupting the biosynthetic pathway of aromatic amino acids, essential for plants

and microorganisms. Enzyme CP4 EPSPS, expressed in the genetically modified plant, features low affinity to glyphosate, so that development remains normal in these plants, even in the presence of the herbicide. Each of the genetically modified corn parental, TC1507, MON810, and NK603 used to obtain the stacked event, was previously analyzed by CTNBio either separately or in combinations, and were held to be safe for human and animal health and the environment. Expression levels of proteins Cry1F, Cry1Ab, PAT and CP4 EPSPS were assessed in pollen, leaves, stalk, root and grain of the stacked TC1507 x MON810 x NK603 corn with no records of significant differences between the values noted in comparisons with individual events. Assessment of injury symptoms of herbicides on the stacked corn event showed that it displays a behavior similar to individual events TC1507 and NK603 in treatments with herbicides recommended for each one (glufosinate ammonium and glyphosate, respectively) and that in exposure to the mix of active ingredients, its tolerance is significantly higher when compared to the conventional control. Efficiency in controlling target pests was assessed through observation of consumption of tissues of foliar disks by neonate larvae of *Ostrinia nubilalis*, the European Corn Borer, and the results showed that there is no significant statistical difference between the isolate events TC1507 and MON810 and the combination of events TC1507 x MON810 x NK603. Composition analysis of grain and forage also showed that there is no significant difference between the stacked corn TC1507 x MON810 x NK603 and the conventional control. As whole, the results revealed an absence of unexpected effects coming from unforeseen interactions on combining, through conventional crossing, the three events TC1507, MON810 and NK603. For the foregoing, the conclusion is that cultivation and consumption of TC1507 x MON810 x NK603 corn is neither a potential cause of significant degradation to the environment nor a risk to human and animal health. Therefore, there are

no restrictions to the use of this corn and its derivatives. CTNBio determines that the post-commercial release monitoring shall be conducted in commercial farming and not in experimental ones. The areas selected for monitoring shall not be isolated from the remaining ones, possess borders or any other situation contrary to commercial usage. Monitoring shall be conducted in a comparative model between the conventional and the GMO, and collection of date shall be made by sampling. Monitoring shall be conducted in biomes that are representative of the main GMO cultivation areas and, whenever possible, include different types of producers. Monitoring shall be conducted for a minimum period of five years. The reports submitted shall be detailed so as to include information on all activities held on pre-sowing and sowing, on its performance, informing the activities held in the area of monitoring during the agricultural cycle, on harvesting activities and climate conditions. Any aggravation of human and animal health shall be followed by the official adverse effect notification systems such as SINEPS – *Sistema de Notificação de Eventos Adversos Relacionados a Produtos de Saúde,* the Health Products Adverse Effect Notification System, as regulated by ANVISA. The analytic methods, results obtained and their interpretations shall be developed in line with the principles of independence and transparency, except in case of commercial confidentiality previously justified and defined as such. Regarding genes *cp4 epsps* and *pat,* which grant the plant resistance to herbicides, the following shall be subject of monitoring: nutritional state and sanity of GM plants; chemical and physical soil attributes related to fertility and other basic pedologic characteristics; soil microbial diversity; soil diaspore banks; community of invading plants; development of resistance to herbicide in invading plants; herbicide residues in the soil, grains and aerial part, and gene flow of the two inserted genes. Regarding genes *cry1Ab* e *cry1F*, which grant resistance to insects, the following shall be monitored: impact on

target and non-target insects; impact on soil indicator invertebrates not belonging to the Insecta Class; residues of insecticide proteins in decomposing organic matter, soil and watercourses close to monitoring area; development of resistance among target insects and flow of the two inserted genes.

TECHNICAL OPINION

I. GMO Identification

GMO designation:	**TC1507 x MON 810 x NK603 corn**
Applicant:	**Du Pont do Brasil S.A., Divisão Pioneer Sementes.**
Species:	***Zea mays L.***
Characteristic inserted:	**Tolerance to glyphosate and ammonium glufosinate herbicides and tolerance to insects of the *Lepidoptera* Order.**
Characteristic introduction method:	**TC1507 x MON 810 x NK603 corn, classified as Risk I Class, was developed through classical genetic improvement by sexual crossing between genetically modified lineages.**
Proposed use:	**Cultivation, human and animal consumption, manipulation, transport, discarding, import and export as well as any other activities related to this corn and progenies derived thereof.**

II. General Information

Zea mayz L. corn is a species of the Gramineae family, tribe Maydae, subfamily Panicoidae, which is segregated within the sub-genus *Zea*, featuring a chromosome number 2n = 20,21,22,24[(1)]. The closest feral species of corn is teosinte, found in Mexico and in some regions of Central America, where it may cross with cultivated corn in production fields.

