

461/2015

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:



**Ministry of Science Technology and Innovation
MCTI**

**National Biosafety Technical Commission
CTNBio**

Office of the Executive Secretary

SPO, Área 05, Quadra 03, Bloco B

70610-200 Brasília, Distrito Federal, ☎ +55 61 3411 5634 • 📠 +55 61 3317-7475 • secretariactnbio@mcti.gov.br

**Ministério da
Ciência, Tecnologia
e Inovação**



Technical Opinion no. 4408/2015

Proceedings: 01200.000202/2014-71

Applicant: FuturaGene do Brasil Tecnologia Ltda.

CQB: 325/11

CNPJ: 12.777.326.984/0001-09

Address: Avenida Dr. José Lembo 1010, Jardim Bela Vista, Itapetininga, SP

CIBio Chairman: Eduardo José de Melo

Title of Application: Commercial Release of genetically modified eucalyptus – Event H421

Meeting: 181st CTNBio Regular Meeting, held on 04.09.2015

CTNBio, following examination of a request for commercial release of genetically modified eucalyptus, was favorable to the **GRANTING** of the request in the terms of this technical

opinion.

FuturaGene do Brasil Tecnologia Ltda. requested CTNBio to issue a technical opinion on biosafety of a genetically modified eucalyptus to increase the volume of wood for the purpose of release in the environment, marketing, consumption and any other activities related to the GMO and progenies derived therefrom. Event H421 on hybrid *Eucalyptus Grandis x Eucalyptus urophylla* expresses two proteins in all tissues of the plant. Protein Cel1, the gene of which comes from *Arabidopsis thaliana*, promotes faster growth of the plant. Protein NPTII confers resistance to aminoglycoside antibiotics (neomycin, kanamycin, etc.), which is important in the transformant selective process. Eucalyptus is not a native species in Brazil, has no center of secondary diversity within the country, nor sylvan relatives with which it may cross, failing, besides, to present invasiveness in areas apt for planting of the species. In addition, eucalyptus is pollinized by insects that typically do not transport pollen to very large distances. Transportation by the wind never moves the pollen over one thousand meters. In this context, all applicable concerns were deeply analyzed regarding direct risks of introducing this GMO in the environment and brought to light in documents forwarded to CTNBio, through active search of publications, by the applicant, by participants of the public hearing and by independent publications. The most relevant final examination was conducted in the *Apis mellifera* bee. Hypotheses linking identified harms to damages were built and the conclusion was for existence of insignificant risks only.

CTNBio examined reports submitted by applicant and the scientific independent literature. Scientific studies conducted to assess biosafety, agronomic and phenotypic characteristics and part of this GMO risk assessment, included literature on regions representative for the culture of eucalyptus in the Brazilian territory.

Analyzed the data, CTNBio reached the conclusion that there are no restrictions to the use of this eucalyptus and derivatives thereto. Pursuant to Article 1 of Law no. 11460, of March 21,

461/2015

2007, “research and cultivation of genetically modified organisms in indigenous lands and conservation area units are forbidden”. Regarding the reach of Article 14 of Law no. 22205/05, CTNBio considered the request compliant to applicable rules and legislation targeted to secure biosafety of the environment, agriculture and human and animal health.

TECHNICAL OPINION

I. GMO Identification

GMO designation:	Event H421 of eucalyptus
Species:	Hybrid of <i>Eucalyptus grandis</i> x <i>Eucalyptus urophylla</i>
Inserted Characteristics:	Increased growth and production of wood
Method used for Insertion:	transformation mediated by <i>Agrobacterium tumefaciens</i>
Proposed use:	Planting, essays, tests, seeding, crossing, transportation, storage, discarding, marketing and any other activities related to the use of eucalyptus plants.
Proteins expressed:	Cel1 (β -1,4 endoglucanase) and NPTII (neomycinphosphotransferase type II)

II. General Information

Event H4421 in hybrid *Eucalyptus grandis* x *Eucalyptus urophylla* expresses two proteins in two tissues of the plant:

- (a) Enzyme β -1,4 endoglucanase (Cel1), a product of gene *cel1* isolated from *Arabidopsis thaliana* (The *Arabidopsis thaliana* Information Resource, 2014). Cel1 is a glycosil hydrolase (β -1,4 endoglucanase) that links to the non-crystalline cellulose and to

xyloglucans promoting relaxation of the cell wall during the phases of expansion and elongation of the plant cell. Protein Cel1, formed by 492 amino acids has a molecular weight of 54 kDa, is highly conserved among plants, including those plants used as food (rice, maize, soybean, beans, potato, cucumber, etc.). Therefore it is a widely distributed protein in nature, consumed as food by different parts of the food chain (from insects to mammals). The protein has a fast turnover and its intracellular construction is only significant in young tissues.

- (b) Protein NPTII (neomycin phosphotransferase type II), of 52 kDa, is an enzyme that grants resistance to certain antibiotics such as neomycin and kanamycin. The enzyme is the product of gene *nptII* of *Escherichia coli* (ISAAA, 2014) and is expressed in all tissues of the plant. It shall be noted that these antibiotics are not used in agriculture for disease control.

Summarizing, the genetically modified eucalyptus displays one functional copy for the expression of protein Cel1, belonging to the family of glycosyl hydrolases, influencing wood growth. This way, one expects an increase in the volume of biomass per area. Gene NPTII was inserted as a marker, granting resistance to antibiotics of the aminoglycoside spectrum. Both proteins are expressed in different biochemical pathways, with no hint of interaction between them.

The insertion proved to be stable along the crossings in progenies analyzed through PCR techniques, displaying a pattern typically Mendelian. The genetic transformation failed to change the botanical standards of eucalyptus and, for being an exotic species, it is not likely that that plant may change its silvicultural behavior in the different Brazilian biomes.

Cel1 and NPTII protein expression quantification was conducted in tissues of eucalyptus H421 produced in laboratory (tissue culture) and in samples collected in field essays. Results of the

essays show that genes *cel1* and *nptii* are transcribed in low proportion in all tissues analyzed (culture tissues, young and mature leaves, branches, roots, pollen grains). Due to their high identity and similarity with endoglucanases present in eucalyptus, it was impossible to obtain antibodies sufficient to protein Cel1, destined to laboratory essays, besides the fact that the endoglucanase displays concentration below the limit of quantification of available methods, except for samples coming from tissue cultures. This way, differential semi-quantitative analyses were conducted through Western blot, using protein extracts of event H421 and of the conventional control clone SP530, with antibodies that link to the total of endoglucanases, a group where enzyme Cel1 is inserted. The average endoglucanase concentration in young leaves was 27,4 and 22,87 µg/g of fresh tissue for the genetically modified event and for the SP5300 clone, respectively. Regarding mature leaves collected in the fields, the endoglucanases displayed concentration of 18,87 µg/g of fresh tissue for the genetically modified event and 22.33 µg/g for the SP530 clone. Average amounts of protein NPTII expressed (in ng/g of fresh tissue) in the loci assessed were 5.3 in pollen, 16.7 in young leaves, 22.2 in mature leaves and 27.2 in roots. Endoglucanase concentrations were below 0.35% of total proteins extracted from tissues of H421 eucalyptus, less than 0.05% in relation to the total composition of tissues of the genetically modified event, while concentrations of NPTII never reached 0.003% of total proteins extracted. No significant difference was measured in Cel1 expression of the transformed material (event H421) given the low expression of the gene.

According to data shown in the application documents, and in line with what would be expected in what regards the transformation with the inserted genes, the transformed variety of eucalyptus fails to display changes in behavior, except the faster growth resulting from expression of gene *cel1* and resistance to certain antibiotics of the neomycin group, due to expression of gene *nptii* (which alters the behavior of the plant in laboratory only, during the selection process of transformants).

