Ministry of Science and Technology-MCT
National Technical Biosafety Commission-CTNBio

TECHNICAL OPINION No.1255/2008.

Process No.:01200.002109/2000-04
Petitioner: Syngenta Seeds Ltda.
CNPJ: 049.156.326/0001-00
Address: Av. das Nações Unidas, 1801 – 4º andar - São Paulo – SP – CEP 04795-900
Subject: Commercial Release of Genetically Modified Corn
Previous Extract: Communication No.115/2000 published on Jul/31/2000.
Meeting: 106th Ordinary Meeting of CTNBio that took place on September 20th, 2007.
Decision: GRANTED

After appreciation of request for Technical Opinion for commercial liberation of genetically modified corn resistant to insects of the Lepidoptera order (Bt11 corn, Event Bt11), as well as all the progenies coming from the transformation event Bt11, and its derivatives of lineages crossings, and non—transgenic populations of corn with lineages bearing event Bt11, CTNBio decided to GRANT it, on the terms of this conclusive technical opinion.
Syngenta Seeds Ltda. requested from CTNBio a Technical Opinion for the free registration, use, essays, tests, seeding, transportation, storage, commercialization, consume, importation, liberation and discard of corn (Zea mays, L.) resistant to insects of the Lepidoptera order – Corn Bt11. This corn was genetically modified through the insertion of plasmid pZO1502 containing a fusion of gene cry1A(Btk) with gene pat. The event of transgenic Bt11 corn was obtained through the direct transfer of DNA in protoplasts of lineage H8540 of corn, deriving from embryo cells in culture in suspension treated with enzymes for degradation of cellular wall. It contains the synthetic gene Btk, that comes from Bacillus thuringiensis var. kurstaki, that codifies á-endotoxin Cry 1Ab, that enables the translation of á-endotoxin lethal to insects that ingest these cells, particularly those of Lepidopterus order, and gene pat, derived from Streptomycin viridochromogenes cepa Tu494, and the codifier of the phosphinotricin enzyme N-acetyltransferase (PAT). For Cry1A(b), the highest expression levels were observed on leaves, with 27 to 33 μg/g of fresh tissue. Levels 5 to 10 times lower were observed in straw tissues, stem and

grains. For PAT, the amount described on leaves is of the order of 44 ng/g of fresh tissue. Half of this value was found on panicles, and 10 times less in style-stigmas. No allergenic or toxic effects were pointed out coming from genetically modified plants and grains. Genetically modified proteins are degraded by digestion of food, by gastric fluids and by bacteria present on human being and animals' gastrointestinal treat. Due to plants bigger production to the attack of insects, and, particularly, of Bt11 corn spikes, there are less toxins of fungus origin in grains, reducing the possibility of intoxications of human beings and animals. Proteins Cry and PAT do not become volatile, nor are absorbed by the epidermis, and, therefore, it would not be justifiable to evaluate the toxicity of such proteins through inhaling or via dermis. No unintentional meaningful biological change occurred on the composition, or on the nutritious value of the grain, and of the Bt11 corn sawdust, as a consequence of Cry1A(b), and pat transgene expression, suggesting, then, that Bt11 corn is substantially equivalent in nutritious composition to the respective isogenic hybrid not genetically modified and commercial hybrids of corn. The dispersion of corn seeds is easily controlled, once corn domestication eliminated the ancestral mechanisms of seeds dispersion, and pollen movement is the only effective escape mean of corn plants genes. The horizontal gene flow between Bt and other species, even those that are very related, have almost no probability of occurrence, for sylvan species related to corn do not naturally occur in Brazil. The coexistence between conventional corns cultivations (improved or creoles), and transgenic cultivation is possible from the agronomic point of view, and for that, one should observe the disposition on Normative Resolution No. 4 of CTNBio. Once B. thuringiensis is a soil microorganisms, the exposition of live organisms, and of the environment to this bacteria, or to any element extracted from it, is an event that abundantly occurs in nature, not resulting in meaningful risk for the soil micro biota. However, even if genic flow occurs between Bt11 corn plants and the creoles varieties, differences of the gene flow in relation to any other existing allele in plants are expected. In sum, the gene or allele will only stay in the population if the gene flow is continuous, with relatively high frequency, and if there is any adaptation advantage. In the Brazilian environment, where sexually compatible native species do not occur, or are known, the risk that Bt11 corn execute or promote the invasion of uncultivated, and cultivated areas does not exist. The ingestion of endotoxin Cry1A(b) by worms of Spodoptera frugiperda, Helicoverpa zea and Ditrea saccharalis with alkaline

digestive environment will promote its death through the interaction of the protein with the receptors of cellular surface of intestinal cells of these insects, promoting the opening of the pores, and the invasion of the microorganisms in the intestinal treat. Thus, insects' death derives from the osmotic unbalance promoted by the toxin, and by septicemia deriving from the invasion of microorganisms into the intestinal flora. Meaningful differences were not observed between the populations of ladybugs, carabidae, cincidelidae and spiders, neither of parasitoid of H. zea, Trichogramma sp., when Bt11 plants were compared to their genetically unmodified isogenic lineage. Bt11 hybrids were efficient for the control of the evaluated plague-lepidopteron, and superior for the profit agronomic parameters of grains and of bitter grains. For the other evaluated agronomic parameters (plants height, insertion height of spikes, date of male and female flowering, note for diseases, percentage of erect plants, kind of grain, grain color), Bt11 hybrids presented performance statistically equals to the respective isogenic not GM hybrids, confirming the equivalence of agronomic performance between B11 hybrids, and the non GM isogenic in conditions of the culture cultivation in Brazil. In Brazil, nowadays, there is an indiscriminate use of insecticides, and even a mixture of chemical products, to try to control insects, especially S. frugiperda. The use of Bt technology in Brazil may contribute for the reduction of the use of insecticides, and, consequently, reduce the impacts of the use of such agro toxics in the environment, in human and animal's health, and it may also indirectly help on the preservation of untargeted organisms' populations, and benefic insects, facilitating the integrated handling of crop plagues. The use of genetically modified plants resistant to insects present positive repercussions also in the aspects related to the acquisition and use of chemical insecticides, to meaningfully reduce the pollution provoked by industrial rejects, and by the use of water used on pulverizations, besides avoiding man, food, rivers and springs contamination deriving from the use, transportation and storage of insecticides. Before the foregoing, one can conclude that the cultivation and consume of Bt11 corn is not the potential cause of meaningful degradation of the environment, or of risks to human and animal's health. For these reasons, there are no restrictions to the use of this corn, or its derivatives. The petitioner should conduct monitoring after the commercial release on the terms of Normative Resolution No.3 of CTNBio. In accordance with what is established on art. 1 of law 11.460, of March 21st, 2007, "it is vetoed the research and cultivation of organisms genetically modified on indigenous lands and