Corn has a history of over eight thousand year in the Americas, and is cultivated since the pre-Columbian period. It is one of the best scientifically characterized higher plants, being today the cultivated species reaching the highest degree of domestication and may only survive

when cultivated by man[2]. Currently there are about 3000 identified races of corn and, within each race, thousands of cultivars.

One of the most important food sources in the world, corn is an input for a range of food products, rations and industrial products. Brazil is one of the largest world producers of corn, which is planted practically all over the national territory[3].

Insects occur more abundantly in the tropics than in temperate regions, where damages caused by such animals are more severe. Among the more important corn pests there is the armyworm, *Spodoptera frugiperda*. Cruz and collaborators[4] estimated the yearly losses caused by infestation of *Spodoptera frugiperda* in about 400 million Dollars in Brazil. Other lepidopteran insects also are important corn pests, such as the corn earworm, *Helicoverpa zea*, and sugarcane borer, *Diatraea Saccharilis*.

The main measure aimed at controlling insects in corn culture has been the use of insecticides. In some areas of the Brazilian Central-West region, dozens of insecticide sprays are needed in a single cycle of culture. Another measure for controlling pests would be the use of resistant cultivars.

The number of cultivars using stacked events is growing around the world. This represents a trend towards meeting the demand of producers when combining two characteristics of agronomic importance in a same hybrid. Following this line, different hybrids of corn containing stacked events through classical genetic improvement are approved in several countries[5].

The combination of two events targeted to control pests of the same order intends to add the scope of action of the two proteins coming from *Bacillus thuringiensis*. The combination is one additional tool to Management of Pest Resistance to individual proteins. The combination of two proteins that grant tolerance to different herbicide molecules will provide greater flexibility in controlling plant pests, and may also be used in the Management of Resistance of

these pests.

III. Description of GMO and Expressed Proteins

Insect resistant and tolerant to glufosinate ammonium and glyphosate corn TC1507 x MON810 x NK603, was produced through the conventional improvement technique, by crossing TC1507 (Herculex I) corn event with MON810 corn event and NK603 (Roundup Ready 2)corn event. The event obtaining by gene stacking, TC1507 x MON810 x NK603, inherited *cry1Ab* gene from its parental MON810, *cry1F* and *pat* genes from its parental TC1507 and *cp4 epsps* gene from its parental NK603. Stability of inserts in crossings containing the three events was confirmed by Southern blot [(6)].

Parental MON810[(7)] was obtained by biolistic technique and its molecular characterization showed integration in a single copy of gene *cry1Ab* with its regulatory sequences, with no trace of additional sequences of the vector or of the antibiotic resistance marker gene. Gene *cry1Ab* was isolated from *Bacillus thuringiensis* subsp. *Kurstaki*, with its nucleotide sequence integrated to the MON810 corn genome to adjust the codons use, while maintaining unchanged the coded protein sequence.

Bacillus thuringiensis (Bt) is a gram-positive bacterium of the *Bacillaceae* family that produces, at the moment of sporulation, crystalline protein inclusions. The inclusions contains Δ-endotoxins, or Cry, proteins that currently form a 300 member family, classified in 49 groups[(8)]. The general mechanism of the insecticide activity of Cry proteins is well known[(9 to 13)]. The proteins are produced in the form of protoxins and are transformed into toxic peptides in the insect's guts by alkaline pH and proteases action. The active toxin interacts with specific receptors in the intestine epithelial cells, leading to pore formation and cell lyse, causing insect death by inanition and scepticemia[(9, 10, 14, 15, 16, 17)]. Cry insecticide proteins are extremely

selective for target insects of the Lepidoptera Order[18 to 22] and they possess, in their intestines, specific receptors for these proteins. Mammals and other non-target organisms fail to possess such binding sites, and therefore are not affected by the Bt protein[23 to 26]. *Bacillus thuringiensis* occurs in soils, dust, feces, insects, bird nests, silkworm creation and natural vegetation[10]. Including parts of plants used as food, with distinct biologic properties able to produce insecticide proteins with specificity for certain agriculture damaging insects. Numerous varieties of *Bacillus thuringiensis* have been used as microbial insecticides. Subspecies *aizawai* is commercially used to control *Galleria millonella* larvae and several other caterpillars, especially the diamond back moth *Plutella xystorella*[27].