461/2015

This eucalyptus shall have the same use than the conventional plant. Cultivation areas shall be among those that show good conditions for the plant.

Pursuant to Law 11105/2005, CTNBio is committed to assessing the risks that genetically modified organisms may present to the environment, biodiversity and human and animal health. Therefore, the comprehensive protection targets designated by the Brazilian Federal Constitution are reflected in this law. CTNBio has the duty of identifying what constituents of the environment may be affected by the organism studied and use the results to develop risk hypothesis and draw conclusions on the product safety. For this purpose, the guidelines of risk assessment set by the Biodiversity Convention (<http://www.cbd.int/doc/meetings/bs/mop-06-13-add1-en.pdf>) and the Office of the Australian Gene Technology Regulator (OGTR) ([http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/\(Content/raffinal5-toc\)](http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/(Content/raffinal5-toc))) are valuable and complete the approach prescribed in CTNBio Ruling Resolution no. 05.

Genetically modified eucalyptus event H421 is being assessed under containment since it was obtained and in fields in the case of Brazil, in planned releases into the environment passed by CTNBio since 2006, showing that adequate management and intensive cultivation can yield wood for both, production of biomass for energy and production of fibers.

Experiments were conducted in four Brazilian regions where this eucalyptus is likely to be cultivated: Angatuba, São Paulo; Araraquara, São Paulo; Caravelas, Bahia; and Passagem Franca, Piauí. In these regions, event H421 was compared to conventional eucalyptus SP350. Experiments were installed with six repetitions in each field and were assessed in cycles of 2 to 3 years when targeted to production of energy biomass and in a range from 5 to 7 years to production of cellulose and paper. Besides assessments of performance and management, these experiments were examined for environmental risk in the context of the application for commercial release.

III. Aspects Related to Human and Animal Health

Futuragene genetically modified eucalyptus event H421 produces more wood because of gene *cel1* isolated from *Arabidopsis thaliana*, which codes for expression of enzyme β -1,4 endoglucanase (Cel1), one glycosyl hydrolase that links to non-crystalline cellulose and xyloglucans promoting relaxation of cell walls during the phases of expansion and elongation of the plant cell. Protein Cel1 is a polypeptide of 492 amino acids with an estimated molecular weight of 54 kDa.

Event features in addition gene *nptII*, which codes for protein NPTII (neomycin phosphotransferase type II), having resistance action on the action of antibiotics such as neomycin and kanamycin. Enzyme NPTII is a modified protein of *Escherichia coli*. Gene *nptII* used in transformation of plants is derived from transposon Tn5 of *Escherichia coli* (Beck *et al.*, 1982), an enterobacterium that is present in the intestinal flora of humans. Protein NPTII is produced by different prokaryotic microorganisms found ubiquitously in the environment, both in aquatic and terrestrial habitats and in human and animal intestinal microflora. The action of protein NPTII is well characterized and culminates with inactivation of aminoglycoside antibiotics, such as neomycin, gentamycin, paromomycin and kanamycin A, B and C. Protein NPTII is degraded in human and animal gastrointestinal tract. The use of selection marker genes, such as gene *nptII* is essential in the selection of cells transformed in the process of genetic modification (Horsch *et al.*, 1984; DeBlock *et al.*, 1984).

Molecular analysis (Southern blot, sequencing and PCR) depict the insertion of a single copy in the expression cassettes of genes *cel1* and *nptII* of the eucalyptus genome, confirmed by the Mendelian segregation pattern in progenies. Sequencing of event H421 showed that constructs present in the vector were preserved in the transformation and that there was neither gene disruption nor structure disruption of the parental eucalyptus genome expression. Stability of integrated DNA was shown by PCR, where the constructs inserted in the DNA, as well as their

insertion loci, were maintained in the tested progenies, obtained in controlled crossings with different species and conventional commercial varieties.

Studies show that there was insertion of a single functional copy of genes *cel1* and *nptII* expression cassettes in a single locus of the eucalyptus genome.

Proteins expressed in eucalyptus H421 do not display toxic activities on other organisms. *In silico* assessment of toxicity and allergenicity with renowned databanks failed to indicate that proteins Cel1 and NPTII are similar or homologous to known allergens and toxins.

Expression levels of proteins in plant tissues (culture tissues, young and mature leaves, branches, roots, pollen grains) as well as in products of honey bees (honey, pollen balls) showed to be quite low. Concentration of endoglucanases in young leaves of event H421 was 27.4µg/g of fresh tissue, as against 22.67µg/g of fresh tissue in conventional clone SP530. In mature leaves, endoglucanases showed concentration of 18,87µg/g of fresh tissue in eucalyptus H421 and 22.33µg/g of fresh tissue in the conventional isolate of mature leaves. Average quantities of protein NPTII expressed (in µg/g of fresh tissue) were 5.3ng/g in pollen, 16.7 in young leaves, 22.2 in mature leaves and 27.2 in roots.

IV. Safety to Human and Animal Health

Eucalyptus is an arboreal species with no known record of use as food in Brazil and other countries, neither directly nor processed, is not consumed by humans or animals that serve as food to man or other domestic animals.

Arabidopsis thaliana, the donor organism of the *cel1* gene, is not a species possessing evidence of being pathogenic or toxic to human and animals and is not customarily allergenic, being analytic and toxicological tests unjustifiable. *Arabidopsis thaliana* became a model plant for genetic and plant development studies by having its genome entirely sequenced. The

organism is a member of the Brassicaceae family, which includes species cultivated and consumed in human food such as cabbage, broccolis and radish, all with a safe record of use.

Protein Cel1 can be found, in very similar forms and with strong identity, in a number of raw (cucumber, tomato, grape, apple) and processed food (cocoa, soybean, maize) consumed by humans and animals. In addition, some hydrolases, such as β -glucanases of *Aspergillus niger* and *Bacillus subtilis*, have been used for a long time in alimentary processing and are included in the food category; noxious effects have not been documented (Pariza and Foster, 1983; in Franck Oberaspach and Keller, 1997).

Gene *neo* (or *nptII*) and its product, enzyme NPTII, has been largely used as a selection marker in transforming human, plant and animal cells and in experiments of gene therapy in humans. The protein has no toxic effect to living beings, is naturally present in the environment and found in microorganisms in foodstuff or in food that colonize the human digestive system (Fuchs *et al.*, 1993). The European Food Safety Authority – EFSA, the Australian Food Safety Authority – OGTR) and the United States Food and Drug Agency – FDA reached a conclusion that the use of gene *nptII* as a selection marker in genetically modified plants – GM (and foodstuffs or rations derived thereto) fails to represent a risk to human and animal health and to the environment. CTNBio has already passed commercial releases of events containing this same selection marker.

Risk assessments were conducted in Piauí, Bahia and São Paulo. Centesimal composition analyses show bromatological equivalence between H421 eucalyptus tissues and conventional eucalyptus. Phenotype and ecological interaction assessments conducted in H421 eucalyptus showed that this genetically modified event has no potential different from its conventional equivalent to be an invading plant, or even to disseminate or perpetuate its genes or to impact other plant or animal organisms in the environment.

461/2015

Toxicity analyses conducted by exposure to leaves and pollen to indicator organisms showed that H421 eucalyptus fails to cause adverse effects on aquatic species, such as fish (*Danio rerio*) and micro-crustaceans (*Daphnia similis*), and land species, such as bee honey (*Apis*) and different species of native bees with no sting, granting these species the same conditions of exposure granted by cultivation of conventional eucalyptus. Toxicity assessment in honeybees was conducted for distances of 400m, 1km and 85km of the cultivation locus.

