NATIONAL ENVIRONMENTAL POLICY ACT DECISION
AND
FINDING OF NO SIGNIFICANT IMPACT

Permit application 11-052-101rm
received from ArborGen LLC

Field testing of genetically engineered
Eucalyptus grandis* X *Eucalyptus urophylla

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services

United States Department of Agriculture (USDA) and the Animal and Plant Health Inspection Service (APHIS) have developed a decision document to comply with the requirements of the National Environmental Policy Act of 1969, as amended, the Council of Environmental Quality's (CEQ) regulations implementing NEPA, and the USDA and APHIS NEPA implementing regulations and procedures. This NEPA decision document is intended to state APHIS' NEPA decision and present the rationale for its selection.

In accordance with APHIS procedures implementing the NEPA Regulations (7 CFR part 372), APHIS has prepared an Environmental Assessment (EA) to evaluate and determine if there are any potentially significant impacts to the quality of the human environment in response to a permit application (11-052-101rm) received from ArborGen for the confined environmental release and interstate movement. If issued, the permit would authorize the planting of genetically engineered (GE) Eucalyptus hybrid clone (*Eucalyptus grandis* x *Eucalyptus urophylla)* to support proposed field research studies on six research sites in Alabama, Florida, Mississippi, and South Carolina. The permit application requests that APHIS permit all the trees except the trees on two of the sites in South Carolina to flower. The purpose of the confined environmental release is for ArborGen to assess the effectiveness of introduced gene constructs which are intended to confer cold tolerance; to test the efficacy of genes to alter lignin biosynthesis; to test the efficacy of genes to alter growth; and to test the efficacy of genes designed to alter flowering. In addition the trees have been engineered with a selectable marker gene which confers resistance to the antibiotic kanamycin. ArborGen has also requested under this permit application that all genetically engineered Eucalyptus trees authorized under permits 09-070-101rm and 11-041-101rm be incorporated into this new permit and allowed to flower. New trees will also be moved interstate from ArborGen's nursery and greenhouse facilities located in South Carolina to the release locations identified in the permit application where they will be planted.

The EA assesses the potential impacts of APHIS issuing or denying a confined environmental release and interstate movement permit with supplemental permit conditions to allow the interstate movement, planting, field testing and flowering of a GE *Eucalyptus* hybrid clone (*Eucalyptus grandis* x *Eucalyptus urophylla)* engineered to express various genes at 6 confined field site locations within in the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres and analyzes the potential environmental and social effects that result from the proposed action and the alternatives. The proposed action of USDA APHIS,

Biotechnology Regulatory Services (BRS) is to issue the APHIS confined field release and movement permit for a hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla* with supplemental permit conditions (*see* Appendices VI and VII of the EA). The movement authorization would be valid for one year and the release would be valid for a three-year period. Comments from the public involvement process were reviewed for substantive issues which were considered in developing this NEPA decision.

Since 1986, the United States government has regulated GE organisms pursuant to a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework) (51 FR 23302, 57 FR 22984). The Coordinated Framework, published by the Office of Science and Technology Policy, describes the comprehensive federal regulatory policy for ensuring the safety of biotechnology research and products and explains how federal agencies will use existing Federal statutes in a manner to ensure public health and environmental safety while maintaining regulatory flexibility to avoid impeding the growth of the biotechnology industry. The Coordinated Framework is based on several important guiding principles: (1) agencies should define those transgenic organisms subject to review to the extent permitted by their respective statutory authorities; (2) agencies are required to focus on the characteristics and risks of the biotechnology product, not the process by which it is created; and (3) agencies are mandated to exercise oversight of GE organisms only when there is evidence of "unreasonable" risk.

The Coordinated Framework explains the regulatory roles and authorities for the three major agencies involved in regulating GE organisms: USDA's APHIS, the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA).

APHIS is responsible for regulating GE organisms and plants under the plant pest provisions in the Plant Protection Act of 2000, as amended (7 USC § 7701 et seq.) to ensure that they do not pose a plant pest risk to the environment. APHIS regulations at Title 7 of the Code of Federal Regulations (CFR) part 340, which were promulgated pursuant to authority granted by the Plant Protection Act, as amended (7 United States Code (U.S.C.) 7701–7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR 340.2) and is also considered a plant pest. A GE organism is also regulated under Part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have information to determine if the GE organism is unlikely to pose a plant pest risk.

When APHIS receives an application for a permit for environmental release and movement, the application is evaluated to determine whether the environmental release and movement, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

> *Administrative action on applications.* After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requested by APHIS, a permit shall be granted or denied (7 CFR 340.4(e)).

The applicant has provided the required information associated with this request in the permit application. This information has been reviewed by APHIS-BRS and is analyzed in the EA.

FDA regulates under the authority of the Federal Food, Drug and Cosmetic Act. The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992 (57 FR 22984-23005). Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. The EPA regulates plant-incorporated protectants under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and certain biological control organisms under the Toxic Substances Control Act (TSCA). This GE *Eucalyptus* hybrid is not used for food or feed purposes and does not contain any genetically engineered pesticides or tolerance to herbicides.

Public Involvement

In a notice published in the *Federal Register* on February 10, 2012, (77 FR28 7123-7124, Docket No. APHIS-2011-0130), APHIS announced the availability of an EA for public review and comment for a proposed controlled field release of a genetically engineered clone of a *Eucalyptus* hybrid. Comments on the environmental assessment were required to be received on or before March 12, 2012. APHIS received a total of 246 comments during the 30-day comment period. All respondents expressed concerns about the permit or opposed granting the permit. Comment documents may be viewed at http://www.regulations.gov/#!searchResults;rpp=25;po=0;s=APHIS%25E2%2580%25932011%25E2%2580%25930130. All comments were analyzed to identify new issues, alternatives, or information. Responses to the substantive comments are included as an attachment to this Finding of No Significant Impact.

Major Issues Addressed in the EA

The EA describes the alternatives considered and evaluated using the identified issues. Issues considered in the EA were developed based on APHIS' determination to issue a confined environmental release and interstate movement permit with supplemental permit conditions to allow the interstate movement, planting, field testing and flowering of a GE Eucalyptus hybrid clone (Eucalyptus grandis x Eucalyptus urophylla) engineered to express various genes at 6 confined field site locations within the States of Alabama, Florida, Mississippi, and South Carolina. The following issues were identified as important to the scope of the analysis (40 CFR 1508.25):

- Interstate Movement of the trees under permit
- Alteration in Susceptibility to Disease or Insects – Potential of the *Eucalyptus* to Harbor Plant Pests
- Expression of the Gene Products, New Enzymes, or Changes to Plant Metabolism - Risk of the Gene Products on the Environment
- Alteration in Weediness characteristics – Potential of the Engineered *Eucalyptus* to be Invasive
- Possibility of Gene Flow Within the Field Test
- Possibility of Gene Flow Outside of the Field Test
- Possibility of Vegetative Propagation / Persistence Outside of the Field Test

- Potential of the *Eucalyptus* in the Field Tests to Become an Invasive Species that Threatens Native Plant and Animal Communities
- Impact on Existing Agricultural Practices
- Potential Impacts to Wildlife
- Potential Impacts by Fire
- Potential Impacts to Human Health
- Transfer of Genetic Information to Organisms with which it Cannot Interbreed - Horizontal Gene Transfer to Other Organisms
- Potential Effects of Growing *Eucalyptus* on Soil Hydrology
- Potential Allelopathic Effects of *Eucalyptus*
- Risks to Threatened and Endangered Species
- Impacts on Unique Characteristics of Protected Areas

Affected Environment:
The confined field tests would take place on land controlled by ArborGen or through private contracts established by ArborGen for field testing. The exact locations are claimed as CBI and have been submitted as part of the APHIS permit application, however the States and Counties where the confined field tests would occur are Baldwin and Escambia Counties, Alabama; Highlands County, Florida; Pearl River County Mississippi; and Berkeley and Dorchester Counties, South Carolina. Under the permit application submitted by ArborGen, there are six research sites where trees would be planted. A total of 14.7 acres (all 6 sites combined) is being requested by the applicant. Five of these locations currently have active APHIS permits (08-011-106rm, 08-014-101rm, 09-070-10rm, 10-112-101r, and 11-041-101rm) for environmental release of GE *Eucalyptus* hybrid clone (*Eucalyptus grandis* x *Eucalyptus urophylla)* in Alabama, Florida, Mississippi, and South Carolina. The remaining site in Dorchester County, South Carolina has been listed in previous APHIS permits/notifications as a holding area for transgenic trees. ArborGen is requesting that trees be allowed to flower at four locations in Alabama, Florida and Mississippi. At the two locations in Berkeley and Dorchester Counties, SC, they have requested to release trees in containers and have indicated they will not allow these trees to flower at these locations. The size of each individual confined field test sites identified in ArborGen's permit application ranges from 0.5 to 7.7 acres, which is less than the 20 acres analyzed in APHIS EA and subsequent FONSI prepared for Permits 08-011-106rm, 08-014-101rm and 10-112-101r (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and (http://www.aphis.usda.gov/brs/aphisdocs/08_014101r_ea.pdf). For the five research sites that are currently authorized by APHIS to plant GE *Eucalyptus* hybrid clone (*Eucalyptus grandis* x *Eucalyptus urophylla)*, ArborGen has indicated in their permit application that any new planting authorized by APHIS under 11-052-101rm would not expand the current field site locations beyond the areas currently authorized by APHIS.