TC1507 corn parental was obtained by the biolistic technique and had the *cry1F* and *pat* genes inserted in its genome, together with regulatory sequences. Molecular characterization of this event shows that it contains an almost complete copy of the DNA insertion used in the transformation and a limited number of non-functional arrangements of the sequence linked to such almost complete insertion. The insecticide protein coded by the synthetic transgene *cry1F* is identical in the 1-605 amino acid sequence of the residual 1,174 amino acids of the natural Cry1F, except for one single substitution of amino acid residues. The codons for the 569 C-terminal amino acids of the complete protoxin correspond to those codons eliminated by the alkaline proteases in the intestine of the insect during the formation of the Cry1F active toxin[10,29]. The optimized *cry1F* codes therefore for a truncated Cry1F protein that is identical to the active core of the *Bacillus thuringiensis var. aizawai* complete protein and to the microbial Cry1F complete protein, of the MR872 strain, expressed in *Pseudomonas fluorescens*. The three sequences of Cry1F protein are identical for the first 605 amino acids, except for the substitution of leucine at position 604 of the plant Cry1F protein. MR872 is the chimera strain

of *Pseudomonas fluorescens* used to produce truncated Cry1F microbial material to toxicological tests.

Field and laboratory tests showed absence of interaction between Cry1F (inherited from parental TC1507) and Cry1Ab (inherited from parental MON810) expressed in TC1507 x MON810 x NK603 stacked corn, being their effect considered to be added to each other in pest control, that is to say, there is independence between events as far as insect control is concerned. There were not changes in composition or expression of the proteins when contrasted to individual events. The same happens with PAT and CP4 EPSPS proteins.

Gene *pat* inserted in event TC507 is a synthetic version of the natural *pat* of the *Streptomyces viridochromogenes*[30], possessing 552 bp and codes for PAT (phosphinothricin acetyltransferase), with 183 amino acids. The synthetic version was produced in order to change codon polarization of guanine and cytosine (G+C) to a level more representative of the plant DNA. PAT protein, is able to chemically inactivate herbicides derived from phosphinothricin, such as glufosinate ammonium, making resistant the cells and plants containing it[32 to 34].

NK6T03 corn parental[35] was produced through biolistic and contains two *cp4 epsps* gene expression cassettes (derived from *Agrobacterium tumefaciens* strain CP4) with its respective regulatory sequences. The nucleotide sequence of one of the *cp4 epsps* gene differs from the original sequence used in the process of nucleotide transformation. One of the nucleotide changes was neutral, that is to say, fails to cause change in the coded protein sequence, and the other resulted in substitution of one amino acid in position 214, so that the coded protein came to be named CP4 EPSPS L214P. The EPSPS enzyme belongs to the shikimic acid pathway (precursor of tyrosine, phenylalanine and tryptophan aromatic amino acid biosynthesis).

Though the shikimic acid pathway and EPSPS proteins do not occur in mammals, fish, birds, reptiles and insects, they are important in plants. Aromatic molecules, all derived from the shikimic acid, are calculated to represent at least 35% of a plant dry weight[33]. The CP4 EPSPS protein expressed in genetically modified plants tolerant to glyphosate is functionally identical to the endogenous plant EPSPS protein, except for the reduced affinity it has for the glyphosate herbicide[37]. In conventional plants, glyphosate binds to the EPSPS enzyme and blocks the shikimic acid biosynthesis, preventing formation of aromatic amino acids and secondary metabolites[36,38]. In glyphosate tolerant genetically modified plants, aromatic amino acids and other metabolites needed to the plant development continue to be produced by the CP4 EPSPS protein activity[39,40].