Physical and chemical analyses of different honeys produced in areas featuring genetically modified trees and in area planted with conventional trees showed that there is no significant difference for all parameters analyzed. Total protein content of honeys analyzed, both coming from areas with transformed trees (0.43%) and coming from conventional area (0.33%) are very low, being only part of the protein due to presence of pollen in honeys. Cel1 protein concentration expressed in event H421 in honey and pollen balls is so low that detection methods available for its measurement were not able to quantify it. For NPTII, concentrations were among the lowest levels found when compared to other tissues of the plant, such as leaves, roots and pollen. A pertinent observation is that man and animals do not absorb proteins, but amino acids and, therefore, concerns related to toxic effects in the consumption of the transgene's proteins in such tiny amount are unfounded.

There is another concept that we may introduce, the TTC – Threshold of Toxicologic Concern. The concept, found in details in Kroes *et al.*, 2004, is being increasingly used by the European Community, specifically by the following committees: Scientific Committee on Consumer Safety (SCCS), Scientific Committee on Health and Environment Risks (SHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). According to the TCC concept, substances that are present in foodstuffs may be held as safe up to the following doses:

- 1.5µg/individual: General threshold. Substances with positive Ames test or structural

461/2015

alerts shall be assessed on a case by case basis;

- 1.5µg/individual/d: Threshold for chemical substances with no structural alert to carcinogenicity or negative results in mutagenicity tests (Ames' Test);
- 45µg/individual/d: Threshold for chemical substances with no structural alert to carcinogenicity or negative results in mutagenicity tests (Ames' Test) and LD₅₀ (median lethal dose) > 1.000 mg/kg of body weight.

Considering the negligible amount of pollen proteins that are present in honey, we may, in this case, use TTC in a way where there is not necessary conduct this product test in animals.

Independent analyses conducted in the Project "*Criação do CDA Eucalyptus: Centro Colaborador em Defesa Agropecuária para a Biossegurança Relativa a Plantas GM de Eucalipto*" ("Establishment of CDA *Eucalyptus*: Collaborating Center in Agriculture and Livestock Defense for Biosafety Related to eucalyptus GM plants"), a document prepared by the Agriculture and Livestock Defense Secretary (SDA), the Ministry of Agriculture, Livestock and Supply (MAPA); jointly with the National Scientific and Technologic Development Council (CNPq), the Ministry of Science and Technology (MCT); between December, 2008 and June 2012 with the purpose of developing and adjusting protocols of assessment, guidelines for comparison and analyses services related to biosafety of plants and plant genetically modified derivatives, strengthen conclusions obtained by applicant in assessing the biosafety of H421 eucalyptus. Among the results of the Project, comparing genetically modified and non-genetically modified derivatives the following shall be noticed: (i) equivalence of composition in essential oils extracted from leaves and their *in vitro* effect on phytopathogenic fungi; (ii) evidence of equivalence (absence) of *in vitro* toxic effects of honey and foliar extracts on human colon cells and on the development of bees (*Apis mellifera*) larvae, as well as on the anatomy of adult insects of the species; (iii) establishment of native bee small colonies in laboratory for testing the toxicity of

461/2015

derivatives (pollen and honey) of genetically modified and non-genetically modified plants; (iv) verification of equivalence of honey samples produced in genetically modified and non-genetically modified areas.

The data found in the project turned possible the following conclusions:

- Eucalyptus event H421 was molecularly characterized and maintenance of integrity of the inherited genic construct; to wit, that it is a single and intact insert, integrated in a single locus of the eucalyptus genome, where all the gene elements remained intact, and no locus of plasmid pB1121 present in the transformation event generated;
- The eucalyptus is not used in human or animal food, though the inserted proteins are easily digested;
- There is no indication that proteins Cel1 and NPTII present in H421 eucalyptus may cause allergy or intoxication in humans and animals;
- Expression levels of exogenous proteins expressed in event H421 are extremely low in plant tissues and undetectable in honey and pollen balls of the beehive.
- No pleiotropic (multiple effects of a single gene) or epistatic (presence of one allele that inhibits the action of another one) effects were identified in eucalyptus H421; being the expected characteristics of increased production of wood the only observable effect.

Following the above observations, the conclusion is that from the viewpoint of human and animal health, eucalyptus event H421 is as safe as its conventional equivalent.

IV.1 Gene flow and Honey

One issue raised during the public hearing held to discuss eucalyptus H421 relates to production of honey, since eucalyptus, according to a recurrent argument of the audience, is “the main source of nectar and pollen for Brazilian apiculture”. Two papers present studies on eucalyptus gene flow, that is to say, on the distance the bees can take the pollen: Pacheco *et al.* (1996) and Campinhos *et al.*, (1998). In the first studies were conducted up to a distance of 300m and reached a conclusion that “*Apis mellifera* L. is effective in promoting crossed pollination in *Eucalyptus saligna*, being greater the activity at a distance limited to 100 meters of distance, gradually decreasing up to the studied distance of 300 meters”. The second study uses alloenzymes as markers to estimate the degree of natural hybridization between *Eucalyptus grandis* and *Eucalyptus urophylla*. Genotypes were found that were not present in the seed nursery, to wit 14.4% of the genotypes came from populations localized 400m distant from the study area. Extrapolation is the use of regression models to estimate the value of the dependent variable (response) for a given value of the independent variable (explanatory) that are outside the observed values of the explanatory variable. This leads to unreliable and sometimes ludicrous values (Larose & Larose, 2014). Even using the extrapolation of the regression model presented in the study by Pacheco *et al.* (1996) and by Campinhos *et al.* (1998), the distances in which the gene flows equal zero are 756m and 467m, respectively. The study submitted by applicant assessed the gene flow up to a distance of 1592m, reaching a conclusion that at a distance of 0 (zero) meter the flow was found to be 30.8%. This value keeps decreasing with the distance, with observations of 14.6% at 10m, 13.1% at 25m, 11.5% at 50m, 11.2% at 100m, 5.0% at 200m, 5.4% at 300m 1.5% at 400m, tending to 0,0% at a distance nearing 600m. This value is close to the ones extrapolated of published papers.

In 2013, Brazil has exported about 16 thousand tons of honey, and about 78% of this total was exported to the United States of America (ABAMEL, 2014). There is a concern about the loss of honey market value due to presence of transgenic material in the product. Article 2 of Decree no. 4680, of April 24, 2003 defines, in the marketing of foodstuff and alimentary ingredient

461/2015

goods targeted to human or animal consumption that either contain or are produced from genetically transformed organisms, presence of GMO above one percent of the good this transgenic nature of the product shall be informed to consumers.

However, analyses conducted in honey collected in beehives within the tillage of H421 eucalyptus, Cel1 protein was not detected and the content of protein NPTII was 3.9% of honey, which is way below the 1% defined in the Decree 4680 for the product to be labeled as transgenic. Law no. 10831, of December 23 2003, regulated by Decree no. 6323, of December 27, 2007, making provisions on organic agriculture, considers, in its Article 1, elimination in the use of genetically modified organisms in the agriculture and livestock organic production system. Regulations on production of organic honey are defined in Ruling Instructions no. 46, of October 6, 2011, and no. 17, of June 18, 2014. Article 8(IX) of RI 46 it is defined that *Plano de Manejo Orgânico*, the Organic Management Plan shall include measures to prevent and mitigate the risks of external contamination, including GMO and derivatives. However, RI 17, in its chapter on honey production, particularly in Article 21(V) defines that the organic production system of honeybees shall use “only non-genetically modified honeybees”. The 20th Edition of *Diretrizes para o Padrão de Qualidade Orgânico IBD – Associação de Certificação Instituto Biodinâmico*, the Directives of IBD – Biodynamic Institute Certification Association Organic Quality Standard, of August, 2014, an organization responsible for over 100 certifications of organic honey producers, in subparagraphs 4.2 and 4.3 defines that only products containing not less than 95% of certified ingredients of organic agriculture may use the seal *IBD CERTIFICAÇÕES Orgânico*, the Organic IBD CERTIFICATIONS. Special labeling requirements apply to products that use in their composition at least 95% to 70% of organic raw materials. The products’ mail labels may display the notice “made with organic ingredients”. The Animal Health Sector Committee, of the National Organic Standards Board, the body that recommends to the United States of America Department of Agriculture the rules to be followed in the production of honey, in a document dated as of December 27, 2010,

does not restricts the use of transgenic material. Therefore, the concerns raised in the public hearing on destination of honey in Brazil are apparently unfounded.

