

NEPA ESA Decision Summary for Permit 08-077-104r

Based on a review of Permit #08-077-104r, the following determinations were made:

- The planting will consist of approximately 1.35 acres of Hybrid Poplar, *Populus nigra* x *P. maximowiczii* and *P. tremula* x *P. alba* in Tipton County, Indiana. Based on the method of transformation (*Agrobacterium*-mediated), only the genetic construct that is designed to be expressed in the Poplar lines is expected to be inserted into the plant DNA. The genetically engineered Poplar lines are engineered to express genes that detoxify halogenated compounds and a selectable marker gene.
- No plant pest vectors or vector agents are expected to be associated with the transformed poplar lines as a result of the transformation process. The introduced genes are not expected to cause disease symptoms in poplar and will not result in the production of infectious agents. Some of the constructs containing the gene of interest and selectable marker genes also contain a non-coding region derived from a plant pest that has been safely used to regulate the expression of transgenes in many plants. Neither the genes encoding the desired traits, nor the regulatory elements controlling their expression, have any inherent plant pest characteristics, nor are they likely to pose a plant pest risk.
- *Populus* is a member of the family Salicaceae. The genus *Populus* is made up of multiple Sections. *Populus tremula* (European aspen) and *Populus alba* (white poplar or silver poplar) are in the Section Populus. *P. nigra* (Black Poplar) is in the section Aigeiros and *P. maximowiczii* is in the Section Tacamahaca. *Populus alba* and *P. tremula* are native to central and northern Europe, N. Africa and central Asia. *P. nigra* is native to Europe and western Asia. *P. maximowiczii* is native to NE China and Japan. These hybrids have been used in numerous breeding programs in the United States and are planted in a number of States.
- Poplars are normally dioecious (having male flowers and female flowers on different plants) and obligatory outcrossers. Flowers are in pendulous catkins, anemophilous; only rarely are staminate and pistillate flowers on the same tree, or the flowers perfect. The parental variety for the transformation events is female, so no pollen will be produced by the transgenic trees. The catkins of poplars produce no nectar; wind is the agent of pollination. The trees will not flower until they reach maturity, which is variable according to their location. Generally flowering normally does not occur until age 10 – 15 (Braatne 1996), with the earliest flowering reported between ages 5 - 10. Nevertheless, the applicant will monitor for flower formation and will prevent any seed dispersal by removing any flowers that form or removing the trees if it becomes too difficult to remove flowers.
- Poplar seeds lack dormancy. Once seeds are shed from the tree they will germinate immediately after dispersal or will die. Seeds are viable for only a few days unless given special storage conditions. No seeds will be allowed to form in these field tests.
- Poplars can also proliferate from root suckering. Poplars sprout vigorously from the stump and root collar. Coppicing (regrowth in certain species by cutting the stem to near ground level) occurs occasionally on young aspen. Reproduction from adventitious shoots on roots (root suckers) is common in many species, although less frequent in those in the *Aigeiros* and *Leucoides* sections. Any root suckers that may form will be easy to detect in a managed plantation. If these appear, the applicant will remove them by mechanical means. At the

termination of the field test the cut stumps will be treated with herbicide to prevent re-sprouting and the field test will be monitored for one year for any resprouting. The applicant will be required to confirm that no resprouting occurs for a two year period before the site can be returned to general use. If volunteers or stump sprouts are still emerging at the end of the first year, a second year will be added to the monitoring period to ensure that no shoots are continuing to be produced.

- After termination of the field trial, the site will be monitored for volunteer plants. Eastern Cottonwood trees are the only poplar native to this region, but it can be easily differentiated from the transgenic trees by differences in leaf type. Eastern Cottonwood has very leathery, flat, shiny deltoid-shaped leaf with crenate (round to blunt teeth) leaf edges. The transgenic hybrids have leaves that are thinner, more papery and lack luster, are lighter green and have an attenuated tip with a wavy margin. Any non-transgenic poplar genotype that is planted on this site after the transgenic field trial is terminated will be clearly distinguishable from the transgenics based on leaf morphology.