The genetically modified parental corns TC1507, MON810 and NK603 that originated the combined event were previously reviewed by CTNBio and released for marketing purposes after being held as safe to human and animal health as the conventional corn (Technical Opinions no. 1679/2008, 1100/2007, 1596/2008). Besides Brazil, the events have been either individually or stacked in different countries[5]. There is no record of adverse effects resulting from consumption of TC1507 x MON810 x NK603 corn.

Events of genetically modified corn, containing one or more of these parental corns, were already released to marketing purposes in Brazil, such as MON810 x NK603 (Opinion no. 2041/2009, of 09.28.2009) and TC1507 x NK603 (Opinion no.2053/2009, of 10.16.2009), MON89034 x NK603 (Opinion 2725/2010, of 11.23.2010) and more recently MON89034 x TC1507 x NK603 (Opinion no. 2753, of 12.17.2010).

IV. Aspects Related to Human and Animal Health

In order to examine whether any unforeseen interaction takes place in the combination

through crossing of events TC1507, MON810 and NK603, a series of tests were conducted to measure the usual parameters used in registering individual events and well known for all cases.

In order to analyze recombinant protein expressions in the stacked corn, plants containing individual events TC1507, MON810 and NK603, as well as a combination of the three events, were distributed in a causative form in three locations. Samples of pollen, leaves, stalk and root in R1 development stage (appearance of stylo stigmas) and, in development stage R6 (physiologic maturing) grain samples were taken. To ensure genetic purity of the grain samples, all plants were manually self-fecundated. Presence of events was confirmed by real-time PCR (Polymerase Chain Reaction). Ten samples of each type (each such sample representing one plant) were taken from each treatment and protein concentration determination (conducted by specific quantitative ELISA methods) recorded no significant difference between the combined event TC1507 x MON810 x NK603 and individual events[41].

Composition analysis of forage and grain was also conducted. For this purpose, field essays with two treatments and three repetitions by location were performed in five locations. Treatments were: control and combination of events TC1507 x MON810 x NK603. Samples of gain and forage were collected and sent to the laboratory, where they were analyzed for: Gross Protein, Total Ashes, Calcium, Phosphorus, Ethereal Extract, Raw Fiber, Acid Detergent Fiber, Neutral Detergent Fiber and Soluble Carbohydrates. Statistically significant differences also failed to be found in the assessed criteria between conventional and combined TC1507 x MON810 x NK603 corn[42].

Parental TC1507, MON810 and NK603 maize were extensively assessed regarding security for human and animal consumption during the analysis for release performed by CTNBio. Besides,

there are worldwide records of safe use of these events, and harmful effects attributed to their consumption are unknown up to this moment.

Crystalline Bt proteins of insecticide action are known to feature a high degree of specificity in its toxicity for a small group of related insects within one or two taxonomic orders. There is no proof that crystallized insecticide proteins originated in *Bacillus thuringiensis* have harmful effects to human or animal health[43 to 45]. Safety for consumption of genetically modified plants is supported by a multidisciplinary approach adopted during the phase of alimentary safety test[46].

Studies of subchronic acute toxicity were conducted over the past 40 years and established Bt microbial product safety in controlling pest insects damaging several farms. There are products with microbial insecticides passed by regulating agencies within Brazil. Other studies with Bt plants show that Cry proteins are not toxic for human beings and fail to present any allergenic potential.

Allergens must be stable in peptic and tryptic digestion and in the acidity conditions of the human digestive system in such a way that they may reach and pass through the intestinal mucosa to produce a response to the allergenic food. Cry1F is easily degradable in a simulated digestive fluid, being almost proteolyzed after one minute in simulated gastric conditions at a molar relation of 100:1 (Cry1F:pepsine)[47], minimizing any potential absorption by the intestinal mucosa when consumed.

Potential toxicity to humans and animals of protein Cry1F was examined in an oral toxicological study in rats[48]. The highest dose submitted in the essay was 5050 mg/kg PV. When adjusting the purity of the essay material (11,4%), the dose was 576 mg of Cry1Fper kg of corporal weight. During the study, observations on mortality and/or clinical or behavioral symptoms

were recorded, corporal weight was taken and necropsies performed. There was no mortality recorded during the study and adverse clinical symptoms and necropsy results were not noticed. The range of doses used in this study failed to cause mortality among the individuals assessed in the essay and, therefore, it was not possible to determine DL50 of protein Cry1F.

