Union of Concerned Scientists
Citizens and Scientists for Environmental Solutions

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Regulatory Analysis and Development
USDA APHIS PPD
Station 3A-03.8
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To whom it may concern:

The Union of Concerned Scientists (UCS) appreciates the opportunity to comment on the environmental assessment (EA) prepared by the Animal and Plant Health Inspection Service (APHIS) for a proposed field test of genetically engineered (GE) hybrid eucalyptus.[1] The trees in this proposed field test contain two unidentified cold-tolerance genes, an undisclosed gene for reduced flower development, and an undisclosed selectable marker, under permit application 06-325-111r. The permit would allow growth and flowering for three years, after which the applicant would be allowed to request an extension of the permit.

UCS, the leading science-based nonprofit working for a healthy environment and a safer world, combines independent scientific research and citizen action to develop innovative, practical solutions and secure responsible changes in government policy, corporate practices, and consumer choices. A major goal of UCS's Food and Environment Program is to strengthen the regulatory system that applies to products of agricultural biotechnology.

Significance of Proposed Trial

This proposed field test is important for several reasons.

- As the first test we are aware of to allow flowering and possible seed development in a GE forest tree species, it will set precedents for risk assessment.
- Forest trees are relatively undomesticated, and as such are capable of surviving and spreading in suitable environments without human assistance. They differ in this respect from most crop plants, which do not thrive in the environment without human cultivation. Therefore, forest tree species pose a risk of gene flow beyond the test site by outcrossing or seed escape.
- Because they are often important members of ecosystems, they may have far-ranging impacts if they escape and spread.
- Finally, as an introduced species, this GE hybrid may have the capacity to become invasive, thereby seriously disrupting native ecosystems, as has been the case with several other eucalyptus species introduced into California.

Inadequacies in APHIS's Environmental Assessment

APHIS has concluded in its EA that the proposed field test "…should not present a risk of introduction and dissemination of a plant pest and should not have a significant impact on the quality of the human environment" (EA, p. 5).

UCS disagrees with APHIS's risk assessment for two reasons. First, the types of genes involved could facilitate gene flow and spread of these transgenic trees into the environment, and second, APHIS has not supported its conclusions about gene flow or environmental impact with substantive data. Instead of data, APHIS bases its conclusions largely on poorly supported inference and selective interpretation of information about eucalyptus species. Although APHIS proposes several conditions to prevent gene flow, these conditions are inadequate to ensure that gene flow will not occur.

We are particularly troubled that APHIS seems to have neglected admonishments from recent federal lawsuits that criticized the agency for lax assessment of gene flow risks. APHIS also continues to ignore the assessment of the National Research Council (NRC) of the National Academy of Sciences, which criticized the agency for lack of rigor in its risk assessments.[2] The NRC criticized APHIS for frequently concluding that the absence of risk data meant that no risk existed. The NRC pointed out that the absence of evidence about a risk is not the same as evidence of the absence of a risk. In other words, when data about a risk are lacking, it is improper to conclude, as APHIS continues to do, that the risk is insignificant. In continuing to draw conclusions of no risk based on little or no data, APHIS has not fulfilled its responsibilities under the National Environmental Protection Act (NEPA) to take a "hard look" at the risks from the proposed field test.

We also are disappointed that APHIS has allowed the applicant to declare the identity of the genes to be confidential business information (CBI) without providing any reasons for doing so. It is not possible for the public to provide completely informed comments, and thus fulfill the purpose of these comments, when critical information such as gene names is withheld.

It is especially difficult to understand how a selectable marker gene that APHIS acknowledges has been used in several commercialized transgenic crops could be claimed as CBI. Risk from different markers can differ substantially. For example, an herbicide-tolerance marker could reduce the ability to control escaped GE eucalyptus, while resistance to an antibiotic like kanamycin would not. Use of a glyphosate-tolerance gene as a marker would carry even greater risk, because glyphosate is widely used to control invasive plants – especially in and near wetlands.

Based on these reasons and our detailed assessment below, UCS recommends that the proposed field tests not be allowed, or at least the trees not be allowed to flower, until data are acquired that absolve the field test of significant risk.

UCS Analysis of the Risk of Escape of Transgenic *Eucalyptus* Beyond the Test Plot

Contribution of Cold-Tolerance Genes to Invasiveness or Other Environmental Harm

APHIS concludes that cold-tolerance genes are "not expected" to alter the weediness of the trees. This claim is made suspect by APHIS admission that susceptibility to cold limits the establishment of these trees in most of the southern United States. APHIS arrives at its conclusion by claiming that the cold-tolerance genes would not be expected to affect (enhance) the reproductive biology of the trees, and thereby overcome "difficulty" that these trees have in becoming established without human intervention.