areas of conservation units". In the ambit of competences of art. 14 of Law 11.105/05, CTNBio considered that the request fulfills the norms and the pertinent legislation that aim at guaranteeing biosafety of the environment, of agriculture and of human and animal's health.

CTNBio's TECHNICAL OPINION

I. GMO Identification
Designation of GMO: Bt11 Corn
Petitioner: Syngenta Seeds Ltda.
Species: Zea mays L.
Inserted Characteristics: Resistance to insects of Lepidoptera order
Method of characteristic introduction: Direct transformation of protoplasts
Proposed Use: Silage and grains production for human and animal consume of GMO, and its derivatives.
II. General Information
Corn Zea mays L. is a species from the Gramineae family, Maydae tribe, Panicoideae family. Corn is a separate species within Zea sub-gender, with chromosome number 2n = 20, 21, 22, 24 (26). The sylvan species closer to corn is teosinte, found in Mexico, and in some places in Central America, where it can be crossed with corn cultivated in production fields. The corn produced can also be crossed with the most distant genre Tripsacum. This crossing, however, occurs with great difficulty and results on sterile-male progeny.
Corn history is over eight thousand years old in the Americas, being cultivated since the pre-Colombian period. It is one of the superior plants best scientifically characterized, being, nowadays, the cultivated species that reached the highest degree of domestication, and only survives in nature when it is cultivated by men(4). Today, there are around 300 races of corn, and within each race, thousands of crops.
Corn is one of the most important sources of food in the world, and is raw material for the production of a wide range of food products, rations and industrial products. Brazil is the third biggest corn producer in the world with a production of approximately 35 million tons in 2005, behind only of the United States of America (282 million tons), and China (139 million tons)(29). In Brazil, corn is basically planted in two crops (summer plantation, and small crop), and it is cultivated practically all over the national territory, being 92% of the production concentrated in the South (47% of production), Southeast

(21% of production) and Center-West (24% of production) (19). In the productive chain of swine, and poultry, approximately 70 to 80% of the corn produced in Brazil is consumed.

It is known that the occurrence of insects in the tropics is bigger than the one in tempered climate regions, and that damages caused are more accentuated. Among the most important corn plagues, one can highlight Spodoptera frugiperda. Cruz et al.(21) estimated that the loss in Brazil, due to the infestation by S. frugiperda was around 400 million dollars per year. From 1999, it was observed an increase on the occurrence of S. frugiperda, and consequently there was increment on the harms. Other species of Lepidoptera order are also important plagues for corn cultivation, such as Helicoverpa zea, and Diatrea saccharalis). It is estimated that these three species may cause damages of up to 34% on corn grains production.

The main insects control measure on corn culture has been the insecticides use. In some areas of the Brazilian center-West regions, for example, dozens of pulverizations with insecticides are necessary in only one culture cycle. Another plague control measure would be the use of resistant cultivars. The acquisition of cultivars resistant to insects through classic genetic improvement has not obtained the hoped success. In the case of S. frugiperda, many attempts have been made with limited success(77).

Brazil is the third biggest consumer of agricultural defensives in the world. Nowadays, we have 142 agro toxics registered for corn, 107 only for worms. There are already many cases of resistance for the constant and indiscriminate use of insecticides in corn culture in Brazil. Besides, one of the factors that affects agriculturists' health the most in Brazil is the use of agricultural defensives responsible for the intoxication of a million people every year (2).

Bt11 genetically modified corn presents characteristics that confer resistance, on the same plant, to insects, and to glufosinate of ammonium herbicide, and resists to the main plagues of Lepidoptera Order that affect corn culture in Brazil, such as S. frugiperda, and H.zea. The genes introduced codify an incomplete form of Bt insecticide protein, obtained from cepa HD-1 of the soil bacteria Bacillus thuringiensis var. kurstaki (btk), and an enzyme (phosphinotricin-N-acetyl transferase, PAT), that confers tolerance to glufosinate of ammonium herbicide, also obtained from a soil bacteria, Streptomyces viridochromogenes. Varieties of corn containing Cry proteins have been used in many countries in the world, and there is no information that hybrids of corn containing cry genes have caused damage to the environment, or to human

and other animals' health. Corn Bt11 is commercialized in 16 countries (Argentina, Australia, Canada, China, European Union, Japan, Korea, Mexico, Philippines, Russia, South Africa, Switzerland, Taiwan, The United Kingdom, The United States of America, and Uruguay), being commercially cultivated in the United states (1996), Canada (1996), Japan (1996), South Africa (2003), Philippines (2005), Argentina (2001) and Uruguay (2004).

In Brazil many necessary experiments were conducted, and enough studies were made to convince CTNBio's members about the biosafety of the event in study. In the risk analysis, the molecular characterization should be considered, taking also in consideration studies carried out regarding the constitutional, agronomic, and physiologic characterization, of this event itself. The long experience with traditional methods of plants improvement, the experience of over three decades in research, and more than one decade of commercialization of transgenic varieties in the world, besides the advancement in the knowledge about the structure and dynamics of genomes, indicating if a certain gene, or characteristics is safe, signal that the process of genetic engineering on its own presents little potential for arising unexpected consequences that would not be identified, or eliminated during the process of genetically modified varieties development (8).