Safety of Cry proteins for human and animal food was confirmed by several authors, including Xu and his collaborators (2009)[49], who obtained results similar to previous authors. Cry1Ab/Ac protein was rapidly degraded in gastric and intestinal fluids, and failed to display adverse effects in mice submitted to an acute dose of 5g (Cry1Ab/Ac)/kg of corporal weight. Once again, the records show that there is neither sequence homology with known allergens or toxins, nor N-glycosylation sites, confirming the idea that no damage will emerge from including the protein Cry1Ab/Ac in human food or animal ration.

Another recent 28 day study in rats, performed by Onose and collaborators (2008)[50], showed that no adverse effect may be attributed to food containing Cry1Ab, since administration of a diet containing Cry1Ab protein has no significance on any physiologic or biochemical parameter, except for a lower level of AST in serum of animals that have received such corn when compared with the control. However, no change in weight or histopathological changes was recorded for organs such as heart, liver and kidney. Besides, in general, higher AST serum levels are recorded with tissue injury, but the interpretation of relatively small changes of AST in toxicological studies shall be carefully made since the range of this parameter's change may be large in healthy animals. Reduction of AST in experiments, therefore, cannot be held as toxicologically significant. In addition, Paul and his collaborators (2009)[51] studied the Cry1Ab protein degradation in the digestion of milk cows. Results indicated that Cry1Ab protein is increasingly degraded during the digestion by these animals in small fragments of 42 kDa,

34 kDa and 17 kDa.

Protein PAT, also present in event TC1507, fails to pose potential risks to human health according to acute oral toxicity and *in vitro* digestibility[52,53]. PAT protein, as expressed in event TC1507 was degraded to undetectable levels within the five seconds following introduction of a simulated gastric fluid containing pepsin, therefore minimizing the protein potential to be absorbed by the intestinal mucosa when consumed. A study of oral acute toxicity in rats with the PAT protein expressed in TC1507 corn, fed with 6000mg/kg of the product containing about 5000mg of PAT protein per kilogram of corporal weight[52] failed to record difference between treated animals and control animals as far as organs weight and histopathology and pathology results are concerned. The results in event TC1507 confirmed previous toxicity studies of PAT protein in lineages of corn tolerant to glufosinate ammonium, where it was clear that the protein is rapidly denatured by heat or very low pH[54,55].

Studies developed by Heroued and collaborators in 2005[56], showed that PAT protein is highly specific and fails to feature characteristics associated to food toxins or allergens, displays sequence homology below 35% and no continuous sequences of eight amino acids with known allergens, as shown by *in silico* tests and in addition has no N-glycosylation sites. Similarly, the authors showed that PAT protein is rapidly degraded in experiments of gastric and intestinal fluid simulations with pancreatine and pepsine. Additionally, no mortality or toxicity was recorded when 1 or 10 mg of PAT/kg of corporal weight by intravenous injection in mice, therefore confirming that the PAT protein fails to display acute toxicity (safety factor > 1000) and does not cause adverse effects in mice after intravenous administration in high doses for a period larger than two weeks. The conclusion is that no harmful effect generated by inclusion of PAT protein in human or animal food may be expected.

PAT protein is enzymatically active and is highly substrate-specific. When there is no adequate substrate, either in the corn plants or in human and animal diets, where the PAT protein may react, the use of the product is safe.

The donor organism of the *cp4 epsps* gene, the *A. tumefaciens* strain CP4, is a bacterium commonly found in the soil that infects susceptible plants and there is no scientific evidence indicating that it may cause adverse effects in human beings and animals. The *Agrobacterium* species are not pathogenic to human beings or animals and there is no record that the *epsps* gene may be a determinant of pathogenicity associated with *Agrobacterium* in plants. Due to its function, the EPSPS are essential to the normal growth of plants and microorganisms, possessing no toxicity associated to this protein family that has a long history of environmental and alimentary safety (35).