Considering that the a route to possible damages is very unlikely and that the expected damage is small, one may conclude that the risk for bees and honey production is negligible.

V. Environmental Aspects

In all procedures for release into the environment, the constituents quality of soil, quality of water (yet no the abundance, which depend on human use), valued organisms in agriculture, iconic species and native species with which the genetically modified organism may produce a fertile offspring are regarded as biodiversity representatives. One shall additionally bear in mind the cross border movement of the living GMO whenever this GMO may affect the biodiversity of the host country.

In order for these constituents, still very comprehensive, to be translated in *final assessment points*, indispensable to the establishment and tests of risk hypothesis, it becomes necessary, at the outset, to extract from the eucalyptus biology and from observation of phenotypic changes linked to the transformation of the event, the elements that would enable discarding certain constituents representative of biodiversity. Eucalyptus biology is conveniently described by OECD (2014), among other scientific literature of comparable value. Other perils may be derived from the public perception of risk and are commented in this opinion.

Eucalyptus originated in Australia, with a number of secondary diversity center in Asia. There are no species sexually compatible with eucalyptus in Brazil. The plant is pollinized by insects and, though the pollen may be transported by wind, this is not the species reproduction mechanism. Insects that pollinize eucalyptus in Brazil are mostly bees, the flight range of which is lower than 1,000m. Each mature plant is able to produce large quantities of seeds, yet eucalyptuses are commercially propagated through seminal or clonal seeding and not by direct

seeding in the fields.

Although eucalyptus exhibits certain characteristics of invading plant, in general it fails to behave accordingly (Silva *et al.*, 2010b). The hybrid employed in this event was never reported to be an invader. Only the *Eucalyptus grandis* species may behave as an invading plant, though it does not exhibit this behavior in Brazil. According to *Comunicado Técnico 83*, the Technical Notice 83, by Embrapa Florestas (Embrapa, 2002), eucalyptus was introduced in Brazil in 1904, with the initial purpose of meeting the demand for firewood, posts and railway sleepers for the Brazilian Southeastern region. Later, in the fifties, eucalyptus came to be used also as raw material in the production of cellulose and paper, charcoal and fiber or particle sheet. It shall be stressed that after over one hundred years of cultivation in different Brazilian edaphoclimatic conditions, eucalyptus failed to exhibit invasive behavior.

Eucalyptus is not used as foodstuff in Brazil, and leaves, branches and seeds of the plant are not consumed by protected animals of the Brazilian fauna. Honey made by bees based on eucalyptus flowers is collected and marketed in Brazil and also exported. This honey may contain small amounts of proteins NPTII and Cel1, depending on presence of the transgenic event's pollen. The alimentary innocuousness of such honey was assessed by CTNBio. The issue of impact on pollinating insects imply selecting a final point of assessment. Given its abundance and importance in pollinating commercial species, the bee *Apis mellifera* was elected as the final assessment point.

Areas where eucalyptus is planted are customarily surrounded or bordered by legal environment conservation areas and, in some cases, with collections of surfacing waters. Part of the material coming from eucalyptus trees and also exudates may reach the soil, subsoil or in surrounding collections of water. This is already the case in conventional eucalyptus tillage and there is no reason to imagine that protein Cel1 if *Arabidopsis thaliana*, present in tiny amounts in the eucalyptus plant tissues, maintained within plants, may negatively impact in

461/2015

the quality of soil and waters. Protein NPTII shall be also promptly degraded in the environment and its antimicrobial action could only be felt in high concentrations on an environment rich in bacteria. Degradability of the two proteins and their impact on different indicator organisms have already been studied, especially for protein NPTII, including by the applicant.

A comparative analysis of biomass degradation was conducted and failed to indicate difference in speed and amount of residues between the GM eucalyptus and its conventional counterpart.

Applicant submitted studies of indicator species, such as two aquatic species (fish and Daphnia), a soil decompositor (earthworm), microorganisms and species of beneficial insects (such as honeybees and stingless native bees). Honey decomposition studies failed to show significant differences and also to find any difference in colony decomposition, being therefore unlikely that the inserted proteins may cause any impact on such insects, as already reported on expression, quantification and effects of the protein.

It shall be noted that applicant submitted plant material to different independent laboratories, belonging to the project “CDA Eucalyptus: Collaborating Center for Agriculture and Livestock Biosafety Related to Eucalyptus Genetically Modified Plants”, sponsored by MAPA, MCTI and CNPq, which forwarded data on:

- Proof of morphologic equivalence and anatomic of pollen, seeds, flowers and fruits; germination of pollen and seeds and development of seedlings;
- Composition equivalence in essential oils of leaf extracts and their effects on *in vitro* phytopathogenic fungi;
- Proof of equivalence (or absence) of toxic effects in honey and leaf extracts on *in vitro* cells of human colon and on development of bee larvae (*Apis mellifera*) as well as on

461/2015

the anatomy of adult insects of the species;

- Establishment of mini-colonies of native bees for toxicity of derivatives (pollen and honey) of GM and non-GM plants;
- Proof of bromatologic equivalence of samples of honey produced in areas of GM and non-GM trees;
- Definition of conditions for detection of GM samples by PCR and qRT-PCR;
- Beginning of comparative studies on transcriptomic and proteomic with a view to determine equivalence in transcripts and peptides in leaves and branches de GM and non-GM Eucalyptus.

No negative effect was recorded. Based on the characteristics of the event, the eucalyptus biology and the intended use of the GM variety, one may conclude that the only protection target that could suffer a direct impact from the GMO other than the impact caused by the conventional variety is the pollinator. As shown, proteins inserted, their degradability and effects, quantified in honey and plant tissues, evidence that event H421 has no identifiable effect on *Apis mellifera*.

VI. Public Hearing

During the public hearing, several other threats were brought by the perception of actors in the Brazilian and international scenario. Most hazards bear no relation to the direct GMO impact, yet supposedly they do bear this relation to the monoculture or the technology associated to the event.

Among the issues raised in explanations, the possibility of a gene that determines increase in wood productivity causing an effect on plant metabolism was examined. It must be

emphasized that after assessment of the event for several years, it became possible to say that changes are insignificant and, moreover, that no change in characteristics linked to behavior of the plant was observed, being this an important factor that emerged during the selection phase of elite events for commercial release, similar to what happens with elite events obtained by classical genetic improvement.