III. Description of GMO and Expressed Proteins

Bt11 corn was genetically modified through the insertion of plasmid pZ01502 containing the fusion of gene cry1A (Btk) with gene pat. This corn expresses gene cry1A(b), derived from the soil bacteria B. thuringiensis subsp. kurstaki, lineage HD-1.

B. thuringiensis (Bt) is a gram positive bacteria of Bacillaceae family that produces, at the moment of its sporulation, crystalline proteic inclusions. These inclusions contain proteins called á-endotoxins, which nowadays form a family of 300 members, classified in 49 groups(20). They are produced under the form of prototoxins that are transformed into toxic peptides in the insect's intestines, through the action of intestinal alkaline pH, and of proteases. The active toxin causes the destruction of epithelial cells, and the death of the larvae(47,23). B. thuringiensis may be considered the biological agent of greatest potential for the control of forests, agricultural plague-insects, and vectors of diseases, thanks to the specificity of the á-endotoxins to insects and target-invertebrate, and its innocuousness to vertebrates and to the environment, including benefic insects and natural enemies(43), making this agent a keycomponent in strategies of integrated handling of plagues(59).

The event of Bt11 transgenic corn was obtained through the direct transfer of DNA(nu) in protoplasts of lineage H8540 of corn, derived from embryo cells in culture in suspension treated with enzymes for degradation of the cell wall, and it contains DNA sequences inserted into the cell genome, according to the following description. The synthetic gene Btk codifies á-endotoxin cry1Ab. The objective of use of Btk genic cassette is to allow, in vegetable cells, the transcription of RNA, and the translation of lethal á-endotoxin to insects that ingest these cells, particularly those of the Lepidoptera order, such as the ones of Spodoptera, Helicoverpa and Diatrea genre (61, 17, 34). Modifications on the original sequence of Btk were carried out in order to alter some codons of preferential use in bacteria for the preferential pattern of vegetable codons, as well as the truncation, that is, the reduction of the size of the codifying sequence, in order to produce a more effectively toxic version to targeted-insects. The synthetic nucleotide sequence, on the truncation version did not alter the polypeptide sequence of codified protein on the considered region. The final sequence of gene Btk(1845 pb) illustrated on the process allows for its immediate comparison with the original sequences of cry1A(b) of B. thuringiensis var.kurstaki available at GenBank with those under the access codes AYB47289, and AFO59670, among others (65,71,39). Gene Btk is regulated by two nucleotide sequences upstream, constituted by the promoter RNA35S of mosaic virus of cauliflower (35S CaMV), isolated CM1841 with 514 pb, and the intron sequence IV56 of gene of 1S (Adh1S) desidrogenase alcohol of corn, with 412 pb. With these regulating elements the transcription of gene Btk has its potential increased in vegetable cells. As terminating sequence, the cassette of expression has a terminal region of 270 pb of gene of nopalina-syntase (3'-nos) of T-DNA of Aggrobacterium tumefciens. All the regulating elements of the transcription have a function widely described in scientific literature (45, 35, 48). In the case of a insecticide toxin without known, or described enzymatic activity, one cannot expect metabolic alterations deriving from the expression of Btk in vegetable cells. The measures of general metabolic contents reinforce the idea that, if any chemical alteration occurs due to genetic transformation, it is not perceptible through sensible methods of analysis, such as, for example, spectroscopy of near infra-red (NIRS).

Another component of Bt11 corn is gene pat, derived from Streptomycin viridochromogenes cepa Tu494 and codifier of phosphinotricin N-acetyltransferase (PAT) enzyme. The original sequence was modified to reduce the content G/C and alter the beginning of the translation GTG to ATG, in

order to enable, and optimize the syntheses of the original protein. The final version of pat gene has 558 pb. Again, a 551 pb sequence of the promoter 35S of CaMV (isolated Cabbs), and the intron sequence IVS2 of 178 pb of gene adhS1 of corn were used to promote and increase the transcription of pat gene. Sequence 3'-nos of 220 pb was used as a terminator element of transgenes. This cassette allows, then, the syntheses of the recombinant protein PAT, capable of chemically inactivate herbicides deriving from phosphinotricin, such as glufosinate of ammonium, making cells and vegetables that contain it resistant to it. Pat Enzyme has described and well-known activity (32, 57, 70). Bt11 corn has framework of plasmid pUC18, including the origin of replication, and places of recognition of endonucleases that allow the adaptation of sequences. These vector DNA fragments have 1520 pb of extension and there is no evidence that they are expressed on vegetable cells(7). The final version of the plasmid used on the genetic transformation of corn was called pZ01502, and has 6,120 pb, including all the cassettes and elements of DNA described above. This plasmid was destitute from the gene of bacteria resistance to antibiotics derived from penicillin, such as ampicillin (gene ampR), originally present on the parental form pUC18.

Hybridizations of Southern blots and amplifications through chain reaction of DNA-polymerase (PCR) were presented to demonstrate the integration of DNA fragment on vegetable genome, the number of gene copies, the presence, or absence of other DNA elements, and the location of transgene. The results presented corroborate to the statements of the proponent that one transgenic copy was integrated to a long arm of chromosome 8 of the corn originally transformed, and, part of it, transferred to the progenies in hemizygote, initially, and hemizygote on final versions of parental lineages for the production of hybrids. The location of the insertion was defined by linking molecular markers of RFLP type (polymorphisms as big as fragments of DNA generated by hydrolysis with endo-nucleases of restrictions). These essays demonstrated, also, the presence of transgenes cry1A(b), pat, and of the origin of replication of pUC18. Finally, such analysis allow for the conclusion that none of the lineages or hybrids derived from the initial event Bt11 contain gene ampR.