All the confined field test sites listed in the permit application by ArborGen are either on privately owned and managed plantation forests and agricultural farm lands or experimental research stations managed by academic institutions and industry, and have been in managed agricultural production for more than 5 years. The standard agricultural and silvicultural practices for land preparation, planting, irrigation, and harvesting of plants have been routinely used on these sites. Sites that include managed pastures have had intense activity including the use of heavy machinery for general upkeep, irrigation, fertilization, controlled grazing and management of grasses. Standard silvicultural practices would be used at these sites for the duration of the field tests. Surveys conducted by the applicant at each of these locations indicate

that there are not any old growth forests or undisturbed natural areas in the immediate surroundings of the test sites. ArborGen has indicated that the trees would be planted on individual research sites ranging from 0.5 up to 7.7 acres, depending on the location with planting density ranging from 300 - 600 trees per acre[1].

The EA analyzes the potential environmental consequences of a proposal for APHIS to issue a confined environmental release and interstate movement permit with supplemental permit conditions to allow the interstate movement, planting, field testing and flowering of a GE Eucalyptus hybrid clone (Eucalyptus grandis x Eucalyptus urophylla) engineered to express various genes at 6 confined field site locations within the States of Alabama, Florida, Mississippi, and South Carolina. Based upon the permit application submitted by ArborGen, two alternatives are considered and analyzed in the EA: (1) deny the permit and (2) issue the APHIS permit.

Alternative A: No Action – Deny the Permit

Under the No Action alternative APHIS would deny the permit application (11-052-101rm) submitted by ArborGen. ArborGen would not be authorized to move and plant the GE *Eucalyptus* trees with new constructs and allow them to reach maturity and flower. Confined field release under permits 09-070-101rm and 11-041-101rm would continue to not allow flowering.

There are currently six active permits under which ArborGen is authorized by APHIS to grow GE *Eucalyptus* which include 32 unique locations within 7 States: 08-011-106rm, 08-014-101rm, 09-070-101rm, 10-112-101r, 11-041-101rm and 11-201-103r. No plantings at any of the 32 locations are authorized by APHIS to exceed 20 acres in size. Trees are allowed to flower under four permits: 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r (two of the locations in Berkeley and Dorchester Counties, SC trees are not authorized to flower). Under permits 09-070-101rm and 11-041-101rm trees are not authorized to flower. As of September 2011, ArborGen has reported to APHIS that they are growing a total of approximately 67 acres of trees on 18 of the 32 permitted locations. Under the No Action alternative, ArborGen could continue to grow GE *Eucalyptus* as authorized by APHIS under these six active permits.

Alternative B: Preferred Alternative – Issue the APHIS Permit

The Preferred Alternative is to approve ArborGen permit application request (11-052-101rm) and issue the APHIS confined field release and movement permit for a hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla* with supplemental permit conditions (*see* Appendices VI and VII of the EA). The movement authorization would be valid for one year and the release would be valid for a three-year period. The permit will need to be renewed by ArborGen and subsequently approved by APHIS to allow the transgenic *Eucalyptus* plants to remain in the ground beyond the three-year time period specified in the permit application.

Under this alternative, APHIS would issue a confined environmental release permit to ArborGen in accordance with 7 CFR part 340 to allow the movement, planting, field testing and flowering

[1] Planting density typically refers to the number of trees per acre. Planting densities can vary greatly depending upon the tree species and the environment, but densities of short rotation hardwood trees in the southeastern US are typically in the range of 300–800 trees per acre. Therefore sites ranging from 10 to 20 acres can have from 3000 to 16,000 total trees planted in the ground. Twenty acres, as defined by forest plantation standards in the southeast, is considered a small planting.

of a GE *Eucalyptus* hybrid clone engineered to express various genes at 6 confined field site locations within in the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres. The permit would allow all the trees except the trees on two of the sites in South Carolina to flower. All genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm would be incorporated into this new permit and allowed to flower. This permit would authorize ArborGen to move and plant the GE *Eucalyptus* trees with new constructs and allow them to reach maturity and flower. Under this alternative, the applicant would be allowed to gather data on performance of the transgenic trees over a multiyear period and the efficacy of the genes in a wide variety of environments for multiple years. This alternative would allow the safe development and use of GE organisms under the mission of BRS.

Environmental Consequences of APHIS' Selected Action

The EA contains a full analysis of the alternatives to which we refer the reader for specific details. The following table briefly summarizes the results for each of the issues fully analyzed in the Environmental Consequences section of the EA.

Table 1. *Issue*	***Alternative A*** ***No Action*** ***Deny the Permit***	***Alternative B*** ***Preferred Alternative*** ***Issue APHIS Permit***
Interstate Movement of the trees under permit	No increased risk	No increased risk
Alteration in Susceptibility to Disease or Insects – Potential of the *Eucalyptus* to Harbor Plant Pests	No increased risk	No increased risk
Expression of the Gene Products, New Enzymes, or Changes to Plant Metabolism - Risk of the Gene Products on the Environment	No expected risk	No expected risk
Alteration in Weediness characteristics – Potential of the Engineered *Eucalyptus* to be Invasive	Not likely	Not likely
Possibility of Gene Flow within the Field Test	Minimal	Minimal
Possibility of Gene Flow Outside of the Field Test	Minimal	Minimal
Possibility of Vegetative Propagation / Persistence Outside of the Field Test	Not likely	Not likely
Potential of the *Eucalyptus* in the Field Tests to Become an Invasive Species that Threatens Native Plant and Animal Communities	Not likely	Not likely
Impact on Existing Agricultural Practices	No change	No change
Potential Impacts to Wildlife	No adverse impacts	No adverse impacts
Potential Impacts by Fire	Minimal risk	Minimal risk
Potential Impacts to Human Health	Negligible	Negligible
Transfer of Genetic Information to Organisms with which it Cannot Interbreed - Horizontal Gene Transfer to Other Organisms	No risk	No risk
Potential Effects of Growing *Eucalyptus* on Soil Hydrology	No negative impacts	No negative impacts
Potential Allelopathic Effects of *Eucalyptus*	Minimal	Minimal
Risks to Threatened and Endangered Species	No effect	No effect
Impacts on Unique Characteristics of Protected Areas	No effect	No effect

Finding of No Significant Impact

The analysis in the EA indicates that there will not be a significant impact, individually or cumulatively, on the quality of the human environment as a result of this proposed action. I agree with this conclusion and therefore find that an EIS need not be prepared. This NEPA determination is based on the following context and intensity factors (40 CFR 1508.27):

Context – The term "context" recognizes potentially affected resources, as well as the location and setting in which the environmental impact would occur. This action would be limited to the interstate movement and environmental release of a GE Eucalyptus hybrid clone at 6 well dispersed confined field site locations in the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres and has limited potential to affect resources outside of the confined field test sites. Supplemental permit conditions (*see* Appendices VI and VII of the EA) established for the permit will effectively limit the reproductive capabilities and establishment of this GE *Eucalyptus* outside the confined field trial locations and reduce the possibility of unintended exposure from moving GE Eucalyptus trees to be negligible to non-existent.

Intensity – Intensity is a measure of the degree or severity of an impact based upon the ten factors. The following factors were used as a basis for this decision:

1. *Impacts that may be both beneficial and adverse.*
   According to the applicant, genetically engineered cold tolerant *Eucalyptus* would enable the production of this hardwood species for pulping and for biofuel applications in managed plantation forests in the southeastern U.S. APHIS issuance of the interstate movement and confined field release permit would allow ArborGen to conduct research to assess the efficacy of the introduced cold tolerance genes, genes to alter lignin biosynthesis in *Eucalyptus*, genes to alter growth and to research mechanisms for altered fertility. The confined release of the trees at six well dispersed confined field site locations in the States of Alabama, Florida, Mississippi, and South Carolina will allow the applicant to obtain data on performance of the transgenic trees and the efficacy of the inserted genes in a wide variety of environments. The establishment and growth of these small confined field tests will not have any impact on existing agricultural practices because they are solely for research purposes. Current agricultural practices will essentially remain unchanged. As identified by the applicant, the field sites that are being proposed under the permit have been used as forest tree plantations, pastures, or for forestry and agriculture research and are specifically designed for field testing crop plants or forest trees. As discussed in Section V of the EA, APHIS considers the possibility of unintended exposure from moving GE Eucalyptus trees under the proposed action to be negligible to non-existent.
2. *The degree to which the proposed action affects public health or safety.*
   The proposed action to issue the APHIS interstate movement and confined field release permit should not pose an unnecessary risk to human health and therefore would have no significant impacts on human health. This GE *Eucalyptus* hybrid is not used for food or feed purposes and does not contain any genetically engineered pesticides or tolerance to herbicides. As described in Section V of the EA and in the response to comments, the risk that these field trials will result in a higher incidence of a fungal pathogen in the U.S. and thereby pose a risk to human health is considered to be negligible.

