NEPA Decision Worksheet

Permit #	05-061-01r
Institution	University of Kentucky
Organism	Tobacco
Category	Pharmaceutical- for treatment of Phenylketoneurea
Gene	Phenyalanine Ammonia Lyase from Arabidopsis thaliana

1. Confinement	
Confinement and mitigation conditions have been reviewed and determined to be adequate	x[1]
2. Threatened or Endangered Species or its habitat	
Resident or migratory in counties and harm to threatened or endangered species or habitat is likely	
Resident or migratory in counties and harm to threatened or endangered species is unlikely	
None observed in area (no harm to threatened and endangered species)	x
New or Novel	
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior EA	
Not new and prior EA	x
4. New or Novel Trait (gene product)	
Never used in a field trial	
Not new but no prior EA	
Not new and prior EA	x
Raises new issues	
5. Cumulative Effects	
Cumulative effects likely	
Cumulative effects possible	
Cumulative effects unlikely	x
6. Plant Pollination	
Primarily bee or insect pollinated crop	
Primarily wind pollinated food or feed crop	
Primarily self fertilized food or feed crop	
Non-food or feed crop	x
7. Effects on Food/Feed Supply	
Known allergen, antinutritive, oral toxicant	
Food safety not established	x
GRAS status or approved food additive for native protein	
GRAS status or approved food additive for plant produced protein	
8. Isolation Distance	
AOSCA standard for crop	1,320 feet
Proposed isolation distance	x[2]
9. Scale	
>100 acres/trait/crop/institution/year	
50-99 acres/trait/crop/institution/year	
10-49 acres/trait/crop/institution/year	
<10 acres/trait/crop/institution/year	x
10. Effects (positive or negative) on other species	
Significant effects expected/observed	
Minimal, non-cumulative effects expected/observed	
No effects expected/observed	x
11. Sexually Compatible Relatives	
Relatives within dispersal distance	
Relatives not within dispersal distance	x
12. Seed Dormancy	
>3 years	
3 years	
2 years	
<2 years	x
13. Persistence in environment	
Crop can naturalize	
Crop can persist 3-5 years without human intervention	
Crop does not persist without intervention	x
14. Comments	

[1] Biocontainment – Chloroplast transformation was employed. This strategy eliminates transgene dispersal through pollen in tobacco where chloroplasts are maternally inherited (Biological Confinement of Genetically Engineered Organisms 2004; National Research Council)

[2] - All plants will be topped by hand prior to flowering to limit any pollen and seed production. An isolation distance of 1,320 feet will be maintained between the regulated tobacco and any non-regulated tobacco plots. At least 2,640 feet (1/2 mile) isolation distance will be kept from any flowering tobacco. All tobacco between the 1,320feet and the 2,640 feet distance will be topped and not used for seed production. The closest tobacco that will be allowed to openly flower and produce seed is over 1 mile from the field test. One field that is 0.9 miles from the field test will be allowed to flower and the only seeds to be used will be from plants that are bagged to prevent outcrossing.

Additional supporting documentation is found in the summary risk assessment completed on May 18, 2005

/s/ _____

Rudaina Alrefai, Ph.D.

Biotechnologist

Biotechnology Regulatory Programs

Animal and Plant Health Inspection Service

NEPA Decision Summary of Permit 05-061-01r

On the basis of review of permit 05-061-01r, APHIS concludes that controlled field testing of the genetically engineered tobacco plants described in this application will not present any risk of plant pest introduction, will have no significant impact on non-target organisms and on the threatened and endangered species, and therefore constitutes a confined field trial. Furthermore, if the field test is performed with mitigating conditions outlined here and in the permit, the risk to human health and the environment will be exceedingly low.