However, there is no accepted definition of invasiveness that requires such traits to "…affect the reproductive biology such as seed production or vegetative reproduction capabilities" (EA, p. 14), as APHIS suggests. Predictions about invasiveness based on a few characteristics of an introduced species are not reliable. On the other hand, some species of *Eucalyptus* are invasive in the United States, and several others have become established. The fact that several *Eucalyptus* species have been able to grow in the wild in the United States should dictate a more careful analysis by APHIS. APHIS cites no data on the competitiveness of this hybrid with native flora in Alabama, nor any references in the scientific literature that supports its contention that these hybrids would not be competitive or invasive in Alabama if they were to survive the cold.

The EA notes that "The species that ArborGen wishes to allow to flower under this permit have not been categorized as invasive..." (EA, p.10). However, in so stating, APHIS provides no discussion about how and where the parent species have been tested for invasiveness, or where these species have been grown, and for how long. Without this information, APHIS's position is unsupported by needed data. Moreover, it is unclear whether any pertinent data exist to support it. Furthermore, general discussion about *Eucalyptus* species is inadequate, because invasiveness may be dependent on the specific characteristics of the biotic and abiotic environment where a species is grown. For example, most invasive species are not invasive in their native environments, and it is only when introduced to new environments that they become invasive. It is unsupportable for APHIS to dismiss the possibility that this hybrid could become invasive if it could survive the cold without performing a thorough analysis of its competitiveness. All that is known is that one barrier to possible invasiveness or establishment would be removed by the addition of the cold-tolerance genes, i.e., the current inability to survive the cold.

APHIS is setting too narrow a standard for potential environmental harm by focusing only on the possible invasiveness of GE *Eucalyptus*. Transgenic cold-tolerant *Eucalyptus* would not necessarily have to become invasive or noxious, as the EA seems to imply, to potentially harm the environment under NEPA. The establishment of these transgenic plants, which could be virtually permanent or very expensive to eliminate if not detected quickly, could harm the environment by other means, without becoming invasive. For example, many native herbivores may be unable to consume the foliage of these trees, and thus the displacement of native trees by *Eucalyptus* would cause herbivores to lose food sources. Other herbivores may be harmed by consuming this foliage. Alternatively, some native herbivores may be selectively favored by their ability to feed on *Eucalyptus* foliage, and thereby increase to undesirable levels. The presence of permanently established transgenic plants in the environment may also be considered harmful under NEPA, as

held in a recent federal court ruling concerning genetically engineered creeping bentgrass.[a] A proper assessment of harm pursuant to NEPA must include analyses of all reasonably foreseeable potential environmental impacts.[b]

APHIS discusses whether the transgenes may produce toxic substances, and concludes otherwise, without providing any supporting data. APHIS fails to consider that the trees themselves, independent of transgenes, could be harmful, and that the transgenes could directly facilitate the establishment of possibly harmful tree species in Alabama. In preparing this EA, APHIS did not take a "hard look" at the potential impacts of the approval, as it must to comply with NEPA.[c]

There is at least one precedent for cold tolerance as a contributor to invasiveness. In Britain, the introduced Rhododendron, *R. ponticum*, is a destructively invasive species whose range is believed to have been enhanced by acquiring cold tolerance from another introduced *Rhododendron* species.[3, 4] *R. ponticum* comes from the Iberian Peninsula and is not well adapted to the colder climate of Britain, especially some of the colder regions of the British Isles. *R. ponticum* has been shown to have acquired genes from the cold-tolerant species, *R. catawbiense*. Hybrid *R. ponticum* now causes destruction of native heath ecosystems,[5] with mitigation costing millions of dollars. Despite these data, there is no evaluation in the EA of cold tolerance contributing to invasiveness or harm.

Dispersal and Establishment of GE Hybrid Eucalyptus Seed

The EA claims that production of seeds, dispersal of seeds, and establishment of transgenic plants beyond the test site are highly unlikely, and therefore there is no significant risk of gene flow or escape. None of these arguments is supported by data, without which they are inadequate.

APHIS claims that *Eucalyptus* has a high level of self-incompatibility, which it expects to significantly limit crossing, and therefore seed production, within the test plot. However, self-compatibility is highly dependant on the specific clone, with fertility levels varying substantially. For example, Pound et al. note that self incompatibility in three individual *E. globulus* trees (the species in which most of this work has been done) varied from 76% to 100%.[6] Without data showing the actual level of self-compatibility in the transgenic clone to be grown in the proposed field test, APHIS's claim has no basis.