3. *Unique characteristics of the geographic area such as proximity to historic or cultural resources, park lands, prime farmlands, wetlands, wild and scenic rivers, or ecologically critical areas.*

   There are no unique characteristics of geographic area such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas that would be significantly affected. As described in Section V and Appendix V of the EA, issuance of the permit to allow flowering of these additional constructs is not expected to impact unique characteristics of protected areas such as park lands, wetlands, wild and scenic areas, or ecologically critical areas. All the confined field test sites listed in the permit application are either on privately owned and managed plantation forests and agricultural farm lands or experimental research stations managed by academic institutions and industry. The standard agricultural and silvicultural practices for land preparation, planting, irrigation, and harvesting of plants have been routinely used on these sites. Surveys conducted by the applicant at each of these locations indicate that there are not any old growth forests or undisturbed natural areas in the immediate surroundings of the test sites. In addition, supplemental permit conditions (*see* Appendix VII of the EA) established for the permit will effectively limit the reproductive capabilities and establishment of this GE *Eucalyptus* outside the confined field trial locations and reduce the possibility of unintended exposure from moving GE Eucalyptus trees to be negligible to non-existent.

4. *The degree to which the effects on the quality of the human environment are likely to be highly controversial.*

   The effects on the quality of the human environment are not highly controversial. Although there is some opposition to APHIS issuing the interstate movement and confined field release permit, this action is not highly controversial in terms of size, nature or effect. This action would be limited to 6 well dispersed confined field site locations in the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres on individual research sites ranging from 0.5 up to 7.7 acres, depending on the location with planting density ranging from 300 - 600 trees per acre. During the public comment period, APHIS received comments opposing APHIS' issuance of the permit. No new issues, alternatives or substantive new information were identified in any of the comments received by APHIS. APHIS has addressed substantive comments in the response to public comments document attached to this FONSI based on scientific evidence found in peer-reviewed, scholarly, and scientific journals.

5. *The degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks.*

   The effects of the proposed action to issue the APHIS interstate movement and confined field release permit are not highly uncertain and do not involve unique or unknown risks. Based on the analysis documented in the EA, the effects on the human environment would not be significant. APHIS has no evidence for any unknown risks of this GE hybrid plant species when released for confined field testing or from moving GE Eucalyptus trees. This GE *Eucalyptus* hybrid has been previously released for confined field testing under APHIS issued notifications and permits in similar geographic locations without any known adverse effects. As described in Section V of the EA and response to comments, the issuance of the confined field release permit to allow the applicant to plant additional trees and to allow the trees to flower at four of the six locations does not present any unforeseen risks. Since there is no increase in the number of sites or acreage where trees will be allowed to reach maturity and flower, over those already authorized by APHIS to flower under permits 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r with similar supplemental permit conditions,

APHIS has concluded that there is no substantial greater risk of loss of confinement and risk to the environment from the proposed action. As presented in Appendix I of the EA, information that has been collected by the applicant and provided to APHIS as part of ArborGen's annual reporting requirements under the existing permits that allow flowering confirmed that cold tolerant translines grown in these field tests did not produce any pollen, that the applicant has not observed any volunteer seedlings in or around the test sites where trees have flowered and produced seed capsules, and monthly field test monitoring observations have not identified any differences in diseases and insects or other non-target organisms between the transgenic and non-transgenic trees in the field test. Based on the analysis and information provided in the EA and supporting permit application, the new genes that are engineered into the trees should not pose any greater risk of loss of confinement and risk to the environment than those that are currently authorized by APHIS for confined field release. The addition of new genes to increase cold tolerance, alter lignin and growth should not compromise the ability of these to remain confined field tests. Adherence to the supplemental permit conditions established for the permit will effectively limit any potential adverse impacts to the human environment. As discussed in Section V of the EA, APHIS considers the possibility of unintended exposure from moving GE Eucalyptus trees under the proposed action to be negligible to non-existent.

6. *The degree to which the action may establish a precedent for future actions with significant effects or represents a decision in principle about a future consideration.*

   The proposed action would not establish a precedent for future actions with significant effects or represent a decision in principle about a future decision. Similar to past permit applications reviewed and approved by APHIS, the decision on whether or not to issue a permit for interstate movement and confined environmental release will be based upon information provided in the permit application. APHIS regulations at 7 CFR part 340, regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. In accordance with these regulations, when APHIS receives an application for a permit for environmental release or movement, the application is evaluated to determine whether the environmental release or movement, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The applicant has provided the information associated with this request in the permit application and APHIS now must make a determination to either grant or deny the permit. Each permit application that APHIS receives undergoes this independent review to determine if APHIS should grant or deny the individual permit.

7. *Whether the action is related to other actions with individually insignificant but cumulatively significant impacts.*

   No significant cumulative effects were identified through this assessment. As discussed in the cumulative effects analysis presented in Section V of the EA, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the release sites or the ecosystem in which they are situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

8. *The degree to which the action may adversely affect districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places or may cause loss or destruction of significant scientific, cultural, or historical resources.*

The issuance of the interstate movement and confined field release permit would have no impact on districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places, nor would they likely cause any loss or destruction of significant scientific, cultural, or historical resources. All the confined field test sites listed in the permit application are either on privately owned managed plantation forests and agricultural farm lands or experimental research stations managed by academic institutions and industry. The standard agricultural and silvicultural practices for land preparation, planting, irrigation, and harvesting of plants have been routinely used on these sites. Similar types of agricultural and silvicultural practices would be used by the applicant for managing trees associated with the permit. In addition, supplemental permit conditions (*see* Appendix VII of the EA) established for the permit will effectively limit the reproductive capabilities and establishment of this GE *Eucalyptus* hybrid outside the confined field trial locations and reduce the possibility of unintended exposure from moving GE Eucalyptus trees to be negligible to non-existent.

9. *The degree to which the action may adversely affect an endangered or threatened species or its habitat that has been determined to be critical under the Endangered Species Act of 1973.*

   APHIS evaluated the potential for negative effects on federal threatened and endangered species as listed by the U.S. Fish and Wildlife Service from the issuance of the interstate movement and confined field release permit and determined that the movement and confined environmental release of this GE *Eucalyptus* hybrid would have no effect on federally listed threatened or endangered species or species proposed for listing, or on designated critical habitat or habitat proposed for designation *(see* section on Threatened and Endangered Species in Section V and Appendix IV of the EA).

10. *Whether the action threatens a violation of Federal, State, or local law or requirements imposed for the protection of the environment.*

    The proposed action would be in compliance with all federal, state, and local laws. The proposed action to issue the APHIS interstate movement and confined field release permit would be carried out in accordance with 7 CFR part 340. This GE *Eucalyptus* hybrid is not used for food or feed purposes and does not contain any genetically engineered pesticides or tolerance to herbicides. There are no other Federal, state, or local permits that are needed prior to the implementation of this action.

NEPA Decision and Rationale

I have carefully reviewed the EA prepared for this NEPA determination and the input from the public involvement process and have selected Alternative B-Issue the APHIS Permit. This alternative would fulfill APHIS' statutory mission and responsibilities to allow the safe development and use of genetically engineered organisms consistent with the plant pest provisions of the Plant Protection Act.

As stated in the CEQ regulations, "the agency's preferred alternative is the alternative which the agency believes would fulfill its statutory mission and responsibilities, giving consideration to economic, environmental, technical and other factors." The preferred alternative has been selected for implementation based on consideration of a number of environmental, regulatory, and social factors. Based upon our evaluation and analysis, Alternative B is selected because (1) it allows APHIS to fulfill its statutory mission to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of genetically engineered organisms; and (2) it allows APHIS to fulfill its regulatory

obligations. Therefore, it is my decision to implement the preferred alternative as described in the EA. Based on all of the analysis and reasons above, I have determined that there would be no significant impact to the quality of the human environment from the implementation of the chosen alternative (Alternative B) and therefore, no EIS needs to be prepared.