Results presented by the proponent regarding the analysis of the presence of cry1A(b) and pat, as well as the pattern of resistance to glufosinate and to S. frugiperda worms, have demonstrated that genes Btk and pat are closely linked, and that both are inherited as loci simple dominants on Bt11 corn lineage. The segregation data match the Mendelian pattern on the proportion 3:1 for

heterozygote progeny.
The proof of the presence of recombinant proteins in different vegetables tissues was executed through imunodetection of Cry1A(b) and PAT. For the first protein, higher levels were observed on leaves, with 27 to 33 μg/g of fresh tissue. Levels 5 to 10 times lower were observed in straw tissues, stem and grains. For PAT, the amount described on leaves is around 44 ng/g of fresh tissue. Half of this value was found in panicles, and 10 times less in style-stigmas.
IV. Aspects Related to Human and Animals' Health
The evaluation of foods safety derived from genetically modified raw material is based on risk analysis, scientific methodology that encompasses the phases of evaluation, management and risk communication. On the risk evaluation phase one looks for the qualitative and quantitative characterization of potential adverse effects, having as base the concept of substantial equivalence for the identification of eventual differences between the new food and its conventional correspondent. The Principle of Substantial Equivalence is key concept on the evaluation process of innocuousness of foods coming from new technologies (27).
To evaluate safety of genetically modified food raw material, or its equivalence to conventional food, it is recommended that four main elements are analyzed, more specifically: (1) parental variety, that is, the plant that originated the new genetically modified raw material; (2) the transformation process, including the characterization of the construction used, and of the resulting event; (3) the product of the inserted gene, and the potential of toxicity and allergenicity, and, finally; (4) the composition of the new variety deriving from the genetic transformation. The group of data of these analyses should allow for the identification and characterization of the potential adverse effects associated to the new raw material consume, subsidizing the phases of management and risk communication.
According to the petitioner, corn Bt11 derives from the transformation of common Zea mays, a species profoundly characterized, and about which there is solid safety background for human consume. Information about identity, origin and chemical composition have been reported, being attached to the process publication copy that provides abundant data regarding its composition, highlighting the naturally observed variations on the presence of nutrients(73). The characterization of Bt11 corn, and its products of expression were extensively analyzed, according to item III of this technical opinion.
The state of the art on the evaluation of toxicity

preconizes the use of essays of animal experimentation, as scientific form of qualitative and quantitative characterization of potential adverse effects to human health caused by the exposition to environmental intoxicating substance, or present in foods. Thus, whenever viable, toxicological essays of xenobiotic in experimentation animals are executed, administrated them through exposition via that allow extrapolating the results observed in animals to humans. This extrapolation allows to establish IDA (Acceptable Daily Ingestion), or Reference Dose that means the dose of this substance to which an individual may expose himself daily without observing negative effects deriving form such exposition.

Thus, the study of protein Btk of corn Bt11 was conducted through acute oral via in rats, besides the digestibility essays. On the essay of simulated digestion, it was observed that the half-life of the protein is inferior to 30 seconds on the gastric system, and that, in the intestines, the complete chain protein is converted into the central fragment resistant to tripsine. The toxicity study through acute oral via was conducted in rats, and no harmful effects were observed on any of the evaluated doses, being 4, 000 mg/kg of body weight the highest dose tested, which is considered the NOEL of the essay, that is, the highest dose in which no harmful effects are observed, estimating thus, DL50 as being superior to 4,000 mg/kg of body weight. Toxicological classification tables consider low toxicity doses over 2,000 mg/kg of body weight that do not provoke harmful effects on evaluated animals under adequate experimental conditions.

One can conclude that the absence of effects in this essay was related to the low potential of absorption of protein demonstrated on the study of in vitro digestibility, where one could observe its rapid degradation in the gastric fluid of mammals, with less than 4% of activity after two minutes. This essay demonstrated the stability of the protein for 19 hours in the intestinal fluid.

The results show that genetically modified corn on the concentration of up to 4,000 mg/kg was incapable of producing acute toxic effects in rats, and that on the concentration of 11% to 33% on the diet (11g to 33 g/kg of body weight) it was incapable of producing intoxication signs in rats fed for 90 days. The Codes Alimentarius of FAO/WHO(28) uses the following formula for the calculation of IDA = NOEL/FS

where:

· IDA is the biggest amount in mg/kg of a chemical substance that can be ingested per day by the human being, during his whole life, and that does not cause any harm;

· NOEL is the biggest dose of a chemical substance in mg/kg that, if used, does not produce toxic effects on animal species most sensitive to it;
· FS is the safety factor, usually equals to 100 (two order factors 10: the first considering the human being 10 times more sensible than the most sensible animal species studied, and the second considering the individual variability within the human species).
In this sense, once the biggest amount of genetically modified corn used in toxicity essays (33,000 mg/kg/day in sub-chronic essay in the rat) did not produce toxic effects, and, considering the impossibility of administrating a bigger amount per day on rats, one can conclude that it is impossible to calculate the NOEL value. In fact, a rat does not ingest 10g/100g per day of body weight of ration, according to Harkness and Wagner's description(36), being impossible to feed it with bigger amount of the product without causing malnutrition due to lack of other normal ration components. Thus, one can understand why there are no IDA values for genetically modified corn. In other words, the level of its possible toxicity, if it exists, is way beyond the maximum amount ingested by any human or animal that, in practice, one can affirm for its absolute innocuousness.
Brake and collaborators(9) compare the nutritional effects of Bt corn to non modified corn in chicken for slaughter. The results showed that the administration of genetically modified corn during 35 days did not interfere with the gain of weight, or with the digestibility characteristics of proteins ingested by the chickens. These results were confirmed, among others, by Taylor et al.(68). Folmer and collaborators(31) compare the nutritional effects of corn Bt with non-modified corn in cattle for slaughter and concluded that the administration of corn Bt did not modify any parameters that indicate food efficiency, or of gain of weight of the treated animals in relation to those of the control group. Sanden and collaborators(58), during a long term study (8 months) in salmons, reported the lack of alterations in the body development, and on tissues of the fish stomach and intestines.
Proteins Cry and PAT have high molecular weight, 65kD and 30 kD, respectively. So, they are not volatile, nor absorbed by the epidermis, and, for these reasons, it is not justifiable to evaluate the toxicity of these proteins through inhaling or dermis via. Additionally, the toxicproteins safety of B. thuringiensis have been proved since the 60's, with the use of microbial insecticides based on Bt (62, 63, 64), even in organic cultures.
The allergenic potential of proteins Cry 1Ab and PAT was investigated using various criteria, including homology of

the sequence of amino acids with allergenic known at the data banks of public domain (Genpept, Swissprot, PIR protein), and no homology was detected(42). On the contrary of known proteic allergenic, studies have demonstrated that proteins Cry1Ab were rapidly inactivated when subjected to simulated gastric fluids of mammals. Similarly, it was noted that protein PAT was rapidly digested in conditions that reproduce human digestion.