Toxicity tests were held with CP4 EPSPS isolated from transformed plants. Harrison and collaborators[57] showed that ingestion of protein doses higher than 1000 times the level found in modified seeds failed to cause any physiologic change in animals essayed. Results of *in vitro* proteolysis also proved that the rapid digestion and safety of the engineered protein keep away any suspected allergenicity. Besides, the protein has no significant amino acid homology with proteins known to be either toxic or allergenic to mammals.

V. Environmental Aspects

As part of the purpose of assessing the appearance of unforeseen interactions resulting from cross-breeding of events TC1507, MON810, and NK603, control efficiency of lepidopteran (European Corn Borer – ECB – *Ostrinia nubilalis*) and coleopteran (Western Corn Rootworm – WCR - *Diabrotica virgifera virgifera*) by the stacked TC1507 x MON810 x NK603 corn, a comparative analysis was performed with the events separately and with conventional corn[58].

Efficiency was measured by recording the consumption of foliar disks consumption by neonate larvae of EBC. The study was carried out using the experimental design of random blocks, with 10 repetitions by treatment and two observations by repetition. The material for each repetition came from a different plant, and the foliar disk was collected from the most recent leave of plants in development stage V4. The essay result indicates that efficiency of insect control was statistically similar between separate events and the stacked corn.

Genetically modified TC1507 x MON810 x NK603 corn was additionally assessed for herbicide injury effects[59]. In this study, the isolated events TC1507, MON810 and NK603, the stacked event TC1507 x MON810 x NK603 and one conventional control were cultivated in a greenhouse under an experimental design of random blocks containing three blocks, each featuring 26 entries. About 12 days from sowing, four different treatments were applied: events TC1507, MON810 and the combination TC1507 x MON810 x NK603 were sprayed with a glufosinate ammonium herbicide in three doses (1x, 16x and 32x the labeled dose) and the control was sprayed with dose 1x; the event mk60 and the combination TC1507 x MON810 x NK603 were sprayed with a glyphosate herbicide in three doses (1x, 26x and 32x the labeled dose) and the control was sprayed with the dose 1x; the combination TC1507 x MON810 x NK603 was sprayed with a tank mix of glufosinate ammonium and glyphosate herbicides in three doses (1x, 16x, and 32x the labeled dose) and the control was sprayed with 1x, or: the four materials were not sprayed. The percentage of injury was visually estimated at days 7, 13 and 21 after the treatment (DAT), where 0% indicating no visible injury and 100% indicating plant death. The height of each plat was also measured from the soil to the top of the higher leaf, on days 13 and 21 DAT, to be used as a plant health indicator. The results were that the combination TC1507 x MON810 x NK603 showed a behavior similar to individual

events TC1507 and NK603 in treatments with herbicides recommended for each one (glufosinate ammonium and glyphosate, respectively) on the three levels of application. Event MON810, in turn, had the same reaction of the conventional control when exposed glufosinate ammonium. Regarding exposure to the mix of active ingredients, the combination TC1507 x MON810 x NK603 displayed tolerance significantly higher than when contrasted to the conventional control. The results showed that there was no unexpected effect regarding tolerance to the herbicide on the combination of these three events in the stacked corn.

The characterization of insecticide activity of the Cry1F protein produced from a microbial source (*Pseudomonas fluorescens*) showed its efficacy against the armyworm (*Spodoptera frugiperda*), sugarcane borer, (*Diatraea Saccharilis*), European Corn Borer (*Ostrinia nubilalis*), corn earworm, (*Helicoverpa zea*), black cutworm (*Agrotis ipsilon*), elasmo caterpillar (*Elasmopolpus lignosellus*), and southwestern corn borer (*Diatrea grandiosella*). No activities were recorded regarding the Cry1F protein against Western Corn Rootworm (*Diabrotica virgifera virgivera*) corn leafhopper (*Dalbulus* maidis) and corn leaf aphid (*Rhopalosiphum maidis*). The equivalence of biochemical characteristics of the protein produced in the plant form and the microbial one was validated by Evans (1998)[(47)]. Studies conducted with protein Cry1Ab also showed its specificity on insects of the Lepidoptera Order[(7)]. Studies performed by Hua and collaborators (2001)[(60)] showed the specificity of Cry proteins through bonding essays in cell vesicles, evidencing the high specificity of this protein complex and insect receptors. Besides, the high specificity of Cry proteins and its rapid degradation in soil are important factors for environmental aspects and its safety has been proven along the history of use of this technology[(61,62)].