Michael C. Gregoire

Michael C. Gregoire
Deputy Administrator Biotechnology Regulatory Services
Animal and Plant Health Inspection Services
U.S. Department of Agriculture

5/2/2012
Date

Attachment
Finding of no significant impact
Response to comments
APHIS No. 11-052-101rm

In a notice published in the Federal Register (77FR28 7123-7124, Docket No. APHIS-2011-0130), APHIS announced the availability of an EA for public review and comment for a proposed controlled field release of a genetically engineered clone of a Eucalyptus hybrid. Comments on the environmental assessment were required to be received on or before March 12, 2012. APHIS received a total of 246 comments during the 30-day comment period. All respondents expressed concerns about the permit or opposed granting the permit. Comment documents may be viewed at http://www.regulations.gov/#!searchResults;rpp=25;po=0;s=APHIS%25E2%2580%25932011%25E2%2580%25930130.

Respondents opposing APHIS' issuance of the permit came from nine non-governmental organizations (NGOs) or individuals apparently representing them, two State government entities, and individuals. The majority of respondents opposing the issuance of the permit came from one NGO, whose members submitted nearly identical form letters with some minor modifications. The NGO also submitted a petition bearing 1,957 signatories that opposed the issuance of the permit. The majority of respondents opposed believe that APHIS failed to comprehensively analyze all relevant issues related to the proposed field trial, that an Environmental Impact Statement should have been prepared and that cumulative impacts were not considered; that there is great potential for these *Eucalyptus* hybrids to become invasive or cross breed with non-transgenic *Eucalyptus* trees; *Eucalyptus* could be a host for *Cryptococcus neoformans gattii*, a fungi that could potentially cause mycoses in humans and animals; that *Eucalyptus* plantations have an impact on hydrology and cause or exacerbate drought situations; *Eucalyptus* trees are known to be an incendiary risk and tend to burn very hot during forest fires that are likely to occur in the drought prone southeastern U.S.; and could have significant impacts on biodiversity. APHIS' responses to these and all relevant comments submitted are as follows:

***Eucalyptus* trees are introduced organisms in the U.S. and are documented as invasive pests in California and Florida. The cold tolerance trait could vastly expand the range of this GE *Eucalyptus* tree–and hence enhance its ability to invade native ecosystems. Experience in California and other parts of the world has clearly demonstrated that when *Eucalyptus* escape, it is next to impossible to eradicate them. Court decisions on genetically engineered perennial organisms including GE bentgrass and GE alfalfa, demonstrate a growing legal foundation around the potential escape of perennial GE organisms, even in field trials.** The above comments were submitted in the form letter.

Response: As covered in the EA, the genes introduced to affect cold tolerance could make the engineered *Eucalyptus* more adapted to cold temperatures in the southern United States, but this trait alone would not impart invasive or weediness characteristics to the engineered plants. The trees could be considered weedy or invasive if they were to produce many seedlings that were readily spread away from the field test sites. Where the non-engineered hybrid *Eucalyptus* (EH1) has been grown in Brazil, on an estimated 400,000 acres planted over 15 years, there has been no indication that large numbers of seedlings are being produced and are becoming invasive

from the commercial plantations. ArborGen is required to report any unusual occurrences, such as increased flowering or seed production to APHIS during the field testing period.

The *Eucalyptus* being released is similar to the Veridian phytomining operation
One commenter compared the release of the *Eucalyptus* under this permit to the Veridian phytomining operation that released Yellowtuft (*Alyssum murale* and *A. corsicum*) in southwest Oregon. The *Alyssum* escaped from cultivation and has been listed as a noxious weed in the State of Oregon. It is now a top priority for eradication in the State.

Response: The situation under which these GE hybrid Eucalyptus trees would be grown and tested is very different from the situation that existed in Oregon under the Veridian project where the Yellowtuft species were allowed to escape from cultivation.

Yellowtuft produces masses of yellow flowers and is a prolific seeder; and its height, from ten to thirty-six inches tall, makes it possible to harvest it by mechanical means. In addition, it is well adapted to the long, hot summers and relatively mild winters of southwest Oregon (http://www.oregonencyclopedia.org/entry/view/alyssum/). As discussed in the EA, the soil conditions in the southeastern U.S. are not conducive to germination and growth of *Eucalyptus* seedlings. The *Alyssum* species used in Oregon produces a large number of seedlings. The serpentine soils where they are being detected are highly conducive to the growth of the species. Supplemental permit conditions (*see* Appendix VII of the EA) established for the permit will effectively limit the reproductive capabilities and establishment of this GE *Eucalyptus* hybrid outside the confined field trial locations and reduce the possibility of unintended exposure from moving GE *Eucalyptus* trees to be negligible to non-existent. Under the supplemental permit conditions established for the permit ArborGen is required to monitor for any seedlings that are produced, remove them and report this to APHIS. The seedlings are easy to detect since they are distinct from other native species and they are readily controlled by the application of EPA approved herbicides. In addition, the GE hybrid *Eucalyptus* trees that would be grown in these confined field tests are similar to those already authorized by APHIS to flower under permits 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r with similar supplemental permit conditions. As presented in Appendix I of the EA, information that has been collected by the applicant and provided to APHIS as part of their annual reporting requirements under the existing permits indicate no volunteer seedlings during the four years that they have been grown and allowed to flower.

State of Florida Official –flowering of trees
An official from the Florida Department of Agriculture and Consumer Services' Division of Plant Industry (DPI) expressed a concern that unlike other permits approved by DPI, these trees will be allowed to flower and that monitoring at the time of flowering and the subsequent information gathered at that time will be crucial to later decisions concerning the release of this cultivar on a larger scale.

Response: APHIS contacted the official and discussed the misunderstanding about the permit with him. APHIS explained that GE *Eucalyptus* trees have been allowed to flower under permits in Florida since 2008 and that reproductive information associated with these permits has been collected by the applicant and provided to APHIS as part of their annual reporting requirements. To date no volunteers have been reported by the applicant. We also discussed the details of the supplemental permit conditions and explained that the permittee is required to scout for and

report the presence of any volunteer seedlings. We also explained that the individual research sites identified in the permit application would be similar in size to the confined field release permits that have been issued by APHIS in Florida. None of which have been over 20 acres in size. The official did not indicate that the agency had had any disagreement or concerns with the analysis provided in the EA.

State of Georgia, District Supervisor Soil & Water District – potential invasive species
This individual was concerned that the *Eucalyptus* that are being field tested could be another invasive species. He states that "until enough controlled tests are run and the American people can know for sure, USDA can control this invasive species, it should not be allowed to be massed planted." He indicates that he is not aware of how many years this has been field tested and under what conditions.

Response: The permit application under consideration by APHIS is for the movement and confined field testing of GE *Eucalyptus* trees on a limited area. APHIS is considering issuing a confined environmental release and interstate movement permit with supplemental permit conditions to allow the interstate movement, planting, field testing and flowering of a GE *Eucalyptus* hybrid clone (Eucalyptus grandis x Eucalyptus urophylla) engineered to express various genes at 6 confined field site locations within the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres. ArborGen has indicated that the trees would be planted on individual research sites ranging from 0.5 up to 7.7 acres, depending on the location with planting density ranging from 300 - 600 trees per acre. The GE hybrid *Eucalyptus* trees that would be grown in these confined field tests are similar to those already authorized by APHIS to flower under permits 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r with similar supplemental permit conditions. As presented in Appendix I of the EA, information that has been collected by the applicant and provided to APHIS as part of their annual reporting requirements under the existing permits have produced no volunteer seedlings during the four years that they have been grown and allowed to flower. As reported by the applicant, these cold tolerant translines grown in these field tests did not produce any pollen, the applicant has not observed any volunteer seedlings in or around the test sites where trees have flowered and produced seed capsules, and monthly field test monitoring observations have not identified any differences in diseases and insects or other non-target organisms between the transgenic and non-transgenic trees in the field test. Based on the analysis and information provided in the EA and supporting permit application, the new genes that are engineered into the trees should not pose any greater risk of loss of confinement and risk to the environment than those that are currently authorized by APHIS for confined field release. The addition of new genes to increase cold tolerance, alter lignin and growth should not compromise the ability of these to remain confined field tests. Adherence to the supplemental permit conditions established for the permit by the applicant will effectively limit any potential adverse impacts to the human environment. As discussed in Section V of the EA, APHIS considers the possibility of unintended exposure from moving GE Eucalyptus trees under the proposed action to be negligible to non-existent. Nothing in the comment indicates that there was any disagreement with the analysis provided in the EA.

Genetic engineering alters genomes in a random and unpredictable fashion
One commenter cited a paper by Latham (2006) which says that "Despite the fact that confidence in the safety and dependability of crop species rests significantly on their genetic integrity, the frequency of transformation-induced mutations and their importance as potential

biosafety hazards are poorly understood." The commenter goes on to state that "Releasing GE trees will mean releasing trees that have never existed before with totally unknown qualities. This is damaging to biodiversity and since they are living organisms this pollution is uncontainable and has the potential to grow over time."