APHIS also refers to unpublished "preliminary experiments" conducted by the applicant in which self-pollinated seed obtained from "this genotype…had abnormal morphology and failed to germinate" (EA, p. 11).[d] These experimental data are also inadequate because they fail to disclose the number of seeds tested. Without the number of tested seed, it is impossible to determine the level of viability. Unless a large number of seeds were tested, there can be no confidence that the viability is low enough to ensure that gene flow is highly unlikely. For example, the several hundred trees of the proposed test may produce millions of seeds (since the level of self incompatibility is

[a] See *International Center for Technology Assessment v. Johanns*, 473 F. Supp. 2d 9 (D. D.C. 2007).
[b] See 40 C.F.R. 1502.4, 1508.8, 1508.18 & 1508.25.
[c] See, e.g., *Save the Yaak v. Block*, 840 F. 2d 714, 717 (9th Cir. 1988).

[d] APHIS fails to say whether they are referring to the clone genotype, or the specific GE genotypes of the proposed field test.

not disclosed, we simply do not know how many). In that case, even low levels of seed viability could result in establishment outside the test plot if such seeds escaped.

APHIS notes that *Eucalyptus* seed is "...very light and small..." but claims that it is "not adapted to wind dispersal" and therefore is expected to "generally" be confined to a radius of about twice the height of the tree (EA, p. 11-12). This assessment relies on references that are not cited as accounting for the effect of high wind speeds on dispersal. Strong winds, such as produced by storms, while not a daily occurrence, are also not rare over a period of several years. It is surprising that APHIS is not considering possible seed dispersal by strong winds because such an event is suspected of causing gene flow of transgenic creeping bentgrass beyond field trial confinement boundaries in Oregon, in addition to cross pollination.[7] It was noted that three of nine escaped transgenic creeping bentgrass plants were likely to be from dispersed seed, with one site 0.4 kilometers from the edge of the control district, and 1.4 kilometers from the nearest creeping bentgrass test plot. Creeping bentgrass seed is also not classically and specifically adapted to wind dispersal, but clearly may be dispersed over significant distances by strong wind. Although not as small as creeping bentgrass seed, the parent *Eucalyptus* species of the hybrid clone are reported to have seed of about 200,000 to over 300,000 per pound. Seed of that size launched from a tree canopy into a high wind could very likely be dispersed far beyond the 100-meter boundary of the field test. Instead of the limited and inadequate data presented about seed dispersal, APHIS should determine how far the seed from these trees could actually be dispersed by strong winds, and how often storms producing high winds may occur in Baldwin County, Alabama, during the period of seed set. There were 10 tropical storms or hurricanes in southern Alabama, including Baldwin County, between 1995 and 2002. There were also 108 tornado events and 180 severe thunderstorms in Baldwin County between 1950 and 2003.[8] According to the National Oceanic and Atmospheric Administration Coastal Services Center "The central Gulf of Mexico [which includes Baldwin County] is one of the more hurricane vulnerable locations along the coastline of the United States."[9]

Finally, the EA claims that it is very difficult for *Eucalyptus* trees to become established by seed, and that they need bare mineral soil (i.e., disturbed soil) to be able to germinate and compete. APHIS argues that, therefore, it is highly unlikely that any seed escaping from the test site could become established. However, establishment in the wild of naturally occurring hybrids of introduced *Eucalyptus* and an indigenous species has been observed.[10] The APHIS assessment is apparently based on general *Eucalyptus* references rather than data about the specific hybrids of this test, and therefore it is not clear that this information is applicable. More important is that seed is a normal route of dispersal for *Eucalyptus* species in the wild. Where adequate sites are available, seedlings may become established and competitive. Disturbed sites including roadsides, field margins, and drainage ditches are not uncommon in rural farming areas such as Baldwin County. Once again, APHIS seems to be ignoring lessons from the Oregon creeping bentgrass field test that led to gene flow. In that case, one of the primary reasons for choosing the site of the so-called "control district" in Central Oregon, in addition to removal from turfgrass seed production areas, was that the semi-arid area around that site was said to be unsuitable for creeping bentgrass establishment, and therefore gene flow. In fact, the many disturbed sites in the creeping bentgrass control district that were suitable for establishment allowed transgenic creeping bentgrass to escape into the environment.[11]

The EA also does not consider the possibility that the hybrids may have transgressive properties relevant to establishment. Although hybrids often have phenotypic traits between the values of the two parents, transgressive traits that exceed either parent are not uncommon.[12] In fact, this property has led to the evolution of new species that grow where neither parent is competitive. Unless transgressiveness is specifically considered and addressed, it cannot be ruled out. Generalizations based only the parent species or on other species of *Eucalyptus* therefore cannot substitute for data on this hybrid.