Biosafety assessment of individual events TC1507, MON810 and NK603 performed by CTNBio at the moment of the commercial release of these events took into consideration environmental aspects and reached a conclusion that they are not potential sources of

significant degradation of the environment, keeping with the biota a relation identical to that of the conventional corn[28,7,35].

Summarizing, corn is an exotic species, with no sylvan sexually compatible kinship in Brazil. Corn is highly domesticated and there are no scientific reasons to foresee the survival of genetically modified and non-genetically modified plants outside a farm environment.

VI. Restrictions to the Use of the GMO and its Derivatives

Pursuant to Article 1 of Law nº 11460, of March 21, 2007 "research and cultivation of genetically modified organisms are not permitted in indigenous lands and in areas of conservation units, except Environment Protection Areas".

The evidence produced in the process and bibliographic references verify the risk level of the transgenic variety as equivalent to that of non-transgenic regarding soil microbiota, as well as other plants and human and animal health. Therefore, cultivation and consumption of corn TC1507 x MON810 x NK603 are not a potential cause of significant environment degradation and do not risk human and animal health. For the foregoing, there are not restrictions to the use of this corn or its derivatives, except for places mentioned by Law nº 11460, of March 21, 2007.

VII. Considerations on Particulars of Different Regions in the Country (Subsidies to Monitoring Bodies)

As established by Law nº 11460, of March 21, 2007, "research and cultivation of genetically modified organisms are not permitted in indigenous lands and areas of conservation units.".

VIII. Conclusion

Considering that corn variety (*Zea* mays) TC1507 x MON810 x NK603 belongs to a well-characterized species, with a solid history of safety for human consumption and that genes *CryAb, Cry1F*, *pat* and *cp4 epsps* introduced in the variety code for proteins ubiquitous in

nature, present in, plants, fungi and microorganisms that are part of the alimentary diet of humans and animals;

Considering that the construct of this genotype used classical genetic improvement and that resulted in the heritage of a stable and functional copy of genes *CryAb, Cry1F*, *pat* and *cp4 epsps*, which grant insect resistance and tolerance to the herbicide ammonium glufosinate ammonium and glyphosate;

Considering that CTNBio assessed the events separately and granted a favorable opinions to their commercial release;

Considering the internationally criteria accepted in the process of risk analysis of genetically modified raw-materials regarding stacked events[(63)]; and

Whereas

1. Southern blot analyses verified maintenance of integrity of genic constructs inherited from parental corns TC1507, MON810 and NK603 during the process of classical genetic improvement;
2. There is no evidence of interaction among metabolic pathways in which Cry1Ab, Cry1F, PAT and CP4 EPSPS proteins act;
3. Expression of proteins Cry1Ab, Cry1F, PAT and CP4 EPSPS on TC1507 x MON810 x NK603 is not significantly different from the expression observed separately in parental events;
4. There is no record that the expressed proteins may cause allergy or intoxication in humans and animals;
5. Composition data of grain and forage failed to display significant differences between the genetically modified variety TC1507 x MON810 x NK603 and the conventional control, suggesting nutritional equivalence between them;

6. As far as insect control, the stacked event TC1507 x MON810 x NK603 was not different from isolate events TC1507 and MON810, though distinct from the conventional control;
7. The combination TC1507 x MON810 x NK603 displayed a behavior similar to individual events TC1507 and NK603 in treatments with herbicides recommended for each of them and, in addition, when exposed to a mixture of active ingredients, the combination TC1507 x MON810 x NK603 showed a tolerance significantly higher when compared to the conventional control;
8. No unexpected events were identified from unforeseen interactions in combining, through conventional mating, of events TC1507, MON810 and NK603;

For the foregoing, the CTNBio plenary reached a conclusion that TC1507 x MON810 x NK603 corn is as safe as its conventional equivalent. Therefore, within the scope of the competences mentioned by Article 4 of Law no. 11105/05, CTNBio finds that the request complies with applicable rules and legislation in effect to secure environment, agriculture, human and animal health biosafety, reaching the conclusion that TC1507 x MON810 x NK603 corn is substantially equivalent to conventional corn, and that its consumption means no harm to human and animal health. As far as the environment is concerned, CTNBio finds that TC1507 x MON810 x NK603 corn is not a potential cause of significant degradation of the environment, keeping with the biota a relation that is identical to that of the conventional corn. Based on technical and scientific reasons, CTNBio reserves the right to review this Technical Opinion at any moment at its discretion.