Okunuki and collaborators(53) showed that the degradation of protein Cry1Ab, after being heated is very fast, and, considering its digestibility in human gastric fluids, they suggested that it should present no allergenic potential, or extremely low one. Batista et al.(5) tested the allergenicity of genetically modified soy and corn in sensitized individuals, comparing it to the one produced by conventional seeds in the same individuals, and showed that the genetically modified products are safe regarding the allergenic potential. Nakajima and collaborators(50) confirm the previous data when they reported the lack of meaningful levels of specific IgE against Cry1Ab in patients' serum with food allergy.

Chowdhury and collaborators(16) studied the destination of intrinsic genes (of corn itself), and recombinants in bullcalves fed with Bt11 corn resistant to insects, and noted the presence of intrinsic and recombinant genes in the fluid of rumen, and in the content of the rectus in the period between five to eighteen hours after being fed. However, recombinant genes were never found in blood cells, or guts, and muscles. Phipps and collaborators(54), in similar work, but with bull-calves fed with ration containing genetically modified soy (gene cp4-epsps) and Bt corn (gene cry1Ab), found fragments of transgenes in the rumen, and in the duodenal digest. There were no traces of transgene in feces, in the blood, or in the animals' milk. Aeschbacher et al.(1), in experiments executed with chicken fed with hybrid Bt corn, did not find any fragment of transgene in the tissues of the muscles, liver, spleen, other organs, flesh or eggs.

A little discussed theme, but with positive impact over human and animal's health is the possibility of having the improvement on grains quality, due to the introduction of Cry toxin in corn. Due to the greater protection of plants to insects attack, and, especially, of Bt11 corn spikes, rotten grains and spikes are extremely reduced when compared to untransformed plants. As a consequence, they diminish the toxins of fungus origin on the grains, reducing the possibility of humans and animals' intoxication. Munkvold et al's(49), and Clements et al's(18) works in 2003 concluded that B11 corn presented reduction on the concentration of fungus in the grains.

Between grains and sawdust, the parameters evaluated presented a similar profile, and within the amplitude used as reference by the International Life Sciences Institute Crop Composition(40). The parameter of total grease percentage by dry weight of grains of Bt11 corn was superior, when compared to the other treatments. However, the fatty acids levels were individually presented within the amplitude published by ILSI(40). The results obtained indicated that no meaningful unintentional biological change occurred on the composition, or on the nutritive value of the grain and of Bt11 corn sawdust, due to the expression of transgenes cry1A(b) and pat, suggesting, then, that Bt11 corn is substantially equivalent in nutritive compositions to the respective isogenic hybrid not genetically modified and commercial hybrids of corn.
From the analysis of residues (proteins) eventually present in food coming from Bt11 corn to be provided to animals and to human beings, one can conclude that none of them have cancer, teratogenic or genotoxic potential. In fact, these proteins do not have any structural similarity with primary or secondary carcinogens, and have no conditions of connecting to human DNA.(15). Finally, the lack of acute, or sub-chronic effects produced by genetically modified corn eliminates, also, any possibility of late neurotoxicity. This toxic effect is exclusive of organophosphorate plaguecide, and does not have any relation to possible residues of Bt11 corn.
Before the foregoing, it is relevant to remind that allergenic or toxic effects coming from genetically modified plants were not found. Genetically modified proteins are degraded by digestion of food, by gastric fluids, and by bacteria present in the gastrointestinal treat of human beings and animals.
V. Environmental and Agronomic Aspects
Corn plants are allogamous and annual, of crossed fecundation and widely pollinated with the help of the wind, insects, gravity and other agents. The introduction of genic elements characterized in Bt11 event did not alter the reproductive characteristics of the plant. Therefore, the same chances of crossed fecundation that occurs between hybrids, and not genetically modified lineages of corn, will occur between plants of Bt11 event, and other corn plants. In Brazil there are no parental species of corn in natural distribution. However, there are populations of creoles corn that can be crossed with genetically modified corns, in case they are planted in the vicinities.
The risk of passing the transgenes to other individuals in nature, and its consequences, mostly in biodiversity is, without any doubt, one of the direct effects that have called the most attention in case of transgenic. The gene

flow may be horizontal, when the exchange of genetic information happens between animals of different species, genetically distant, or vertical when the passage of genetic information occurs between individuals of the same species.

The horizontal gene flow between Bt and other species, even those very related, have almost null probability of occurrence. Sylvan species related to corn do not naturally occur in Brazil. Siqueira and collaborators (66) and Nielsen et al. (51) discuss the possibility of Bt gene of transgenic plant passing to other microorganisms of the soil. The conclusion is that the probability is very remote. Once B. thuringiensis is a soil microorganism, the exposition of live organisms and of the environment to these bacteria, or to any element extracted from it is an event that occurs abundantly in nature, not resulting in meaningful risk for soil micro biota. It would be much more plausible for this gene to pass from B. thuringiensis to other micro-organisms.