Another point discussed was the fact that essays displayed an absence of competitive differential (and consequently invasiveness). This way, concerns were voiced around ability to germinate and establishment of spontaneous, voluntary plants, coming from H421 and its isogenic plant. Questions were raised on possible contempt of ability for development higher than that of H421, after established (characteristics directly related to competitiveness) and on existence of data accounting for H421 being no longer competitive. Answering to this question, it was recognized that invasiveness depends on a series of plant characteristics, but rapid growth is not included among these traits. Though a plant featuring faster growth may be more competitive, it needs invading potential to start with, which is not the case of the hybrid eucalyptus in study. It shall be noticed that no botanical characteristics of eucalyptus was changed, therefore, its reproduction mode and phenological behavior remain unchanged. Two essays on eucalyptus invasiveness through seeds produced in commercial tillage were published (Silva *et al.*, 2011a and Silva *et al.*, 2011b). The first was conducted in 18 different regions where eucalyptus is planted (Minas Gerais, São Paulo, Rio Grande do Sul and Espírito Santo). The results show that inside the plot and on the adjacent area, that is to say to 10m distant from the plot, the number of plantlets found ranged from 37 to 157, respectively. Authors concluded that, though the plants have germinated, eucalyptus plants apparently fail to establish in conditions found in commercial cultivars. Besides, the management system used, keeping grazed the inner part of the plot and the surrounding area, as well as the rotation used, prevent this establishment. Another published paper studies invasiveness of commercial eucalyptus seeds in fragments of native forests, previously artificially seeded with

Eucalyptus grandis or a hybrid of *Eucalyptus grandis* and *Eucalyptus urophylla*. On the 270th day after sowing, no eucalyptus plantlet was recorded in the three loci studied. Applicant also conducted a study reported in the dossier submitted, on two areas under Planned Release into the Environment, located in Angatuba, São Paulo; and Caravelas, Bahia. The study show that there was no significant difference between the percentage of seed germination for eucalyptus H421 and the percentage of germination for the conventional eucalyptus, in field conditions. Essays shown by applicant were conducted in important culture regions. Given the lack of changes on the genetically modified eucalyptus phenological behavior, the impact on Brazilian ecosystems is not likely to be different from the conventional eucalyptus.

Transformation of wood quality for greater content of cellulose versus productivity for cellulose production was assessed. The question was whether the generated data actually support the request for commercial release of H421. Besides, it was questioned whether there are important experiments to be completed and, in the affirmative, whether they could compromise the decision taken. Applicant clarified that the data brought by it and the previously available information on eucalyptus, genes employed and their safety are sufficient to reach a scientifically sound decision. Results extracted from planned releases are among the date brought by applicant, though some results were not completed by the date of submission of the dossier. Consistency of data was, nevertheless, assessed, with special emphasis on data related to the impact on bees, which are important to support the assessment of the risks to a protection target that may actually be impacted. Other data came to support the hypothesis, fully satisfied, that there are no unanticipated effects derived from the transformation operated in this event.

It is worth stressing that at the Conference of Parties to the Convention on Biological Diversity - COP-9 (Decision IX-5), an agreement was reached between the parties that the use of transgenic trees should be approved after studies based on science, under domestic

legislations. Brazil, in this regard, complied with the terms of the agreement, through field and laboratory tests considering the proposed use of the eucalyptus.

The effect of *nptII* was studied in bacteria present both in the intestinal tract of bees and of human beings (in the consumption of honey). It was noted that in other countries antibiotics are regulated for use in controlling diseases in bees. In Brazil, this use is not yet allowed. However, presence of these substances in beehives could select resistant diseases, which could lead to a future tragedy in case a severe disease turns epidemic, a case in which control would be more difficult. As shown, antibiotics for which protein NPTII is effective are not in clinic use. Besides, the protein is easily degradable and expressed in tiny amounts in plant tissues.

Another theme discussed in the hearing relates to the ideal time to reach a conclusion on whether there are not negative or unwanted interactions. Even before experiments start and time length are inferred, performing a risk assessment is mandatory. Risk assessments preceded all planned releases and in part supplied data for the commercial release risk assessment. Considering that the only concrete harm to a protection target that represents biodiversity is represented by consumption of GM pollen by pollinators, the time for conclusion on risks may be small and indeed it was extrapolated in this case. However, the safety of inserted proteins, the use context of eucalyptus and the data analyzed up to now enable a conclusion on the use of this plant.

Another issue analyzed was whether the tests were conducted in an adequate form to enable a conclusion that there are no negative interactions in bees and in the presence of pollen containing GMO material. The information is relevant, taking into consideration that three times more protein Cel1 was detected in pollen than in other tissues. It shall be stressed that the amount of protein by gram was calculated with tissues of different humidity degrees. Besides, the actual amount of the protein to which a bee may be exposed through pollen is

minute, since the total mass of ingested pollen is very small compared with the mass of nectar or of the bee itself. Besides, protein Cel1 is ubiquitous and one of its isoforms is undoubtedly present in pollen of plants that are part of the bee diet. Summarizing, the most important is that this protein is present in so minute amounts in the adult plant that it is undetectable.

Besides, the point was raised that no studies were conducted in animals (mice and rats) for the purpose of assessing stability on digestion and possible (cito)toxic, mutagenic and teratogenic effects. It shall be noted that this is a commercial release of a novel transgenic organism. It shall be emphasized that eucalyptus is not used as food by protected and iconic sylvan animals and therefore the issue of alimentary innocuousness lacks sense in nutritional assessment, remaining the impact on bees being the main issue. For being highly unlikely, the impact on animals that consume honey in non-managed both inside and outside the planted area was also excluded. Protein NPTII was already extensively assessed and there are no robust reasons to discard any non-negligible risk posed by this protein to human and animal health in concentrations common in typical diets. The amount of protein Cel1, as mentioned above, is undetectable in the adult plant and in a number of other plants consumed. Moreover, the proteins are already distributed in nature, with no record of adverse effects caused by their consumption.

Another important fact discussed in the public hearing was the possible increase on the use of water and agrochemicals given the two years reduction in harvest cycle. It was noted that the cutting time is an issue related to technology and management of the tillage, and has no relation with the GMO direct impact in soil. About the faster water consumption by the plants, if it proves to be the case, there is no reason to expect that this increase may imply a considerable amount in consumption.

According to Lima *et al.* (2012), there is evidence that the increase in forest culture growth rate affects positively the consumption of water. The magnitude of this possible impact is strongly

affected by climate and soil in the planting locations. To ease this impact, the paper recommends using forest management strategies. Among these strategies, according to the authors, encouraging effects come from adequate spacing and percentage of occupation of the hydrographic microbasin, and deliberate inclusion of critical areas as protection. Among the results shown in defense of their conclusions, the authors divide the yearly amount of water of atmospheric precipitation in a microbasin reforested with eucalyptus in two parts, one named yearly production of water (Q) and the other, evapotranspiration in the scale of the hydrographic microbasin. Therefore, atmospheric precipitation water has two different destinations: watercourses and atmosphere. The same as in any other agricultural or forestry ecosystem, water does not vanish and shall be always put to use.

Regarding herbicides, a reduction in their use shall take place, since the plants grow faster, cast shadows on the soil and may produce more rapidly substances that can inhibit the growth of competitive plants (allelopathy), reducing the period in which controlling practices are necessary. These aspects relate directly with culture management rather than with GMO safety. It shall be stressed that this technology is not turned to tolerance to herbicides.

The conventional genetic improvement program of eucalyptus has already produced clones with properties that are similar to the ones of the genetically modified plant. This is due to the fact that the clone used in the transformation has about 10 years, being more modern clones significantly more productive. Therefore, water consumption in this genetically modified culture is not different from consumption in any other garden clonal cultivation.

Some participants argued that the studies also lack scientific trustworthiness when indicating a 20% volume increase in wood with the H421 event, since samples average was leveled up, but the change was in the range of 6% to 20% in different locations and also whether there may be clone varieties currently studied that show the same increment without the transgenes. Effective gain in productivity shall be assessed after the commercial release and has no bearing

in risk aspects. It shall be stressed that this technology may be used in the future to elite clones, with similar or even higher productivity when compared to the growth rates found in this event.

