

NEPA Decision Summary for Permit #08-337-105r

Dr. Kristi Snell, the Director of Plant Sciences for Metabolix, Inc., has requested a permit for a small confined field release of genetically engineered tobacco plants at a site in Fayette County, Kentucky. This release includes both *Nicotiana tabacum* and of interspecific hybrids of genetically engineered tobacco.

Based on a review of Permit #08-337-105r, the following determinations were made:

1. The gene constructs proposed for the confined field release are expected to result in tobacco that produces poly-beta-hydroxybutyrate (PHB), a biodegradable polymer that can be produced through the expression of bacterial genes. The construct consists of several bacterially derived genes involved in the synthesis of PHB that are claimed as Confidential Business Information (CBI). The construct also includes a selectable marker gene derived from bacteria encoding an enzyme that gives resistance to commonly used antibiotics. The applicant is also applying to release plants containing a construct with only the selectable marker. Regulatory sequences are derived from native Tobacco genes. The constructs were introduced using a biolistic transformation technique of directed insertion through homologous recombination. Constructs containing similar genes have been approved previously from other applicants and the selectable marker gene has also been approved previously on multiple occasions and in multiple plant species. None of the genes encoding the desired traits or the selectable marker, nor the regulatory elements controlling their expression, have any inherent plant pest characteristics, and they are not likely to pose a plant pest risk.
2. Based on biolistic transformation, only the genetic construct that is designed to be expressed in the genetically engineered tobacco lines is expected to be efficiently and stably inserted into the tobacco genome. No plant pest vectors are expected to be associated with the transformed tobacco lines as a result of the transformation process.
3. The intent of this field release is to produce plant materials containing PHB as part of a pilot study. No seeds from this field trial will be produced, because plants will be harvested before mature seeds are developed. A biocontainment strategy is used that is expected to eliminate concern for gene transfer via pollen. All regulated material will be collected and securely shipped to a confined location or made non-viable. Ultimately all regulated material will be made non-viable.
4. PHB is a biopolymer which is present in all living organisms (Das, et al., 2002. *Biochemistry*, 41:5307-5312). PHB is biodegradable (Kim and Rhee, 2003. *Applied Microbiology and Biotechnology* 61:300-308; Jendrossek and Handrick, 2002. *Annual Review of Microbiology*, 56:403-432). PHB is considered non-toxic and not found to have genotoxicity (Ali, et al., 2008 *Toxicology in Vitro*, 22:57-67). Furthermore, due to the presence of nicotine in the tobacco plant, few organisms consume tobacco. The only reported consumption of field tobacco is occasional foraging by skunks and insects that are plant pests. Because PHB is non-toxic and ubiquitous, in the event of animal feeding, no additional deleterious effect is expected.

5. There is no critical habitat within Fayette County, Kentucky. There are no major rivers in the action area. No toxins are produced by the expression of the introduced genetic material in the regulated article, nor will any pollen containing genetic modifications be released. The regulated material will not be used as food or feed. This field trial will have no effect on threatened or endangered species.
6. The plant species does not raise new issues. APHIS has previously prepared an EA (05-354-01r) for a field release of tobacco expressing pharmaceutical proteins that included similar confinement measures as proposed in this permit. All plants at the test plots will be topped/deflowered to decrease the potential for out-crossing. An isolation distance of at least 1320 feet (the AOSCA standard for production of foundation tobacco seed when flowers are not bagged or removed) between the regulated material and non-regulated tobacco will be maintained, and 2640 feet will be the minimum distance to any flowering tobacco. A distance of 5280 feet will be maintained between the regulated plots and tobacco plants grown for seed. All plots will be harvested before mature seed develops. APHIS will be notified if any flowers have progressed to the seed stage. A 50 foot barrier of tilled soil or grass will be maintained around the regulated material growing in the field. This isolation distance and temporal isolation meets the APHIS guidelines for an industrial trial for tobacco that uses reproductive isolation methods.
7. Tobacco seeds will be germinated in a greenhouse and plantlets will be transplanted into the field. This reduces the possibility of seeds being released into the environment. Personnel who handle the regulated material will receive instruction in all the activities that they carry out involving the regulated material. This training will be documented and the documentation will be made available to APHIS inspectors. This training will encompass conditions stipulated in the permit, the APHIS permit conditions, the APHIS supplemental permit conditions, and the pertinent Federal regulations. Activities related to the field release and movement of the regulated article will be documented. Dedicated facilities will be used for storage of equipment and regulated articles for the duration of the field release.
8. The applicant will survey the plants for flowering, removing them before they mature, it is highly unlikely that any transgenic pollen will be produced during the field trial, and even less likely that transgenic DNA will transfer to other tobacco plants.
9. There is a 50 foot buffer surrounding the field test site. No food or feed will be harvested from the buffer zone and the plants within the buffer zone are not sexually compatible with tobacco. There are no sexually-compatible relatives of tobacco known to exist in the area where the trial will be performed.
10. All plant material will be harvested before mature seed develops, and any flowers found will be destroyed by autoclave or tilled into the soil. Any plant material left after harvest, containing only insignificant amounts of the proteins, will be plowed under the soil surface. The proteins have no known or foreseeable toxic effects, so this method of disposal should have no negative impacts on the environment. Nonetheless, the applicant