The vertical gene flow, at first, has no consequence because most agriculturists do not reuse the collected grains as seeds. The hybrid seeds of F1 generation are acquired every year. However, there is a small contingent of agriculturists of subsistence that keep creoles varieties. Nodari and Guerra(52) argue that the diversity of agricultural species composed of creoles cultivars of corn may be threatened by transgenic. However, it is possible to keep these cultivars, for hybrid corn has been intensively cultivated in Brazil for many decades in the same regions in which most of the creoles cultivars are concentrated and the latter have been kept. On the other hand, even if gene flow occurs between plants of Bt11 corn, and creoles varieties, it is not expected difference of genic flow in relation to any other allele that exists in plants. Discussion in this regard is presented by Ramalho and Silva(59). In sum, gene or allele will only remain in the population if the genic flow is continuous, with a relatively high frequency, and if there is any adaptation advantage. Additionally, the characteristics introduced into event Bt11 would not bring potentially damaging consequences to human, animal's health, or to the environment, due to the considerations made previously, and to the background of safe use in other countries for more than 10 years(12). However, it is necessary to emphasize that the coexistence between conventional cultivars of corn (improved or creoles) and transgenic cultivars of corns is possible from the agronomic point of view(11,46), and one should note the disposition on Normative Resolution No.4 of CTNBio. It is also important to remember that most of indigenous races, creoles populations, ancient and recent

cultivars, as well as exotic cultivars of corn are preserved in Brazil by EMBRAPA, as well as in various institutes of germoplasm preservation in the world. Classical analysis of the genetics presented by the proponent has demonstrated that there is no possibility of distinction between the pollen of Bt11 corn and the pollens of non-transgenic corns. The results pointed out to the fact that heterozygote corn plants for genes cry1A(b) and pat do not produce progenies excess in crossing-test, concluding that Bt11 corn pollen is not more competitive, or efficient in fertilization than the conventional pollen. Comparing the pollen concentrations to 1m of source culture under low to moderate winds, it was estimated that, approximately, 2% of pollen is noted at 60m, 1.1% at 200m, and 0.75-0.5% at 500m of distance. At 10 m of a field, in average, the number of pollen grains per area unit is ten times smaller than the one observed at 1m from the border. Therefore, if the established distances of separation developed for the production of corn seeds are observed, it is expected that the pollen transfer to the adjacent varieties are minimized, being improbable the presence of genetic materials with resistance to insects.

Seeds dispersion is easily controlled, once corn domestication eliminated the ancestral mechanisms of seeds dispersion, and the pollen movement is the only effective mean of corn plants genes escape, thus, in face of the nature of grains, cobs and corn plants, this vegetable survival is limited to the plantation, and harvest cycle made by human being, since it is totally dependent on him for the seeds to germinate after being thrashed. The different vegetable tissues and organs do not have proliferation capacity, being restricted to seeds firmly stuck to the spikes, and protected by straw, that is, only human activity can remove the seeds from the spikes, and guarantee the survival of the vegetable, cycle to cycle. Thus, corn plants are not invasive plants, and their control is easily executed on crops where cultures rotations are conventional practices, with eventual arise of voluntary, or spontaneous plants derived from seeds lost during harvest. In the Brazilian environment, where native species sexually compatible with corn do not occur, or are not known, the risk of Bt11 corn execute, or promote the invasion of uncultivated and cultivated areas does not exist.

With expected effects of transgenic expression, an incomplete version of protein Cry1A(b) is expressed on vegetable tissues. Lepidopterus insects S. frugiperda , H. zea and D. saccharalis are particularly susceptible to the action of this class of á-endotoxins, for they have digestive treat with alkaline pH, what promotes the

solubilization of proteic crystals, and the intestinal receptors specific to them. This endotoxin ingestion by worms with alkaline digestive environment will promote the death of the insects through the interaction of the protein with intestinal receptors of cellular surface, promoting the opening of the pores, and the invasion of microorganisms of the intestinal treat. Thus, the insects' death derives from the osmotic unbalance promoted by the toxin, and by septicemia deriving from the invasion of the intestinal flora by microorganisms(10).

One of the advantages of transgenic plants resistant to insects expressing genes that codify á-endotoxins, or the microbial preparations, when compared to chemical insecticides, is the high specificity to target-species. In fact, no differences were observed among populations of Dermaptera: Forficulidae, Coleopteran: Anthocoridae, Carabidae, Cincidelidae and Araneae. In relation to eggs parasitism of H. zea by Trichogramma sp. (Hymenoptera: Trochogrammatidae), no meaningful differences were observed either when compared to Bt11 plants with their isogenic lineage not genetically modified(30). The results reinforce observations made in other countries and cultures where field studies showed that the abundance and activity of untargeted insects (predators and parasitoids) were similar when plants genetically modified with Bt were compared to non-genetically modified plants. In contrast, crops whose control is made through chemical methods, negative effects are normally observed on the biological control of plagueinsects.

Before the foregoing, one can conclude that the use of Bt plants, and the consequent reduction on the applications of insecticides tend to favor the presence of predators insects and parasitoids of plague-insects(57).

In relation to target-insects, this event was tolerant to the attack of H.zea, almost no damage occurred on spikes, and to the attack of S. frugiperda. In severe infestation conditions of S. frugiperda, the proponent demonstrated that hybrids of Bt11 corn presented productivity extremely higher than that the one of its non-transgenic isogenies.

In fields experiments carried out in 2000 in Uberlândia, MG, Bt11 corn also showed a noted effect over Mocis latipes (plague of Lepidoptera order that feeds from leaves).

According to analysis of factorial variance of the data presented by the proponent, no difference was observed between the hybrids derived from original elite lineages and the derivatives of Bt11 converted lineages selected for the aspects of productivity, humidity on harvest, putting roots on the ground, spikes height, plants height, and thermal units for adornment, or dehiscence of pollen grains. However, meaningful differences were described between the original elite-lineages, and the conversions

Bt11 for the characteristics of putting the stem on the ground, and integrity note. Bt11 corn presented smaller stem breakage than the non-transgenic hybrids, due to the fact that the former is less susceptible to damages on the leaves and on the stem, due to the smaller incidence of plague-lepidopteron. In relation to differences between grains produced by Bt11 corn, and by equivalent conventional corn, the analysis results of spectroscopy of near infra-red (NIRS) have demonstrated that there are no differenced in relation to non-transgenic grains for density, weight of 100 grains, grains size, amid percentage, protein percentage, oil percentage, and fiber percentage.