Response: The engineered *Eucalyptus* trees in the proposed confined field tests have been observed over the course of nine years in the laboratory, greenhouse and in confined field tests to identify any unusual or unintended effects due to the transformation process. As confirmed in field tests conducted by the applicant since 2006, only the traits for which the trees have been transformed have been expressed in these trees. In most cases any transformation-induced mutations would have been eliminated early in the transformation process as trees were regenerated in the laboratory and grown for field testing. The permit application under consideration by APHIS is for the movement and confined field testing of GE Eucalyptus trees in a limited area. APHIS is considering issuing a confined environmental release and interstate movement permit with supplemental permit conditions which includes specific monitoring and reporting requirements. In addition, APHIS would also conduct yearly inspections to look for unintended effects. The GE hybrid *Eucalyptus* trees that would be grown in these confined field tests are similar to those already authorized by APHIS to flower under permits 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r with similar supplemental permit conditions. As presented in Appendix I of the EA, information that has been collected by the applicant and provided to APHIS as part of their annual reporting requirements under the existing permits have not identified any differences in diseases and insects or other non-target organisms between the transgenic and non-transgenic trees in the field test. Given the small size of the confined field test sites identified in the permit application and the supplemental permit conditions that would be imposed on the applicant and the fact that the permits can be revoked at any time by APHIS and the trees removed if any unintended effects are found, APHIS does not anticipate that issuance of the confined environmental release and interstate movement permit would impact biodiversity.

Excessive claims of CBI and failure of ArborGen to reveal critical information

A couple of commenters indicated that the designation of the majority of transgenic modifications as CBI prevents any rational evaluation of the impact of those genes and modified *Eucalyptus* on the environment, humans and animals. And because the locations are claimed as CBI it is impossible to create a creditable environmental assessment for the permit application. Also a commenter indicated that normally consideration would be limited to only those species and critical habitat identified by the United States Fish and Wildlife Service and/or National Marine Fisheries Service likely to be found in the area of the field tests. The commenter believed that, without this information, such consideration is impossible. There was a concern that "bystanders exposed to the CBI GM *Eucalyptus* pollen, dust from decaying leaf detritus and pollution of surface and ground water from polluting plant products" would suffer adverse side effects.

Response: APHIS takes the use and designation of CBI very seriously. Like all Federal agencies, APHIS is subject to the Trade Secrets Act (18 U.S.C. § 1905) (TSA). The TSA prohibits officers and employees of federal agencies from publishing or disclosing trade secrets and other confidential business information "to any extent not authorized by law." Information that is claimed as CBI by applicants is evaluated by APHIS to ensure that the claims are legitimate and APHIS evaluated ArborGen's claims for CBI protection in this instance. It would

be a clear violation of the Act for APHIS to reveal Confidential Business Information to the public that legitimately falls under the Act. Federal employees can be fined and/or imprisoned for violating the TSA. Every gene construct component in the permit submission is evaluated for any plant pest risk as well as any potential effects on the environment. None of the genes that have been engineered into these trees (to affect cold tolerance, lignin formation, growth and pollen formation) are expected to have any effects on "bystanders exposed" to the *Eucalyptus* trees or potentially pollute surface and ground water. No pollen is being produced by the trees in the field tests due to the presence of the barnase gene. In addition a thorough analysis for threatened and endangered species and critical habitat are made for every location and if APHIS determines that there could be a species present at the field test site, appropriate contacts are made with the USFWS. A thorough discussion of potential impacts of the proposed action on human health, water resources and threatened and endangered species are presented in Section V and Appendix IV of the EA.

Concern that the CBF genes could have pleiotropic effects and affect allelopathy
One commenter indicated that the C-Repeat Binding Factor (CBF) genes when over expressed display improvement in cold tolerance, water retention, higher oil gland density, wax deposition and over expression of anthocyanin pigments (Navarro et al., 2011). The gene network influenced by CBF gene modification produced highly pleiotropic. The commenter was concerned that the production of allelochemicals in the modified trees did not appear to have been investigated.

Response: The reference cited in the comment (Navarro et al., 2011) does indicate that the gene can have multiple phenotypic effects. As a transcription factor this is not unexpected. As the publication indicates, the multiple changes observed are consistent with the correlation between cold tolerance and drought tolerance and reinforce the hypothesis that, by triggering metabolic changes directed towards limiting cell dehydration, the DREB transcription factors are partly responsible for these observed correlations. As covered in the EA, allelopathy tends to be an inexact science and many studies in allelopathy are inconclusive and difficult to interpret due to potential interactions with other aspects of the environment. Even though the *Eucalyptus* under this permit could demonstrate allelopathic properties, the presence of any allelochemicals is not going to make the *Eucalyptus* planted under these permits more invasive or present a plant pest risk. Since all these field tests are confined and limited in size, any allelopathic effects should be small. As a standard silvicultural practice, herbicides will also be used within the field test sites and any of their effects on understory vegetation will be as severe or more severe than any allelopathic effects.

Pollen spread
One commenter was concerned about pollen spread from the trees and cites an Australian study that found that remnant populations of *Eucalyptus* were connected by pollen dispersal to pollen sources up to 1.94 kilometers away (Sampson and Byrne, 2008). The concern is that the distance between these GE *Eucalyptus* and other *Eucalyptus* that are 1000 meters away would lead to the spread of the GE trees.

Response: As indicated in the EA, the observations made to date by the applicant on APHIS approved confined field tests that have allowed GE *Eucalyptus* hybrid trees to flower indicate that the flowering of the hybrid does not overlap the flowering of other *Eucalyptus* in Florida including one of the parents of the hybrid, i.e. *E. grandis*. Also given that the transgenic hybrid

is not producing any pollen, there is little if any potential for outcrossing of the hybrid to other species. One of the requirements of the existing permits and proposed permit is that the applicant must report any overlap in flowering if it occurs with other *Eucalyptus* tree species in the area. If any overlap in flowering were to occur, the only seeds that could be formed from crossing would be on trees within the test plots whose female flowers could be susceptible to incoming pollen. Monitoring of any seeds and seedling formation in the permitted plots by the applicant would show if any seedlings were being formed as hybrids from incoming pollen sources. In accordance with the supplemental permit conditions, monitoring for and removal of volunteers within 100m from the edge of transgenic test plot by the applicant would effectively eliminate any seedlings that may be produced and thereby effectively reduce the possibility that any *Eucalyptus* hybrid trees would become established.

Concerns about Cryptococcus

One commenter and the form letter was concerned about the spread of *Cryptococcus neoformans gattii* by the transgenic *Eucalyptus* trees indicating that the spread of this "deadly yeast" is mainly brought about by birds and their feces. The commenter appears to take issue with the statement in the EA that the scale of the field tests is miniscule compared to the vast expanses of native trees that could potentially harbor the pathogen and says that "There was no vast expanse of native species homes for the toxic yeast in the peer reviewed scientific publications."

Response: As covered in the EA, it is well documented in the literature that many trees, other than *Eucalyptus*, can harbor *Cryptococcus* in North America. According to the APHIS report cited in the EA (APHIS, 2004): "In the endemic area of Vancouver Island, trees that have tested positive for *C. gattii* include alder, bitter cherry, cedar, Douglas Fir, and Garry Oak." Many years after its first recovery from clinical specimens, *C. gattii* was isolated from the environment for the first time by sampling of *Eucalyptus camaldulensis* trees (Ellis and Pfeiffer, 1990). This tree species was initially thought to be the exclusive natural habitat of *C. gattii* both in its native Australia and around the world given that the tree has been exported extensively. However now it is clear that more than 50 tree species have been reported as harboring the fungus (Springer and Chaturvedi, 2010). Although *Eucalyptus* is present in many of the areas known to have *C. gattii*-associated cryptococcosis, the actual isolation of *C. gattii* from *Eucalyptus* trees is rare outside Australia, despite extensive sampling. Imported *Eucalyptus* has not been associated with the environmental presence of *C. gattii* in Spain, central Africa, or Canada; and most *Eucalyptus* trees tested in Papua New Guinea, Egypt, and Italy were negative for *C. gattii*. When C. gattii was first associated with cryptococcosis, early environmental surveys for *C. gattii* in imported *Eucalyptus* spp. rarely included other local tree species for testing. Although understandable, this was unfortunate because *C. gattii* is now known to have extensive associations with other tree species (Springer and Chaturvedi, 2010).

C. gattii is clearly not specific to *Eucalyptus* trees in North America and other areas of the world and is most likely being spread by birds and other animals (Springer and Chaturvedi, 2010) throughout temperate tree species since very little *Eucalyptus* is present in these areas. Therefore, the planting GE *Eucalyptus* trees in confined field tests in southeast would not be expected to increase the incidence of the disease any more than planting any other tree species, since many species can host the fungus. Also it is important to note that the recent literature has begun to sort out the difference between *C. neoformans* and *C. gattii*. Until recently, these two fungi were classified as varieties of the same species but are now considered two different species. The pathogen *C. gattii* is believed to be clinically more virulent than *C. neoformans* and

causes more severe symptoms of cryptococcosis. For a review see (Chaturvedi and Chaturvedi, 2011).