APHIS evaluated plant pest impacts related to the transformation method used in this permit and concluded that the DNA inserted into the plants does not have any inherent plant pest characteristics and is not likely to pose a plant pest risk for the following reasons:

- The function of the PAL protein is widely known and the expression of the *Atpal1* gene can be confirmed from the enzymatic activity of the expressed protein in the transformed tobacco plant.
- The PAL protein is normally expressed in tobacco and all other plants and has no known or foreseeable toxic effects on the plant. However, the effect of over-expression of PAL and/or its metabolites have not been explored (Elkind et al., 1990; Way et al., 2002). APHIS is requesting the applicant to provide additional data from the field test.
- A chemical method was used to develop the transgenic tobacco plants, no living organisms such as *Agrobacterium* were used and no plant pest vectors are expected to be associated with the transformed tobacco lines.
- The kanamycin resistance marker gene was eliminated during transformation and did not get inserted into the chloroplast genome. Therefore, the transformation method eliminated the risk for human and animals to develop antibiotic resistance from the expression of this marker gene. However, such risk is significantly low even when the antibiotic resistance marker gene is expressed. Additionally, the Food and Drug Administration (FDA) has previously addressed this issue in the 1992 policy statement on foods derived from new plant varieties (http://www.cfsan.fda.gov/~acrobat/fr920529.pdf) and in the draft guidance that was issued on September 1998 (US FDA/CFSAN Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants.)

APHIS evaluated plant pest impact related to the quarantine and final disposal of transgenic plants and concluded that the field trial is a confined release and has no significant impact on the environment. The following containment measures should be sufficient to prevent any unplanned release of the transgenic plant material or transgenic seed; or the persistence of the transgenic material or its progeny in the environment:

- Dedicated equipment will be used for seeding, transplanting, and harvesting and will be labeled accordingly. This precaution ensures that the transgenic tobacco plants are not inadvertently removed from the field and therefore eliminates dispersal and gene flow of the transgenic tobacco plants.

- Chloroplast transformation used to develop the transgenic tobacco will prevent the transgene dispersal through pollen since chloroplasts are maternally inherited in tobacco (Birkey C. William, 1995).
- The research area contains several field trials that are regulated by APHIS under other permits. Plants in the research plot will be topped by hand prior to flowering to limit any pollen and seed production. Each field trial is labeled to prevent any mixing of the different transgenic events. Any non-transgenic tobacco plants (border rows, control plots, etc.) that are grown in the research plot area will be treated the same as regulated articles including growing, disposal, SOPs, and supplemental permit conditions. These measures will eliminate any possibility of accidental release of the transgenic material.
- Redundant measures are implemented to prevent gene flow through pollen dispersal to any compatible species or by seed dispersal. An isolation distance of 1,320 feet will be maintained between the regulated research plot and any other non-regulated tobacco plots. Additionally, an isolation distance of at least 2,640 feet (1/2 mile) will be kept between the regulated plots and any flowering tobacco plot. All tobacco between the 1,320 feet and the 2,640 feet isolation distance will be topped and not used for seed production. This area will be also monitored 5 days per week during the days when flower buds could first be observed. Flowers if produced within this area will be destroyed and not allowed to mature to produce seeds. Additionally, the applicant will notify APHIS within 24 hours if a flower is found that has progressed to the seed development stage. These measures would further ensure that the transgenes do not enter the commercial tobacco seed supply via cross-pollination or seed dispersal.
- The closest tobacco that will be allowed to openly flower and produce seed is over 1 mile from the field test. One field that is 0.9 miles from the field test will be allowed to flower and only seeds from plants that are bagged will be used. Additionally, the applicant presented a procedure to report to APHIS any unauthorized or accidental release of the transgenic material. These measures would further ensure that the transgenes do not enter the commercial tobacco seed supply.
- Perimeter fallow zone of 50 feet will be maintained around the transgenic test site to ensure that transgenic tobacco are not inadvertently commingled with plants to be used for food or feed. This perimeter area will not contain any plants that are sexually compatible with the transgenic tobacco plants. This area will be sown with grass to prevent soil erosion and will be monitored through out the field trial for any sexually compatible relatives.
- Tobacco is primarily self-pollinating with a 95% frequency of self pollination since tobacco anthers (male organ) split apart to release pollen usually before or as the flower corolla lobes (female organ) expand resulting in self-fertilization of tobacco and limiting outcrossing (Litton and Stokes, 1964). Any outcrossing relies on pollinators that feed on flowers and pollen, such as hummingbirds, bees and other insects with a 0.5-4% frequency of pollination (Free, J.B, 1970). Topping flowers will limit the availability of food for these pollinators thereby, minimize any outcrossing.