Eastern Cottonwood



Hybrids

TRANSGENES

- The genetically engineered Poplar lines are engineered to express a Cytochrome P450 2E1 (CYP2E1) that is involved in metabolism of volatile organic compounds, including, benzene, chloroform, trichloroethylene (TCE), carbon tetrachloride, styrene, toluene, xylenes, methyl chloroform and a selectable marker gene (Doty 2000, Doty 2007, Wang 2004). Cytochrome P450 is a family of enzymes that are involved in the oxidative metabolism and are found in animals, plants, and bacteria. When CYP2E1 is introduced into Poplar, the organic compounds are broken down but not volatilized or diffused into the environment (Doty 2000, Doty 2007, Wang 2004). Cytochrome P450 has been previously release in regulated field trials six times. During the course of these field trials, there have been no reported unintended detrimental effects to non-target organisms. Therefore, field trials of plants expressing CYP2E1 do not raise new issues.
- Neomycin phosphotransferase II confers resistance to aminoglycoside antibiotics, including kanamycin. NPTII was used a selectable marker. The *nptII* gene was present in 29 plant lines that have been deregulated and field tested in hundreds of field trials. During the course of these field trials, there have been no reported unintended detrimental effects to non-target organisms. Therefore, field trials of plants expressing phosphinothricin acetyl transferase do not raise new issues.

THREATENED AND ENDANGERED SPECIES

- BRS has reviewed data provided by the applicant and available in scientific literature and information maintained on the Fish and Wildlife Service's (FWS) website

(<http://www.fws.gov/endangered/wildlife.html#Species>). There are 31 Threatened and Endangered Species (TES) listed (or proposed) for the state of Indiana. (http://ecos.fws.gov/tess_public/StateListingAndOccurrence.do?state=IN), none present in Tipton County, IN. No critical habitat for TES occur in Tipton County, IN.

- The trials will be conducted according to the confinement measures described in the design protocols and in compliance with the processes set forth in 7 CFR 340.4 and all supplemental permit conditions. Therefore, the proposed release does not have the potential to affect "significantly" the quality of the "human environment" as those terms are defined at 40 CFR 1508.27 and 1508.14. Based upon this analysis, BRS has reached the conclusion that the field trial is not likely to have effects on listed threatened or endangered species or their critical habitat, and consequently, consultation with FWS is not required.

References

Braatne JH, Rood SB, Heilman PE (1996) Life history, ecology, and conservation of riparian cottonwoods in North America. Pages 57-85 in: Biology of Populus and its implications for management and conservation. Stettler RF, Bradshaw, Jr. HD, Heilman PE, Hickley, eds. NRC Research Press, Ottawa, Canada.

Doty SL, Shang TQ, Wilson AM, Tangen J, Westergreen AD, Newman LA, Strand SE, Gordon MP (2000) Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2E1.

Doty SL, James CA, Moore AL, Vajzovic A, Singleton GL, Ma C, Khan Z, Xin G, Kang JW, Park JY, Meilan R, Strauss SH, Wilkerson J, Farin F, Strand SE (2007) Enhanced phytoremediation of volatile environmental pollutants with transgenic trees. PNAS 104:16816-16821.

Wang X, Dossett MP, Gordon MP, Strand SE (2004) Fate of carbon tetrachloride during phytoremediation with poplar under controlled field conditions. Environ. Sci. Technol. 38:5744-5749.

For the above reasons, and those documented on the NEPA/ESA worksheet, APHIS has determined that this permit involves a confined field trial of a genetically engineered organisms or products that do NOT involve a new species or organism or novel modification that raises new issues. APHIS has determined that the actions authorized under this permit do NOT have the potential to significantly affect the quality of the human environment. Therefore, approval of this permit is properly categorically excluded from the need to prepare an EA (or EIS) pursuant to 7 CFR 372.5., and none of the exceptions to this categorical exclusion apply.

Signed: _____/s/_____

Michael T. Watson, Ph.D.

Branch Chief, Plants Pests and Protectants

Biotechnology Regulatory Services