CTNBio finds that this activity is not a potential cause of significant degradation to the environment or of harm to human and animal health. Restrictions to the use of the GMO in screen and its derivatives are conditioned to the provisions of Law no. 11460, of March 21, 2007.

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Regarding the post-commercial release monitoring plan, CTNBio determines the instructions below to be followed and performed the technical actions of monitoring as follows:

I) Instructions:

a) Monitoring shall be applied to commercial tillage and not to experimental ones. Areas selected for monitoring shall not be isolated from the remaining areas, have borders or exhibit any other situation away from the commercial standard.

b) Monitoring shall be performed in a model comparing the conventional corn cultivation and the GMO cultivation system, and data collection shall be conducted by sampling.

c) Monitoring shall be conducted in biomes that represent the main areas of GMO culture and, whenever possible, include different types of producers.

d) Monitoring shall continue for a period of at least five years.

e) In all monitored areas, the applicant shall give details of information on all activities conducted in pre-sowing and sowing, on their respective execution, reporting activities conducted in the monitored areas during the culture cycle, on harvesting activities and climate conditions.

f) Monitoring of any harm caused to human and animal health shall be conducted through adverse effects official notification systems, such as SENEPS – *Sistema de Notificação de Eventos Adversos relacionados a Produtos de Saúde*), the Adverse Effects Notification System related to Health Products, regulated by ANVISA.

g) Analytical methods, results achieved and interpretations thereof shall be developed in conformity with principles of independence and transparency, except for aspects of commercial secrecy previously justified and defined as such.

(II) - Monitoring technical actions to be conducted:

1 - As far as genes *pat* and *cp4 epsps*, which grant resistance to herbicides, the following shall be monitored:

a) Nutritional status and sanity of GMO plants.

b) Chemical and physical attributes of the soil related to fertility and other basic pedological characteristics.

c) Soil microbial diversity.

d) Soil diaspore bank.

e) Community of invading plants.

f) Development of herbicide resistance in invading plants.

g) Residues of herbicide in soil, grains and aerial part.

h) Genic flow of inserted genes.

2 - As far as *cry1Ab* and *cry1F* genes, which grant resistance to insects, the following shall be monitored:

a) Impact on target and non-target insects.

b) Impact on non-Insecta Class soil indicator invertebrates.

c) Residues of insecticide proteins in organic matter in decomposition, both in the soil and watercourses near the monitored area.

d) Development of resistance by target insects.

e) Genic flow of the two inserted genes.

CTNBio analysis considered opinions issued by the Commission members, documents delivered to CTNBio Office of the Executive Secretary by the applicant and results of planned releases into the environment. Applicant's studies and independent scientific literature, conducted by third parties, were also considered and consulted.

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Brasília, June 22 2011

Edilson Paiva

CTNBio President

Technical Advisory: Thais Haline Vaz

Dissenting Vote:

CTNBio members, Dr. José Maria Gusman C. Ferraz, Dr. Leonardo Melgarejo, Dr. Solange Teles da Silva, Dr. Graziela Almeida da Siva, Dr. Pedro Canísio Binsfeld and Dr. Rodrigo Roubach voted against the commercial release of TC1507 x MON810 x NK603 corn. Dr. Leonardo Melgarejo, justifying his vote, alleges that the application for the commercial release of TC1507 x MON810 x NK603 corn is not under CTNBio rules of Ruling Resolution no. 05 and, therefore, fails to take into consideration the principle of precaution. Dr. José Maria endorsed Dr. Melgarejo' statement. Dr. Solange Telles justified her vote stating that, for being an organism that is consumed as food, the corn fails to observe Article 10(6), that is to say, assessment of risk to human and animal health, as provided by Ruling Resolution no. 05.

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In Witness Whereof, I have hereunto set my hand and seal in this City of Brasília, Federal District, Brazil, this Wednesday, April 15, 2015.

Fees according to

Official Gazette of 04/15/2011

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Marco Antônio Rochadel

Public Translator