will be expected to consult with appropriate State and Federal regulatory agencies, including the EPA, to ensure the proper disposal of the regulated material.

11. The plot area, including the 50 foot unplanted barrier, will be checked for volunteer plants once a month for 12 months, with weekly monitoring during the optimal seed germination stage. The results of this monitoring will be recorded. All volunteers will be treated as regulated material and will be disposed by autoclave or tilling into the soil. The proposed field site is under 10 acres, and trials of such small size area have been easily monitored and confined to permitted areas, under environmental mitigation measures similar to those specified in the permit application and in the permit application and in the standard and supplemental permit conditions.
12. The confinement measures described in the application and supplemental permit conditions should be sufficient to prevent any unplanned releases of the transgenic plant material or transgenic seed; or the persistence of the transgenic material or its progeny in the environment. Tobacco is not observed to be capable of establishment in unmanaged environments: it is reliant on continuous human intervention for its survival. In previous field tests and applications, seed dormancy in tobacco has not been observed.
13. Regulated materials in this field trial are not intended for food and/or feed. Any use of these products for food or feed must be in compliance with the guidelines published in the Federal Register by the United States Food and Drug Administration - 57 FR 22984, May 29, 1992.
14. Of the 38 animals and 9 plants in Kentucky that are recognized as threatened and endangered (TES) species by the U.S. Fish and Wildlife Services, only four of which are found in Fayette county, none consume tobacco. Therefore these field trials will not harm or have adverse or other significant effects on threatened or endangered species. The applicant has provided NEPA/ESA findings that reiterate No Effect on TES species.

The only past, present, and reasonably foreseeable actions associated with the location for the proposed release are those related to agricultural production. The proposed environmental release is of transgenic Tobacco is a small scale release at a single location for six months. The proposed release site has been used as agricultural land to grow crops for approximately 40 years and this site has been approved for Field Releases of regulated Tobacco in the past. The field release is not expected to significantly alter the agro-ecosystem of the release area. 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 site or the ecosystem in which it is situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

For the above reasons, and those documented on the NEPA/ESA decision document, APHIS has determined that this permit involves a confined field trial of genetically engineered organisms or products that do NOT involve a new species or organism or novel modification that raises new issues. Issuance of this permit qualifies for categorical exclusion status under 7 CFR §

372.5(c)(3)(ii), and none of the exceptions for categorically excluded actions under 7 CFR § 372.5(d) apply to this action because APHIS has determined that all environmental impacts resulting from the issuance of this permit will be insignificant. APHIS has determined that this action does NOT have the potential to significantly affect the quality of the human environment, and neither an environmental assessment nor an environmental impact statement is required.

Signed: _____
Susan Koehler
Branch Chief, Plants Branch
Biotechnology Regulatory Services

Date: _____
JS_/s/_