Concerns about the alteration in susceptibility to diseases or insects
Commenters were concerned that the susceptibility of the trees to insects and disease were not adequately addressed in the EA, particularly the trees that have altered lignin.

Response: The GE hybrid *Eucalyptus* trees that would be grown in these confined field tests are similar to those already authorized by APHIS to flower under permits 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r with similar supplemental permit conditions. As presented in Appendix I of the EA, information that has been collected by the applicant and provided to APHIS as part of their annual reporting requirements under the existing permits have not identified any differences in diseases and insects or other non-target organisms between the transgenic and non-transgenic trees in the field test. Growth measurements have indicated that trees had normal to a moderately reduced growth phenotype. The trees have been visually inspected on a monthly basis for the presence of any insect and disease damage. As reported in the applicant's annual reports, the degree of insect and disease infection has been minimal. Adherence to the supplemental permit conditions established for the permit by the applicant will effectively limit any potential adverse impacts to the human environment. However, if during the tests there is evidence of increase disease or insect susceptibility, the applicant is required to report this to APHIS. The permittee is required to report any such unanticipated effects (including excessive mortality or morbidity) to APHIS under the terms of the permit - *see* 7 CFR 340.4(f)(10)(ii). An assessment of disease and insect susceptibilities is one of the primary reasons the applicant is conducting the confined field testing of the trees.

Kanamycin resistance
One commenter raised an issue about the risks from the kanamycin resistance gene. The commenter indicated that kanamycin is still in clinical use and also kanamycin resistance cross-reacts with new antibiotics.

Response: In 1998, FDA developed draft guidance on the use of antibiotic resistance markers in transgenic plants http://www.fda.gov/food/guidancecomplianceregulatoryinformation/guidancedocuments/biotechnology/ucm096135.htm. In this document, FDA concluded that the likelihood of the transfer of antibiotic resistance is remote. In this draft guidance the FDA stated, "FDA acknowledges that the likelihood of transfer of an antibiotic resistance marker from plants to microorganisms in the gut or in the environment is remote and that, such transfer, if any, would likely be insignificant when compared to transfer between microorganisms, and in most cases, would not add to existing levels of resistance in bacterial populations in any meaningful way." Further, to evaluate safety, the European Food Safety Authority (EFSA) reviewed the antibiotic selection markers used in genetically engineered plants http://www.efsa.europa.eu/en/efsajournal/doc/opinion_gmo_05_en1.pdf. In this 2004 document, various antibiotic resistance genes were assigned into groups based on the criteria of therapeutic use in humans and in animals and presence in the environment; Group I is composed of kanamycin and hygromycin resistance genes. The opinion states that because of the frequency of horizontal transfer plants to other organisms is very rare, previous existence in the environment and the history of use of the kanamycin resistance, that there is no rationale for restricting Group I antibiotics. In addition, the EFSA 2004 document states: Kanamycin is

rarely used today because of its considerable side effects. Only under conditions of multiple mycobacterial resistance to other drugs is kanamycin still used as a reserve tuberculostatic agent. For the same reason as kanamycin, neomycin, which is poorly absorbed orally, is also rarely used intravenously/intramuscularly to treat infections. These antibiotics are rarely administered orally, which minimizes the selective pressure for antibiotic resistance in the gut. Their use in the treatment of humans has been superseded by more effective aminoglycoside antibiotics that are not substrates for APH(3')-II. The antibiotics are rarely used in agriculture or aquaculture and thereby do not provide selective pressure for a possible transfer of the resistance genes from genetically modified plants to soil microorganisms.

Gene for altered fertility

Commenters were concerned about the "dangers of the barnase gene" and stated that it is a known cytotoxic protein that breaks down RNA. "Because they are from a soil bacterium, and unrelated to any mammalian RNAses, they are not susceptible to eukaryotic 3 RNAse inhibitors. Consequently, they are highly toxic, and are actually being engineered currently as a means of killing cancer cells. Although the barnase is being used to prevent pollen formation, this is not 100 [%] effective, and many beneficial insects and other wild-life could well be affected."

Response: As indicated in the EA, the transgenic *Eucalyptus* hybrid identified in the permit application utilizes barnase to produce male sterile plants, reducing the potential for gene flow into the environment. Male sterility is achieved through the localized production of barnase in pollen producing cells. Barnase is a ribonuclease, an enzyme that degrades RNA, thereby regulating protein synthesis. Ribonucleases are highly ubiquitous molecules found in all living cells (Worrall and Luisi, 2007). The transgenic *Eucalyptus* trees identified in the permit application and other permits already authorized by APHIS were engineered using barnase gene mutants with reduced toxicity to minimize tissue damage (Rottmann et al., 2008). The barnase production is controlled by a tissue specific promoter. As with all genes, the gene encoding barnase is present in every cell of the transgenic plant, however, the promoter acts like an "on" switch that controls when and how strongly the gene is expressed. The PrMC2 promoter used by ArborGen, originally identified in pine (*Pinus radiata*), restricts barnase production to the tapetum, a small layer of cells within the male floral organ, or anther (Walden et al., 1999; Hofig et al., 2003; Rottmann, et al., 2008). Within the anther, the tapetum surrounds developing pollen grains. Mariani, et al. (1990) developed transgenic tobacco plants using the barnase gene also controlled by a tapetum-specific promoter (Tap29). They observed that in transgenic plants tapetal cells senesced early in their development, preventing pollen formation. All other floral organs, including the anthers, formed normally proving tapetal cells which could be eliminated without affecting flower development. Similar results were observed using PrMC2 in *Eucalyptus* trees (Rottmann et al., 2008). After several years of research, no pollen has been observed in transgenic *Eucalyptus* trees as reported in the permit application and in annual reports submitted by ArborGen up through 2011 (*see* Appendix I of the EA). As with other ribonucleases, barnase degrades quickly after the destruction of tapetal cells, and does not accumulate within the plant tissues (Mariani et al., 1990); http://www.fda.gov/Food/Biotechnology/Submissions /ucm161162.htm). Therefore barnase is only produced for a short period of time during floral development, is limited to a discrete cell layer within the anthers of the flower, and is rapidly degraded (Mariani et al., 1990; Rottmann et al., 2008; Hofig et al., 2003). In previous studies using transgenic radicchio containing the barnase gene, researchers were unable to detect accumulation of barnase within floral tissue (http://www.fda.gov/Food/Biotechnology/Submissions/ucm161162.htm). Therefore it is highly

unlikely that consumption of, or exposure to, transgenic *Eucalyptus* could contain enough barnase to cause mammalian or insect toxicity. Direct exposure of organ tissue, is also highly unlikely to occur in nature. In addition the FDA has previously reported that consumption of barnase would likely degrade quickly during digestion further reducing the risk of barnase exposure (http://www.fda.gov/Food/Biotechnology/Submissions/ucm161162.htm).

With regard to toxicity of barnase to bees and other pollinating insects, there is no clear evidence indicating pollinators would be adversely affected by ingestion of barnase. Combined with the facts that barnase is only produced during the short period of tapetum formation and is quickly degraded, as well as the lack of pollen produced by the *Eucalyptus* hybrids, it can be concluded the tissue specific production of barnase is unlikely to adversely affect pollinators or other insects. APHIS has made a determination of nonregulated status of three plant species, corn (petitions 95-288-01p, 97-342-01p, 98-349-01p), rapeseed (petitions 98-278-01p, 01-206-01p) and chicory (petition 97-148-01p) since 1995 that have included the barnase gene. APHIS is unaware of any reported cases of mammalian or insect toxicity resulting from barnase consumption or exposure occurring within the past 17 years.

Based on these findings, no hazard or exposure associated with the use of barnase has been identified, therefore APHIS concludes that there is no foreseeable risk associated with the use of barnase in transgenic *Eucalyptus* trees in the proposed confined field tests.

Mode of transformation and hazards of horizontal gene transfer
One commenter expressed concerns about horizontal gene transfer between the Agrobacterium used to transform the plants to wild-type *Agrobacterium* in the soil, and to other bacteria and fungi in the soil, citing a publication by (Knight, 2010).

Response: It is highly unlikely that any Agrobacterium remains on the plants that are generated in the laboratory since antibiotics are used to remove them prior to release into soil. During the *Eucalyptus* transformation process, the transformed tissue is kept on selection medium containing antibiotics to kill any residual Agrobacterium for at least four months, following which individual events are transferred to medium without selection. As indicated by the applicant, Agrobacterium growth has never been observed in the cultures at this stage. ArborGen has also collected vegetative tissue from select tissue cultures as well as field-grown transgenic plants and analyzed for the VirD gene which would indicate the presence of Agrobacterium. The lack of detectable VirD signal in samples indicates that no live or dormant Agrobacterium remains in the transgenic plants.