However, this aspect relates to forest management of the culture and not with safety of the GMO currently assessed by CTNBio.

VII. Determination of Exposure and Damage for Identified Hazards

Taking into account that only the impact on pollinizers is a real threat, that is directly related to the protection target recognized by the country Constitution (biodiversity including protection of insects beneficial to the agro-ecosystem, etc.), this technical opinion considers the indispensable elements for risk ranking, according to internationally agreed procedures, to wit: exposure to danger and damage class (or magnitude). This opinion also draws a damage pathway, from the hypothetical ingestion of transgenic pollen by bees to damages to individuals, beehives or populations. Apart from this pathway, the results contained in the dossier make clear that there is no negative impact of genetically modified pollen on bees.

All molecular data indicate that the event in study has only one copy of the insert, which is also present in pollen. The construct is constituted by:

- (1) The coding sequence of gene *nptII* from *Escherichia coli*, which is directed by a NOS promoter and the termination sequence of transcription and polyadenilation derived from the terminating sequence of 3' of nopaline synthase (nos) of *Agrobacterium tumefaciens*;
- (2) The coding sequence of gene *cel1* from *Arabidopsis thaliana*, which is directed by a promoter of the Strawberry Vein Banding Caulimovirus (SVBV), and the terminating sequence of transcription and polyadenilation derived from the termination sequence

of 3' nopaline synthase (nos) of *Agrobacterium tumefaciens*. This T-DNA was inserted into the eucalyptus genome by transformation with *Agrobacterium tumefaciens*, to express genes *cel1* and *nptII*, resulting in the synthesis of proteins Cel1 and NPTII .

Bees transport the pollen to the beehives in different manners. The pollen may also be transported to beehives by the wind. However, pollen is not food for bees, and is ingested together with nectar or honey. The mass of pollen to which a bee may be exposed is very small. Therefore, the amount of proteins Cel1 and NPTII that may be present in the bee diet is many orders of magnitude lower. Protein Cel1 itself is widely distributed in plants and hence, with a record of contacts with humans and animals, mainly through consumption of *in natura* plants. Thus, in the first step of the damage pathway, we may assume that the likelihood of the bee being exposed to significant amounts of the protein is very low. Protein NPTII is of microbial origin and determines resistance to antibiotics, and this is a reason for the emphasis in the protein risk analysis.

In the second step of the pathway it is worth creating a plausible hypothesis on how the protein behaves in the digestive tract of a bee. Same as with pollen itself, the most probable is that the protein is promptly digested, which actually occurs with the majority of proteins ingested by the insect, the digestive tract of which is rich in proteases. Thus, the likelihood that this protein comes to be distributed, in an active form, over the greater part of the digestive tract is very remote.

In the third step we shall question through which mechanism enzyme NPTII could cause a negative impact on the bee. There is no record in the literature that the enzyme would impact the epithelium of the digestive tract of any organism, but it may inhibit the action of some antibiotics that would otherwise be important in the management of bees in an apiary. It shall be kept in mind that the enzyme has no antibiotic action and fails to kill bacteria. The amount of protein remaining after proteolytic digestion within the digestive tract should not be

sufficient to inhibit the action of antibiotics purposely added to the bee diet in apiaries. Then, the likelihood that antibiotic concentrations capable of inhibiting NPTII are present in the digestive tract of bees that come to graze on flowers of genetically modified eucalyptus is also very small.

In the fourth step, it becomes intuitive asking why the apiarist would use the antibiotic that, even in a very low likelihood, could be inhibited by the presence of NPTII. Therefore, the probability that another antibiotic could be inhibited by NPTII is clearly very small. It shall be kept in mind that both neomycin and kanamycin are not used in the treatment of bacterioses in apiaries, being oxytetracycline the preferred drug and, less frequently, some aminoglycosides, anphenicols and sulfonamides (Monteiro *et al.*,2010). The probability that the whole pathway comes to be realized is therefore very remote.

We may now assess the extension of the damage. In case all paths are realized and the bee master could not control an eventual bacterial infection in his or her bees, there would be a loss of the beehives. The same would not take place in the remaining beehives. The, the damage would be restricted to some few beehives previously affected by a bacterial infection. A damage that does not affect a whole population (in this case damage would be restricted to some boxes of the apiary) can be held to be a minor damage.

Damage pathways similar to this one may be established for non-target arthropods, soil, water, beneficial microorganisms or other protection targets, in case a suspicion based on science might come to happen and the threat materialize itself in damage. Once more, the data shown in the dossier strongly suggest that there are no impacts to soil, waters, microbiota and biota other than those that could be caused by conventional eucalyptus.

A risk hypothesis that has been raised considers the horizontal transfer of gene *nptII* to bacteria. In this case, the transfer would be to the bacteria of the bee alimentary canal

microflora. This hypothesis, however, contradicts what is known of plant and microorganism biology and, above all, what is known of gene organization and expression in these groups of living beings. The probability of transfer of functional genes from plants to microorganisms is extremely low, as shown more than twenty years ago by Fuchs *et al.* (1998). It is not surprise that the transfer of a certain antibiotic resistance marker from genetically modified plants to bacteria was never recorded in natural conditions (EFSA, 2007). Identical conclusions were reached by OGTR (OGTR, 2012), Petersen *et al.* (2014) and many others.

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

Applicant delivered a molecular characterization of the insert with a detailed map of the vector. Assessment of genetic inheritance of genes showed normal segregation, where the segregating plants display normal phenotype in all cases analyzed. No pleiotropic and epistatic effects were recorded in essays with the modified event resulting from insertion of genes.

Applicant fully met, in different levels, the requirements of both domestic and internationally standardized biosafety essays for the correct characterization of the transgene innocuousness.

Based on this information, available in the literature, submitted in the request for commercial release, it is noticeable that crossing risks are low and, in case such risks do happen, expected damages are negligible. Besides, essays conducted by applicant revealed that the event introduced in general fails to change the relation of the plant with the biota when compared with its parental. Regarding the environment, the conclusion is that eucalyptus event H421 is not a potential cause of significant degradation to the environment or harm to human and animal health, keeping with the biota a relation identical to that of the conventional eucalyptus. Restrictions to the use of the GMO in analysis and its derivatives are conditioned to the provisions of Law no. 11460, of March 21, 2007.

IX. Considerations on Particulars of Different Regions of the Country (Subsidies to

461/2015

monitoring agents)

According to the rule of Article 1 of Law 11460, of March 21, 2007, *“research and cultivation of genetically modified organisms in indigenous lands and in conservation unit areas are forbidden, except for Environment Protection Areas”*.

The genetic modification introduced fails to change the basic characteristics of the plant, and therefore the eucalyptus behaves the same as its conventional counterpart in cultivation conditions, except for the characteristics that has been inserted.