Agronomic parameters, and the efficacy on plaguelepidopteron control of Bt11 corn hybrids were compared to isogenic lineages in essays conducted in 5 places: Uberlândia-MG, Ituiutaba-MG, Iraí de Minas-MG, Campo Mourão-PR and Pinhalzinho-SC, in the agricultural harvest of 2005/06. Plants structure, spikes insertion height, male and female flowering date, note for diseases, percentage of erect plants, kind of grains, gains color, humidity content, profit, and rancid grains, were the parameters studied on the agronomic evaluations. For the study of efficacy of the event Bt11 in the control of lepidopteronplague damage of S. frugiperda, of D. saccharalis, and of H. zea were evaluated. Bt11 hybrids were efficient for the control of the evaluated lepidopteron-plague, as well as superior for the agronomic parameters grains profit and rancid grains. According to presented information the favorable differential of performance was mainly related to the efficient protection against the attack of the plagues studied. For the other evaluated agronomic parameters Bt11 hybrids presented performance statistically equals to the respective non GM isogenic hybrids. These results confirm the equivalence of agronomic performance between Bt11 hybrids, and the non GM isogenies in cultivation conditions of the culture in Brazil.

In Brazil nowadays, there is indiscriminate use of insecticides, and even mixture of chemical products, trying to control insects, especially S. frugiperda. With the massive application of these chemical products, an agricultural desert is created in certain regions of Brazil, for the natural enemies of plagues are the first to be eliminated. The frequent application of chemical insecticides contributes for the degradation of the environment, environmental pollution and break of all the ecosystem in corn culture, and even in other cultures in rotation. With the adoption of genetically modified plants resistant to insects, the reduction of insecticides has been considerable in countries that have adopted the

technology for more than ten years. For example, in the United States, producers have obtained reduction of more than 8,000 tons of insecticide active ingredient only in 2001(14,34,33). In China, the applications of insecticides were reduced in an average of 67%, and the reduction in volumes of insecticide active ingredients was reduced in 80%(38). In South Africa the reductions were around 66%(41). Before the foregoing, one can consider that the use of Bt technology in Brazil may contribute for the reduction of the use of insecticides and consequently, reduce impacts of use of these agro toxic in the environment, in the human and animal's health. What's more, the use of Bt technology may have positive impact on the preservation of populations of untargeted organisms and benefic insects, facilitating the integrated handling of crop plagues(69, 37,6). Additionally, the adoption of technologies that reduce pulverization of chemical products in crops may favor acquiring secondary benefits, such as the reduction of use of raw-material on the production of agro toxics, on the conservation of fuel used to produce, distribute and apply such agro toxic, and for the elimination of use necessity and discard of agro toxic cartons(44).

VI. Restriction to the use of GMO and its derivatives:

Studies presented by the petitioner demonstrated that there was no meaningful difference between the hybrids of corn derived from unmodified lineages and Bt11 corn in relation to agronomic characteristics, such as productivity, harvest humidity, putting the root on the ground, spike height, plant height, and others. Besides, there were no meaningful differences in the reproduction way, dissemination or capacity of survival of the genetically modified corn in relation to lineages of unmodified corn. All the evidences presented in the process, and in bibliographic references such as Schuler et al.(60), of Maagd et al. (22), Candas and collaborators(13), Brookes et al. (11), Broderick et al. (10), Sanden et al. (58), Okuniki et al. (53), among others, confirm the risk level of the transgenic variety as equivalent to the non-transgenic varieties in face of the soil micro flora, to untargeted vertebrate and invertebrate animals, as well as to other vegetables, and to human and animal health. Thus, the cultivation and consume of Bt11 corn are not potentially causing meaningful degradation of the environment, or risks to human and animal's health. For these reasons, there is no restriction to the use of this corn, or its derivatives.

After ten years of use in different countries, no problem was detected for the human and animal's health, or for the environment that may be attributed to transgenic corn. It is necessary to emphasize that the lack of negative effects

resulting from transgenic plants cultivation of corn does not mean that they cannot happen. Zero risk to absolute safety does not exist in the biological world, however, there already exists an accumulation of trustworthy scientific information , and a safe background of ten years use that allows us to affirm that Bt11 corn is as safe as its conventional versions. Thus, the petitioner should conduct monitoring after the commercial release on the terms of Normative Resolution No. 3 of CTNBio.

The vertical gene flow for local varieties (called creoles corns) of open pollination is possible, and presents the same risk caused by commercial genotypes available in the market (80% of conventional corn planted in Brazil come from commercial seeds that went through a process of genetic improvement). The coexistence of conventional corns cultivations (improved or creoles), and transgenic cultivations of corns is possible from the agronomic point of view(11, 46), and should follow the disposition on Normative Resolution No. 4 of CTNBio.

VII. Considerations about particularities of different regions of the Country (subsidies to the inspections organs):

In accordance with what is established on art. 1 of Law 11.460, of March 21st, 2007, "it is vetoed the research and cultivation of organisms genetically modified on indigenous lands and areas of conservation units".

VIII. Conclusion

Considering that Bt11 corn derives from the transformation of common corn Z. mays, species profoundly characterized, and about which there is solid safety background for human and animal consume, and that the transformation process gave place to the insertion of a sole copy of the fragment of DNA containing the genetic constructions with genes pat and Btk.

Considering that the safety of corn containing gene pat was exhaustively analyzed by CTNBio on process 01200.005154/1998-36, and, moreover, that on Technical Opinion 987/2007 all aspects related to biosafety of Liberty Link corn were approached.