High flammability of *Eucalyptus*
Several commenters including the form letter noted the high flammability of *Eucalyptus* which allows *Eucalyptus* plantations to be susceptible to initiation or rapid spread of wildfire.

Response: As indicated in the EA, it is not clear that *Eucalyptus* plantations present a greater fire hazard than do the pine plantations which are common in the Southeastern USA. To assess the risk of wildfire in live and dead material, (Núñez Regueira et al., 2002a; Núñez Regueira et al., 2002b) in northern Spain used calorimetry analysis combined with multidimensional assessment of climatic and physical characteristics in plantations of a *Pinus* species (*P. pinaster*) and of a *Eucalyptus* species (*E. globulus*). For pine, the fire risk index for live biomass varied from extremely high in July to little risk in some winter and spring months. Pine residues,

however, varied between no risk and extremely high risk. For *Eucalyptus*, fire risk index for live biomass varied from a middle risk in July to little risk in fall and winter. Dead matter of *Eucalyptus* varied between a middle risk to extremely high risk in August. Ganteaume et al. (2009) undertook a variety of assays and comparisons of fuel bed flammability and firebrand capacity (the material that can ignite the fuel bed). *Pinus* litter flammability was higher than that of *Eucalyptus*, which in turn was higher than that of a hardwood species (*Ulex*). While differences between southeastern pines and the proposed *Eucalyptus* species and these analyses may be important, basic similarities within the genera are likely relevant. For example, studies of different *Eucalyptus* species have shown that despite the differing climactic specificities of *E. globulus* and *E. nitens* and differences of volatile content and solid mass, the total caloric content of wastes after pulping are similar (Perez et al., 2006). Due to the small size of these field trials, and the fact that they are isolated from one another by many miles, there is no reason to believe that they pose any more of a fire risk than do other forest tree plantings

Wildfires in California and Australia

Some of the comments and the form letter cited two large wildfires in arid areas of Australia and California involving *Eucalyptus*. These fires were mentioned as examples of deleterious consequences of *Eucalyptus* plantings.

Response: These fires occurred in areas of extensive plantings of *Eucalyptus*, but it is likely that many factors are important for the frequency and season of occurrence of fires, not just the species of trees within a burned area. One post-fire report on the Oakland, California fire noted that extended drought conditions and freeze-killed *Eucalyptus* contributed large amounts of residue for the fire. However, a wide variety of grasses, brush, trees, and houses combined to provide fuel that sustained this fire, and the fire had not initiated in the *Eucalyptus* stands (FEMA, 1991). As noted, one of the most important risk factors for *Eucalyptus* production is the presence of accumulating fuels within a stand (Núñez Regueira, et al., 2002a). The duration of these field trials will be limited (no more than seven to nine years). ArborGen has seen little accumulation in field trials extending through five years (Les Pearson, ArborGen, personal communication) and consequently little to no accumulating fuel is expected to arise within the proposed confined field trials.

Failure to Consider the Environmental Effects of *Eucalyptus grandis X Eucalyptus urophylla* Pollen on Bee Populations

One commenter was concerned that the EA did not consider the effect of pollen on bee populations. There was a concern that when the flower pollen becomes genetically modified or sterile, the bees will potentially go malnourished and die of illness due to the lack of nutrients and the interruption of the digestive capacity of what they feed on through the summer and over the winter hibernation process. He also indicates that there already exist sufficient acreage and plots to capture and test pollen from GMO *Eucalyptus* and to monitor its effect on bee populations in proximity to them. And if this process is shown to have no deleterious effects on existing bee populations, over a series of years should any proposal to expand the range of GMO *Eucalyptus* be considered.

Response: It is important to note that the trees are not producing any pollen, therefore the studies that are proposed by the commenter cannot be performed. Also, as discussed above and within the EA, there is no reason to believe that the products of the introduced genes would have any negative effects on insects such as bees. ArborGen has observed many bees visiting the

Eucalyptus flowers in the field test because the flowers are very attractive to bees and they feed on the nectar produced by the flowers. As noted above no products (including barnase) produced by the inserted genes would be expected to affect bees. No dead bees have been observed in the vicinity of the field tests.

Unacceptable Environmental Risks: Water Resources
Several comments including the form letter noted the potential impacts of *Eucalyptus* on hydrology. The comments centered on concerns that tree plantations have been documented to deplete ground water and cause or exacerbate drought situations. They also cited the information that the Forest Service provided upon APHIS' request as an appendix to the EA to indicate that the risk is unacceptable.

Response: The proposed action would be limited to 6 well dispersed confined field site locations in the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres on individual research sites ranging from 0.5 up to 7.7 acres. ArborGen has supplied data to APHIS indicating the maximum size of each of the plantings at each site, the individual watersheds where the plantings occur, the area of the watershed, how much of the watershed will be occupied by the field tests, the location of the closest primary and secondary streams, and the location of any critical habitat for Federally listed threatened and endangered species within the watershed. The data provided by ArborGen show that none of the potential planting sites identified in the permit application occupy more than 0.005% of any given watershed.

The potential impacts by Eucalyptus on hydrology relate to the scale of the plantings. The proposed confined field trial plantings are very small in terms of scale in forestry practices and watersheds. Individual forest plantings in the southern U.S. range in size but can typically be up to 120 acres at a single site in a given year (SFI standards: http://www.sfiprogram.org/files/pdf/sfi_requirements_2010-2014.pdf). A 20 acre planting is considered small in these terms. The issue of hydrology is thoroughly addressed in Section V of the EA. As noted in the section under Potential Effects of Growing *Eucalyptus* on Soil Hydrology in the EA, the Forest Service has pointed out that the significance of the impact on groundwater and stream flow will depend greatly on the area extent, size, and spatial distribution of the plantations. For example, a few small (less than 10 hectares, i.e. approximately 25 acres) and well-dispersed plantations may only have very localized impacts and negligible impacts at the watershed scale. Based on the very small footprint of these proposed confined field tests and the weight of evidence, APHIS concludes that the impacts of these field trials on hydrology will be negligible.

Unacceptable environmental risks: Native Flora and Fauna
One commenter noted a comment made on a previous EA for GE *Eucalyptus* permits. The commenter states that "The Georgia Department of Natural Resources, Wildlife Resources Division has recommended that ArborGen's permit requests be denied because the Department has "serious concerns about potential impacts on hydrology, soil chemistry, native biodiversity and ecosystem functions regardless of whether this nonnative hybrid turns out to be invasive in a plantation setting." They further note that *Eucalyptus* plantations "will be extremely inhospitable environments for native flora and fauna." Moreover, noting the high water use for *Eucalyptus* plantations, the Department expressed concern regarding the increased potential for significant impacts on water resources and aquatic communities.

Response: The potential impacts of the proposed action on native flora and fauna is thoroughly analyzed in Section V of the EA and discussed in several of the responses above.

The GE hybrid *Eucalyptus* trees that would be grown in these confined field tests are similar to those already authorized by APHIS to flower under permits 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r with similar supplemental permit conditions. As presented in Appendix I of the EA, information that has been collected by the applicant and provided to APHIS as part of their annual reporting requirements under the existing permits have produced no volunteer seedlings during the four years that they have been grown and allowed to flower. As reported by the applicant, these cold tolerant translines grown in these field tests did not produce any pollen, the applicant has not observed any volunteer seedlings in or around the test sites where trees have flowered and produced seed capsules, and monthly field test monitoring observations have not identified any differences in diseases and insects or other non-target organisms between the transgenic and non-transgenic trees in the field test. Based on the analysis and information provided in the EA and supporting permit application, the new genes that are engineered into the trees should not pose any greater risk of loss of confinement and risk to the environment than those that are currently authorized by APHIS for confined field release. The addition of new genes to increase cold tolerance, alter lignin and growth should not compromise the ability of these to remain confined field tests. Adherence to the supplemental permit conditions established for the permit by the applicant will effectively limit any potential adverse impacts to the human environment. As discussed in Section V of the EA, APHIS considers the possibility of unintended exposure from moving GE Eucalyptus trees under the proposed action to be negligible to non-existent.

Failure to Assess Cumulative Effects of all existing ArborGen permits

One commenter expressed the concern that since there are now six active permits under which ArborGen is authorized by APHIS to grow GE *Eucalyptus* which include 32 unique locations within 7 States where up to a total of 654.7 acres of GE *Eucalyptus* could be planted, that the cumulative impacts of this proposal are substantial and significant and should be considered.