X. Conclusion

Considering that the species eucalyptus is a well characterized plant with a rich history of safety for humans and animals, and that the genes introduced in this variety fail to evidence adverse effects according to the tests conducted; and

Considering that the centesimal composition data fail to point to significant differences between genetically modified and conventional varieties, suggesting equivalence between them; and

Whereas:

1. Proteins inserted are present in plants and microorganisms and different natural mutants, therefore humans and animals have for long time a record of exposure to such proteins; and
2. Molecular analysis evidenced that integrity and stability of the insert was maintained;
3. Analyses of segregation and standard of genetic inheritance are stable; and
4. Agronomic assessment indicate that the insert failed to promote expression of any

461/2015

other characteristics except for the expected one; and

5. Inserted proteins did not exhibit nutritional, immunologic and histologic differences, characterizing innocuousness of the transformation operated and safety of eucalyptus use. Possible allergenic effects resulting from introduction of the selected genes have also been discarded, either by the history of their use in different transformation or the *in silico* analysis of expressed peptides; and
6. Evidences obtained from experimental and observational studies indicate similarity between the genetically modified eucalyptus and its conventional counterpart in toxicological, allergenic and nutritional aspects (considering pollen and bees). There is no evidence of any toxicological risk, indicating that the only consequence of the modification inserted by the genetic manipulation of inserting the genes described was the volumetric increase in wood;

For the foregoing and taking the internationally accepted criteria in the process of analyzing risks in genetically modified raw materials, one may conclude that this eucalyptus is as safe as the conventional plant. Regarding the competences attributed to it by Article 14 of Law no. 11105/05, CTNBio held the application as being in line with applicable rules and legislation to secure biosafety of the environment, agriculture, human and animal health, and reached a conclusion that this eucalyptus is substantially equivalent to the conventional eucalyptus. Regarding environment, CTNBio concluded that eucalyptus event H421 is not a potential cause of significant degradation of the environment, keeping with the biota a relation identical to that of the conventional eucalyptus.

CTNBio holds this activity as being not a potential cause of significant degradation of the environment or cause of harm to human and animal health. Restrictions to the use of the GMO analyzed and its derivatives are conditioned to the provisions of Law no 11460, of March 21,

2007.

CTNBio analysis considered the opinions issued by members of the Commission; documents forwarded by applicant to CTNBio Office of the Executive Secretary; and results of planned released into the environment. Records of the Public Hearing, texts, etc. were also taken into consideration independent scientific studies and publications conducted by applicant and by third parties.

XI. Bibliography

Campinhos EN, Peters-Robinson I, Bertolucci FL, Alfenas AC (1998) – Interspecific hybridization and inbreeding effect in seed from a *Eucalyptus grandis* x *E. urophylla* clonal orchard in Brazil. *Genetics and Molecular Biology*, vol. 21, no. 3, p 1-8.

EFSA (2007) - Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. doi:10.2903/j.efsa.2007.74

EMBRAPA (2002) – Comunicado Técnico 83: Produção e Rentabilidade do Eucalipto em Empresas Florestais (available at http://www.cnpf.embrapa.br/publica/comuntec/edicoes/com_tec83.pdf)

Franck-Oberaspach, S. L.; Keller, B. 1997. Consequences of classical and biotechnological resistance breeding for food toxicology and allergenicity. **Plant Breeding** 116 (1): 1–17, March 1997.

Fuchs RLI, Ream JE, Hammond BG, Naylor MW, Leimgruber RM, Berberich SA (1993) - Safety assessment of the neomycin phosphotransferase II (NPTII) protein. *Biotechnology (N Y)* **11**(13):1543-7.

461/2015

ISAAA (2014) - Gene: nptII. (consulted on 11/2014) - <http://www.isaaa.org/gmapprovaldatabase/gene/default.asp?GeneID=18>

Kroes R, Galli C, Munro I, Schilter B, Tran LA, Walker R, Würtzen G (2000). Threshold of toxicological concern for chemical substances in the diet: A practical tool for assessing the need for toxicity testing. *Food Chem Toxicol* 38: 255-312

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Würtzen G (2004). Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet. *Food Chem Toxicol* 42: 65-83

Kroes R, Renwick AG, Feron V, Galli CL, Gibney M, Greim H, Guy RH, Lhuguenot JC, van de Sandt JJ (2007). Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. *Food and Chemical Toxicol* 45: 2533- 2562

LaroseDT , C. D. Larose CD (2014) - *Discovering Knowledge in Data: An Introduction to Data Mining*. 2a. Edição, John Wiley & Sons, Inc. 319pp.

Lima WP, Laprovitera R, Ferraz, SFB, Rodrigues, CB, Silva, MM (2012) – Forest plantations and water consumption: a strategy for hydrosolidarity. *International Journal of Forestry Research*, Vol. 2012, Artigo ID 908465, 8p.

Monteiro GVTA, Santos HC, Guerreiro RS, Rêgo FLT (2010) - Avaliação da presença de resíduos de tetraciclina em amostras de mel comercializadas no estado da Bahia. *R. Ci. méd. biol.* 9(2):102-107

OECD (2014) – Consensus document on the biology of *Eucalyptus* spp. Series on Harmonisation of Regulatory Oversight in Biotechnology no. 58. (available at

461/2015

[http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2014\)27&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)27&doclanguage=en)).

OGTR (2012) - Risk Assessment Reference: Marker Genes in GM Plants. Acessado em 11/2014. (Disponível em <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/marker-genes-ref-1-htm>)

Pacheco IA, Kageyama, PY, Wiendl FM, Berti Filho, E (1996) – Estudo da dispersão de pólen de *Euycalyptus saligna* (Smith) por abelhas *Apis mellifera* L. utilizando-se o radiofósforo ³²P. IPEF, n. 34, p47-52.

Petersen W, Umbeck P, Hokanson K, Halsey M. (2005) - Biosafety considerations for selectable and scorable markers used in cassava (*Manihot esculenta* Crantz) biotechnology. Environ Biosafety Res. **4**(2):89-102.

Silva PHM, Poggiani F, Mori, ES, Dias, CTS, DiCiero, L (2011a) – Potencial de invasão de eucalipto pelas sementes produzidas nos plantios comerciais. Circular Técnica, IPEF, no. 203, p 1-7.

Silva PHM, Poggiani F, Sebbenn AM, Mori ES (2011b) - Can Eucalyptus invade native forest fragments close to commercial stands? Forest Ecol. Manag. **261**(11): 2075–2080.

The Arabidopsis Information Resource (2014) - Locus: AT1G48930 (consulted on 11/2104). <http://www.arabidopsis.org/servlets/TairObject?type=locus&name=At1g48930>

SCCS, Comunidade Europeia, 2012

http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_092.pdf

XI. Request for private examination

Dr. Paulo Kageyama, a member representing the Ministry of Agriculture Development,

461/2015

requested private examination of the documentation based on four issues:

- i) Impact of H421 eucalyptus in Silvicultural Hydrology;
- ii) Gene flow of H421 eucalyptus and contamination potential;
- iii) Eucalyptus H421 honey and human feeding;
- iv) Eucalyptus H421 and lack of certification by FSC – Forest Stewardship Council.

i) Impact of H421 eucalyptus in Silvicultural Hydrology;

This member emphasized that for long time potential high consumption of water by eucalyptus tillage is questioned and, in the current situation of drought and lack of water, this is an important point to take into account. He called the attention to the alert raised by the United Nations on February 20, 2015 and certainly this concurs to aggravate the situation. The member highlighted that:

“in the initial phase of eucalyptus growth (the first four or five years), a fact verifies both in the origin (Australia) and in Brazilian conditions, where the eucalyptus forests consume more water than the remaining phases, causing, with H421 an increase in water consumption precisely in the most critical phase of its consumption, the initial growing years of the species, in which the transgenic shows its priority.

He asserted that the seven year rotation prescribes a period in which the initial growing phase, up to

the fourth or fifth year followed by a period of diminished growing rate, fails to promote an excessive expenditure of water during the whole rotation. With the combination of several years of seeding, this balance in water consumption becomes clear and evident.