Considering also that:

1. Corn is the species that reached the highest degree of domestication among cultivated plants, being able to survive in nature without human intervention.
2. There is no sylvan species in Brazil with which corn can be crossed, since the closer sylvan species to corn is teosinte, found in Mexico and in some places in Central America, where it can be crossed with corn cultivated in production fields.
3. Protein Cry1Ab was detected in low levels of analyzed tissues, and presented great susceptibility to digestion in

simulations of gastric fluids, not demonstrating acute toxicity in mammals, or similarity with known allergens.
4. Due to the greater protection of plants to insects' attack, particularly, of Bt11 corn spikes, rotten grains and spikes are meaningfully reduced when compared to untransformed plants, consequently, there is reduction of toxins of fungus origins in grains, diminishing the possibility of intoxication of humans and animals.
5. No unintentional meaningful biological change occurred on the composition, or on the nutritious value of the grain and of the Bt11 corn sawdust, due to Cry1A(b) and pat transgene expression, suggesting, then, that Bt11 corn is substantially equivalent in nutritious composition to the respective isogenic hybrid not genetically modified, and to commercial corn hybrids.
6. DNA molecule is a natural food component, not presenting any evidence that such molecule may have adverse effect to men when ingested in food in acceptable quantities (no direct toxic effect).
7. There is no evidence that intact genes of plants may be transferred and functionally integrated to human genome, or to other mammals exposed to this DNA, or foods manufactured with these elements(16).
8. The petitioner answered to all the questionings postulated on Normative Instruction No. 20 of CTNBio, and none of the questions indicate that this corn may present adverse effects on human or animal food.
9. There is no risk of Bt11 corn to execute or promote invasion of uncultivated areas.
10. B. thuringiensis may be considered the biological agent of greatest potential for the control of forests, agricultural plague-insects, and vector of diseases, thanks to the specificity of endotoxins to insects and targetinvertebrate, and its innocuousness to vertebrates and environment, including benefic insects and natural enemies, making this agent a key-component in strategies of integrated handling of plagues.
11. B. thuringiensis cultures are registered in the National Health Surveillance Agency – ANVISA under different formulations for the application in 30 kinds of vegetable cultures for food use.
12. Bio-pesticides based on toxin are widely used as an alternative to chemical insecticides in terms of safety to non-targeted organisms, and when the development of resistance to chemical insecticides occurs.
13. Meaningful differences were not observed between the populations of ladybugs, Carabidae, cincidelidae and spiders, as well as parasitoid of H. zea, Trichogramma sp., when plants Bt11 are compared to their isogenic lineage not genetically modified.

14. One of the advantages of transgenic plants resistant to insects expressing genes that codify endotoxins, or microbial preparations, when compared to chemical insecticides, is the high specificity to target-species.
15. The use of Bt technology in Brazil may contribute for the reduction of the use of insecticides, and, consequently, reduce the impacts of the use of such agro toxics in the environment, in human and animal's health, and it may also indirectly help on the preservation of nontargeted organisms populations and benefic insects, facilitating the integrated handling of crop plagues.
16. The use of genetically modified plants resistant to insects present positive repercussions also in the aspects related to the acquisition and use of chemical insecticides, to meaningfully reduce the pollution provoked by industrial rejects, and by the use of water used on pulverizations, besides avoiding man, food, rivers and springs contamination deriving from the use, transportation and storage of insecticides.
17. The coexistence among cultivations of conventional corns (improved or creoles) and transgenic cultivations of corns is possible from the agronomic point of view, and one should observe the disposition on Normative Resolution No. 4 of CTNBio.
18. Comments, opinions, suggestions and documents resulting from the Public Hearing that took place on March 20th, 2007 did not present relevant scientific fact, substantiated by scientific evidences that compromise environmental safety of human and animals' health of corn Bt11.
19. Attachment III of Cartagena Protocol about Biosafety (Decree 5.705, of February 16th, 2006) says that risks associated to live organisms modified, or to products derived from them, to wit, benefited materials that have as origin a live modified organism, containing new detectable combinations of replicable genetic material obtained through the use of modern biotechnology, should be considered on the context of risks presented by the nonmodified receptors or parental organisms in the probable receptor environment.
20. The historical use of this transgenic variety in the world reveals a great accumulation of trustworthy scientific information that indicate that this variety is as safe for the environment, and for human and animal health, as the varieties of hybrid corns that have been being used.
21. After ten years of use in different countries, no problem was detected for human, animal's health, or to the environment that may be attributed to transgenic corns. It is necessary to emphasize that the lack of negative effects resulting of corn transgenic plants does not mean that they

may not happen. Zero risk and absolute safety does not exist in the biologic world, although there already exist an accumulation of trustworthy scientific information and a safe background of ten years of use that allows us to declare that corn Bt11 is as safe as conventional versions. Thus, the petitioner should conduct monitoring of postcommercial release on the terms Normative Resolution No. 3 of CTNBio.

Before the foregoing, and considering the international criteria accepted on the process of risk analysis of genetically modified raw-material, it is possible to conclude that Bt11 corn, derived from MON810 lineage, is as safe as its conventional equivalent.

CTNBio thinks that the cultivation and consume in commercial scale of Bt11 corn are activities that do not potentially cause meaningful degradation of the environment or aggravations to human and animal health. The use restrictions of the GMO in analysis and its derivatives are conditioned to disposition on Normative Resolution No. 03 and Normative Resolution No. 04 of CTNBio.

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Walter Colli
President of CTNBio
Divergent Vote:
CTNBio's member, Dr. Rubens Onofre Nodari (Environmental Permanent Sector Sub-commission) voted contrarily to the commercial release of Bt11 corn.
The reporter Dr. Fábio Kessler Dal Soglio (Vegetable Permanent Sector Sub-commission) issued contrary opinion to this product approval considering the following points:
1. Problems on the characterization of the genetic transformation event;
2. Insufficient demonstration of safety of Bt11 corn or human and animal consume, and effect on the environment of Brazil;
3. The social and cultural importance of corn in Brazil and negative consequences of the release of transgenic varieties over these dimensions of the Brazilian Rural development, going against the Brazilian legislation of protection of intellectual property of traditional communities and indigenous people;
4. The observation of the Precaution Principle, in accordance with Law 11.105, for the certainty that the release of the transgenic varieties of corn will cause direct impact in traditional, local and creoles varieties of corn, important component of the Brazilian biodiversity, harming, then, the environment.

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