Gene Flow by Cross Pollination with Ornamental *Eucalyptus*

The EA dismisses the risk of gene flow between the field test trees and *Eucalyptus* outside the proposed test site. APHIS claims that there are no "significant plantings" of cold-tolerant species of *Eucalyptus* in Alabama, that ArborGen has determined that there are no eucalyptus trees within 500 meters of the test site, and that the literature about the genus suggests that 300-meter isolation is sufficient to prevent outcrossing. Furthermore, APHIS notes that several cold-tolerant species are in different taxonomic sections of *Eucalyptus*, and therefore unlikely to cross with the test species and produce viable and fertile offspring. APHIS also claims that differences in anthesis between the test trees and other species would mitigate crossing.

The pollen flow isolation distance cited in the EA is inadequate. Literature on adequate isolation distances is in a state of flux for several reasons, especially because previous standards and measurement techniques, and limited data, are often insufficient to accurately determine pollen flow frequencies, particularly under different ambient conditions.[13] For example, contrary to the data cited in the EA, a recent paper on *Eucalyptus nitens* demonstrated significant cross pollination at 300 meters (0.7%), with similar levels at the limit of measurement (1.6 kilometers).[14] These levels would, for example, be unacceptable even for most conventional seed purity standards. Several other studies revealed out-crossing between *E. grandis* and *E. urophilla* at 800 meters and possible hybridization between two other species at 6 kilometers.[15] The 500-meter zone checked by ArborGen is therefore inadequate.

The EA notes the lack of "significant" plantings of *Eucalyptus* in Alabama and cites the U.S. Plants Database for support. However, this database does not claim to be comprehensive and would not account for most ornamental plantings. Therefore, there can be no assurance that plantings of *Eucalyptus* do not occur within pollination distance of the proposed test site.

The EA claims that gene flow between field test trees and cold-tolerant *Eucalyptus* that may be growing in Alabama is unlikely because they are not closely related. The EA suggests that this is demonstrated by the grouping if the different species in different taxonomic sections. Although inter-sectional crosses are often less successful than inter-series crosses, as the EA notes, some inter-sectional crosses may nonetheless produce normal seedlings. In particular, species in some different sections in the subgenus *Symphyomytrus* often form successful crosses. This subgenus contains most of the cold-tolerant species noted in the EA (*E. cinerea, E. gunnii, E. neglecta, E. nova-angelica, and E. macarthurii*), which are in the section *Maidenaria*. Species in *Maidenaria* often can be successfully crossed with species in the section that contain the trees of the field test, *Transversaria*.[16] For example, successful crosses between one of the parent species of the field test hybrid, *E. grandis*, and one of the cold-tolerant species, *E. gunnii*, have been accomplished. The progeny varied from low viability to vigorous. Therefore, contrary to the EA's assertion, there is a

reasonable chance that cold-tolerant *Eucalyptus* species that may be grown in Alabama could form viable crosses with the field test trees, allowing gene flow to occur. Without data specifically excluding this possibility, the EA has no solid basis to show that gene flow is unlikely.

Furthermore, low F_1 progeny fertility is not uncommon in crosses between different species in many plant families, but is often not an absolute barrier to successful hybridization and introgression.[17] Without considerably more experimental data on crosses between the proposed field test trees and possible local *Eucalyptus* plants, it cannot be said with confidence that outcrossing and establishment in the environment could not occur. More data would also be needed to determine how much overlap of anthesis may occur, which can only be determined by knowing specifically about species that may be in the area. Once again, the EA has supplied insufficient data to have confidence that gene flow is unlikely.

Finally, the suggestion that only larger (commercial) plantings of *Eucalyptus* in the region would be "significant" is incorrect. Larger plantings may be more likely to be pollinated by nearby trees, rather than by trees from a more distant field test. On the other hand, a small number of isolated trees may be more likely to receive pollen from a field test because there is less nearby pollen for the test trees to compete with. In addition, to the extent that the local trees are self-incompatible, they may favor outcrossing with field test pollen, depending on the mechanism of incompatibility.

For all of these reasons, APHIS's analysis of the possibility of transgene escape by outcrossing is inadequate.

UCS Conclusion and Recommendation

APHIS makes many poorly supported inferences and assumptions in its EA that, when carefully considered, reveal a weak and inadequate risk assessment. The data and reasoning used by APHIS do not adequately assess the possibility of gene flow, and therefore do not constitute the "hard look" required under NEPA to ensure the protection of the environment. The EA also does not fulfill the recommendations of the NRC for performing rigorous risk assessments, because APHIS continues to prepare EAs with insufficient data.

UCS recommends that APHIS deny a permit to ArborGen until the company submits sufficient data for a rigorous risk assessment and the agency prepares a new EA that takes a "hard look" at risks based on the new data.

Thank you for your consideration of these comments.

Sincerely,

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