As it may be seen, the arguments used in the application related to Event Transgenic Eucalyptus H421, based in larger part on authors contacted by us, this important and complex theme of blue water production seems not being correctly interpreted. The reputation of large scale eucalyptus monoculture, called by the organized community and also by the population and general, many unprintable manes, certainly deriving from scientific arguments submitted by the authors in general, certainly shall promote several claims and possible actions” (transcribed)

ii) Gene flow of H421 eucalyptus and contamination potential;

Dr. Kageyama argues that one must consider that eucalyptus is an allogamous species the main pollinizer of which is the Africanized European bee; that there are large scale forests of the urograndis group, compatible for crossing with eucalyptus H421. He alleges that there are eucalyptus planted forests in small areas of family agriculture for the production of honey and energy and that these eucalyptuses may suffer crossings with the transgenic plant. Based on the foregoing, the member questioned about the distances of pollen dislocation, as against the

work published, using radioisotopes and a recent work conducted by FuturaGene jointly with other laboratories using the genetically modified eucalyptus H421.

Dr. Kageyama suggests that a fresh team discuss the distances proposed by the recently published article and that the study be remade.

Dr. Kageyama argues that, on the issue of transgene eucalyptus gene flow, this would not meet the best interests of the public and silviculture, since its phenotype is targeted to cellulose industry, being this one just a small portion of the use of this culture.

Dr. Kageyama points out that the practice of using monoclonal is recoiling and, in order to increase genetic diversity in each planted plot different varieties are used, in addition to avoiding aspersions of agrochemicals by plane. The use of genetically modified eucalyptus could interfere in this new management practice.

Dr. Kageyama alleges that there is a moratorium proposed for the use of modified trees and that FST does not grant certification in areas containing planted forests of transgenic eucalyptus. Therefore, the proposition is not within the international context.

iii) Eucalyptus H421 honey and human feeding;

Dr. Kageyama argues that Brazil has about 500 thousand apiarists, of which 350 thousand are small producers, with an output of 40 thousand tons per year. Out of the total exported, especially to the United States of America and Europe, 80% are organic honeys (SEBRAS, 2014, Abemel, 2015). Not less than 35% of the Brazilian honey production comes from producer associations from eucalyptus farms beehives which, to be accepted as organic, may not carry any trace of transgenics. The member stresses that eucalyptus is a great attraction for bees, mainly those belonging to *Apis mellifera*. It is taken for granted that beehives associated or close to GM eucalyptus forests will produce honey with transgenic traces. Brazilian honey

comes from bees that feed on native and diversified forests and, supplementary, on eucalyptus plantations (Abemel, 2015); this is the difference of the Brazilian honey and it is precisely what may come to be lost in other countries: batches of honey for export shall be strictly analyzed and, in case they contain any transgenics, the batch will be discarded.

Dr. Kageyama argues that applicant advocates, with no concrete basis, that Brazil should make international rules less strict, setting flexible limits for the traces of transgenic genes, and adds:

“The proposal is on the brink of irresponsibility, taking into account the scale of Brazilian eucalyptus plantations (currently about 5.1 million hectares) and the irreversible dependence in the production of honey and other products such as in natura propolis and pollen. Considering the contamination potential and the way in which the majority of rural producers cultivate eucalyptus in their properties, it would be impossible to avoid collapse of this activity in Brazil, keeping in mind that the company Suzano Papel e Celulose has over 800 thousand hectares of eucalyptus forests in seven states of the Brazilian Federation, which means a gigantic scale taking into account that bees can fly up to 6 kilometers for collecting nectar in an eucalyptus plantation due to the large supply of nectar and pollen (Bastos, E. M., 2014 – Speech delivered at the CTNBio Public Hearing of September, 2014)” (transcribed).

Besides, Dr. Kageyama argues that:

“pollen grains coming from transgenic trees also have in their DNA the gene artificially inserted in the transgenic eucalyptus. Company FuturaGene itself recognizes that the pollen of H421 eucalyptus is viable, and therefore may fertilize flowers of non-transgenic eucalyptuses, generating new eucalyptus trees. Transport of this pollen by bees of wind may reach hundreds of meters and even some kilometers, being this way impossible to avoid mating between trees of transgene eucalyptus and conventional

461/2015

ones, and may even contaminate stocks of potential seeds as well as improved species developed to the production of honey, such as the improved variety Eucalyptus urophylla to produced earlier and earlier large amounts of honey.

According to a study conducted by the Ministry of Agrarian Development, presented during the Public Hearing held by CTNBio, documents supplied by FuturaGene present flaws that fail to enable a conclusion on whether the modified plant in study is or is not safe to bees. It shall be noted that the bee species Apis Mellifera is a generalist when foraging, that is to say, the insect visits the flowers of different species when looking for nectar and is therefore an important pollinizing agent of different plants, including cultures used in human feeding, such as coffee, pumpkin, beans, cucumber, tomato and many others. Therefore, a risk to the health of these insects also represents a risk to the production of foodstuffs.” (transcribed).

According to Dr. Kageyama, there is a possible creation of pathogens resistant to antibiotics, since the eucalyptus has a copy of gene *nptII* in its DNA. Given that the gene codes for enzyme neomycin phosphotransferase, granting resistance to different antibiotics it may be consumed when present in honey. Dr. Kageyama argues that presence of the enzyme in beehives may select pathogens that are resistant to antibiotics, which may cause difficult to control diseases in bees. He adds that the effects of this enzyme on bacteria present in the intestinal tract of bees and humans that consume this honey are unknown.

In his argument, Dr. Kageyama considers that the applicant admits that several essays were not conclusive, since many beehives became lost in the fields and that data coming from honey and impacts in the bee fauna result from controlled experiments in laboratory. He advocates an investigation to be conducted in the beehive locations.

iv) Eucalyptus H421 and lack of certification by FSC – Forest Stewardship Council

461/2015

Dr. Kageyama argues that the majority of paper and cellulose purchasers in the world requires that the industry display a green seal, as the one conceived by the Forest Stewardship Council (FSC). He sustains that the last FSC assembly a directive was ratified in the three chambers, economic, social and environmental, of refusing the commercial use of Transgenic Trees, allowing only the research on the field, contained in Criterium 6.8 (FSC-STD-BRA-01-2014 V1-0 PT) "Use of biologic control agents shall be documented, minimized, monitored and carefully controlled according to domestic legislation and scientific protocols internationally accepted." Use of genetically modified organisms is forbidden according to item 6.8.1, "The Organization shall respect FSC guidelines on abstaining from using use GMO in Forest Management Unit."

Therefore, the Ministry of Agrarian Development considers that the commercial release of eucalyptus fails to be safe and the research procedures are incomplete and unable to foresee or repair damages to animal health, as well as contamination of biodiversity at this moment.

For the foregoing Dr. Kageyama opposes the idea that there is no evidence that H421 eucalyptus fails to cause negative impacts to the environment and human and animal health. He therefore requested that the license for commercial release be rejected.

Dr. Kageyama submits, in addition, an opinion of Professors Walter Paula Lima and Silvio Ferraz ESALQ.USP, entitled "CONSIDERATIONS ON WATER CONSUMPTION BY FOREST PLANTATIONS OF FAST GROWTH SPECIES," as well as an article by Silva, A., and Grattapaglia, D, Forest Ecology and Management (2014).

XII. Dissenting Votes:

The following members voted against the Application:

- Paulo Kageyama – Representative of the Ministry of Agrarian Development;
- Geraldo Miniuci Ferreira Junior – Representative of the Ministry of External Relations;

461/2015

- Suzi Barreto Cavali – Specialist in Family Agriculture.

Brasília, Distrito Federal, 04.09.2015

Edivaldo Domingues Velini

CTNBio President

Advisor: Gutemberg D. Sousa.

[Reverse of the 34 pages of the original document blank.]

In Witness Whereof, I have hereunto set my hand and seal in this City of Brasília,
Federal District, Brazil, this Tuesday, November 17, 2015

Fees according to

Official Gazette of 04/15/2011

Page 73

Marco Antônio Rochadel

Public Translator