Response: A cumulative impacts analysis is provided in Section V of the EA. As identified in the permit application, GE *Eucalyptus* trees with the new constructs would be released at 6 locations. The new permit application does not expand the number of trees or total acreage that could be planted at any of the confined release sites currently approved by APHIS. As described in the EA and other EAs prepared by APHIS for authorizing ArborGen permits (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf and http://www.aphis.usda.gov/brs/aphisdocs/08_014101r_ea.pdf), the 32 field test sites are widely dispersed across the southeastern US. The sites are 900 miles apart from the farthermost east and west plantings. They average approximately 50 miles apart. The closest plantings have at least a mile separation between them, no individual planting is greater than 20 acres in size and there is no reason to assume that there will be any interaction between these distant sites. The small size of the field plots and the distance between test sites spread over thousands of miles indicate no cumulative effects would result from APHIS issuing the proposed field release permits. Therefore the only past, present, and reasonably foreseeable actions associated with the locations for the proposed releases under permit are those related to agricultural or forest tree production. Based on the analysis provided in the EA, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed

action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the release sites or the ecosystem in which they are situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

Failure to Consider a Reasonable Range of Alternatives

One commenter said: "While the DEA contains only two alternatives, it actually only has one: the proposal contained in Arborgen's application. The other alternative is the "no action" alternative, which is to deny the application. It therefore fails to include a range of alternatives. The commenter suggests two additional alternatives should be analyzed in the DEA:

- One which excludes the change in conditions, the allowed flowering, of genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm, and
- One which both excludes the change in conditions, the allowed flowering, of genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm, but also does not allow the flowering of genetically engineered *Eucalyptus* trees at the 6 new sites.

Response: APHIS has prepared this EA in response to confined environmental release permit applications (APHIS Number 11-052-101rm) received from ArborGen LLC. This permit application was submitted in accordance with APHIS' regulations in 7 CFR part 340. In accordance with these regulations when APHIS receives an application for a permit for environmental release, the application is evaluated to determine whether the environmental release, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

> *Administrative action on applications*. After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requested by APHIS, a permit shall be granted or denied (7 CFR 340.4(e)).

The applicant has provided the information associated with this request in the permit application and APHIS now must make a determination to either grant or deny the permit. Since APHIS decision is to either grant or deny the permit application, APHIS analysis of the two alternatives (No Action – Deny the permit and Preferred Alternative – Issue the APHIS Permit) identified in the EA are reasonable. The EA analyzes the potential cumulative effects of the proposed field test locations and has shown that the issuance of the permits will not have a significant impact on the human environment. Further analysis on a site by site basis or over a more restricted space or time will not provide any additional information or analysis that would be necessary to make an informed NEPA decision on the issuance of these permits.

The alternatives suggested by the commenter have been analyzed in the EA as either the No Action Alternative or the Preferred Alternative. As clearly stated in Section V of the EA, GE *Eucalyptus* trees grown under the confined field release permits 09-070-101rm and 11-041-101rm would not be allowed to flower under the No Action Alternative. An additional alternative that analyzes the potential impacts of not allowing GE *Eucalyptus* trees to flower at

any of the proposed 6 confined release sites would not provide any additional information to the decision maker than what is already provided under the Preferred Alternative. In effect, the supplemental permit conditions under the Preferred Alternative will have the same impact on the environment as would an alternative that does not allow the trees to flower. As described in the EA, the supplemental permit conditions (*see* Appendix VII of the EA) established for the permit will effectively limit the reproductive capabilities and establishment of this GE *Eucalyptus* outside the confined field trial locations.

Failure to prepare an EA or EIS on permits 09-070-101rm and 11-041-101rm
A commenter said: "Because permits 09-070-101rm and 11-041-101rm and all previous permits were approved without completing either an EIS or an EA, there has not been an environmental assessment of any sort on the environmental effects of introducing *Eucalyptus grandis X Eucalyptus urophylla*. This includes, but is not limited to critical habitat analysis under ESA, effects on hydrology and effects of pollen on bee populations, as referenced in previous sections of this document. APHIS did not provide public notice or an opportunity for public comments or request an ESA determination from FWS. Therefore the environmental effects of all of these permits must be considered in any environmental analysis, immediate, cumulative or otherwise."

Response: This comment is outside the scope of this EA. APHIS has prepared this EA specifically to evaluate and determine if there are any potentially significant impacts to the human environment in response to a confined environmental release and interstate movement permit application (11-052-101rm) received from ArborGen to authorize the planting of genetically engineered (GE) Eucalyptus hybrid clone (*Eucalyptus grandis* x *Eucalyptus urophylla)* to support proposed field research studies on six research sites in Alabama, Florida, Mississippi, and South Carolina. A discussion of potential impacts associated with permits authorized by APHIS for GE *Eucalyptus* is presented in the cumulative impacts analysis provided in Section V of the EA and the "cumulative effects" response above.

All regulatory actions taken by APHIS, including the authorization of permits 09-070-101rm and 11-041-101rm, are conducted in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. § 4321 et seq.), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372).

REFERENCES:

APHIS (2004). The emergence and colonization of *Cryptococcus gattii* in British Columbia. Emerging disease notice. December 22, 2004. Available online at: <http://www.aphis.usda.gov/vs/ceah/cei/taf/emergingdiseasenotice_files/cryptococcal_disease_britishcolumbia.htm>.

Chaturvedi, V. and S. Chaturvedi (2011). Cryptococcus gattii: a resurgent fungal pathogen. Trends in Microbiology 19: 564-571.

Ellis, D. H. and T. J. Pfeiffer (1990). Natural Habitat of Cryptococcus neoformans var. gatii. Journal of Clinical Microbiology 28: 1642-1644.

FEMA (1991). The East Bay Hills Fire, Oakland-Berkeley, California. U.S. Fire Administration/Technical Report Series. USFA-TR-060/October 1991.

Ganteaume, A., C. Lampin-Maillet, et al. (2009). Spot fires: fuel bed flammability and capability of firebrands to ignite fuel beds. International Journal of Wildland Fire 18: 951-969.

Hofig, K. P., R. L. Moyle, et al. (2003). Expression analysis of four Pinus radiata male cone promoters in the heterologous host Arabidopsis. Planta 217: 858-867.

Knight, C. J., A. M. Bailey, et al. (2010). Investigating Agrobacterium-Mediated Transformation of Verticillium albo-atrum on Plant Surfaces. PLoS ONE 5(10): 1-5.

Latham, J. R., A. K. Wilson, et al. (2006). The Mutational Consequences of Plant Transformation. Journal of Biomedicine and Biotechnology 2006: 1-7.

Mariani, C., M. De Beuckeleer, et al. (1990). Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature 347: 737-741.

McKeand, S. E., B. J. Zobel, et al. (2007). Southern Pine Tree Improvement - A Living Success Story. In, Southern Forest Tree Improvement Conference - 2007. http://www.rngr.net/publications/sftic/2007/southern-pine-tree-improvement-2013-a-living-success-story/?searchterm=McKeand.

Navarro, M., C. Ayax, et al. (2011). Two EguCBF1 genes overexpressed in *Eucalyptus* display a different impact on stress tolerance and plant development. Plant Biotechnology Journal 9: 50-63.

Núñez Regueira, L., J. A. Rodrıguez Añón, et al. (2002a). Determination of risk indices corresponding to eucalyptus in Galicia using bomb calorimetry. Thermochimica Acta 394: 267-278.

Núñez Regueira, L., J. A. Rodrıguez Añón, et al. (2002b). Using bomb calorimetry for determination of risk indices of wildfires originating from pine residues. Thermochimica Acta 394: 291-304.

Perez, S., C. J. Renedo, et al. (2006). Energy evaluation of the *Eucalyptus globulus* and the *Eucalyptus nitens* in the north of Spain (Cantabria). Thermochimica Acta 451: 57-64.

Rottmann, W. H., K. H. Norris-Caneda, et al. (2008). *Reproductive ablation constructs* - Patent number 7,453,025. United States, *ArborGen*, LLC

Sampson, J. F. and M. Byrne (2008). Outcrossing between an agroforestry plantation and remnant native populations of *Eucalyptus loxophleba*. Mol Ecol. 11: 2769-2781.

Sheffield, R. (2009). Planted forests and plantations. Forest resources of the United States, 2007. Gen. Tech. Rep. WO-78. Washington, DC: U.S. Department of Agriculture, Forest Service, Washington Office. 67-69. Eds. W. B. Smith, P. D. Miles, C. H. Perry and S. A. Pugh.

Springer, D. J. and V. Chaturvedi (2010). Projecting global occurrence of Cryptococcus gattii. Emerg. Infect. Dis. 16: 14–20.

Walden, A. R., C. Walter, et al. (1999). Genes expressed in *Pinus radiata* male cones include homologs to anther-specific and pathogenesis response genes. Plant Physiology 121: 1103-1116.

Worrall, J. A. and B. F. Luisi (2007). Information available at cut rates: structure and mechanism of ribonucleases. Current Opinion in Structural Biology 17: 128-137.