- It is unlikely for tobacco to become a weed under most agricultural situations since tobacco is unable to persist in the environment without continuous human intervention and is not reported to be an agricultural weed. The test plots will be monitored weekly for weed, disease, and insect infestation.
- For crops such as tobacco where seed dormancy has not been observed, monitoring for volunteers is conducted for an additional 12 months. Any volunteers that are identified will be destroyed and not allowed to flower despite the fact that these plants will be topped to eliminate flowering.
- The protein produced by the transgenic tobacco plants is expressed in leaves and stems only and the leaf tissue will be removed from the field site at harvest. Any plant material left after harvest will be plowed under the soil surface. Since the protein has no known toxic effects, this method of disposal should have no negative impacts on the environment. Since this permit is for a single field test of 0.25 acres over one year, there are no foreseeable cumulative impacts.

APHIS also evaluated the potential impacts on non-target organisms and concluded that this field release has no significant hazard on field workers, non-target organisms and threatened and endangered species (TES) for the following reasons:

- The PAL protein is not expressed in the flowers or pollen of the tobacco plants, thereby limiting the exposure of non-target organisms to this protein through dispersal of pollen.
- Sequence alignments and homology searches using the BLAST (NCBI) program showed that this protein is similar to other PAL proteins present in plants to which humans are regularly exposed. The sequence homology search also did not show any similarity to known toxins or allergens. Therefore, the PAL protein expressed in the transgenic tobacco plants has no known or foreseeable toxic or allergenic effects to humans or animals.
- Workers are required to wear protective clothing (gloves and long sleeves, as needed and as the weather permits) during harvesting and processing of the regulated article and during purification of the protein from the transgenic plants. This measure will reduce skin exposure to the nicotine in wet tobacco plants and minimize the risk of developing the green tobacco syndrome.
- Only trained employees will perform seeding, transplanting and harvesting of the transgenic tobacco according to Standard Operating Procedures (SOP) that the applicant submitted for APHIS' approval. This will also minimize any accidental release or possible human exposure.
- There are 33 animals (4 mammals, 2 birds, 5 fish, 1 crustacean, and 21 clams) and 9 plants recognized by the U.S. Fish and Wildlife Services as threatened and endangered species (TES) that live in the state of Kentucky http://ecos.fws.gov/tess_public/servlet/gov.doi.tess_public.servlets.UsaLists?state=KY. The animals on the TES list will not be affected by this field release due to lack of toxicity of PAL and its product. Further, as these animals do not feed on tobacco, they also will not be exposed to any transgenic tobacco. Additionally, tobacco will not hybridize with any of the 9 plants on the TES list for the state of Kentucky.

This field release does not involve new species or organisms or novel genes that raise new issues. Many field trials have been performed with transgenic tobacco plants under APHIS authority. APHIS is familiar with the biology of tobacco and methods to manage confined tobacco trials. APHIS has prepared 25 Environmental Risk Assessments (EA) for tobacco and found no significant impact (FONSI) in every case.

For the above reasons, APHIS has determined that (1) pursuant to 7 C.F.R. §372, the field trials proposed under permit #05-061-01r will not significantly affect the physical environment and (2) there are no applicable, extraordinary, or other reasonably foreseeable circumstances under which significant environmental effects could occur given the protective and ameliorative measures specified above. Therefore, this field test is deemed confined within the meaning of 7 C.F.R. §372.5.

References:

- Birkey C. William. 1995. Uniparental inheritance of mitochondrial and chloroplast genes: Mechanism and evolution. *Proc. Natl. Acad. Sci, USA.* **92**: 11331-11338
- Elkind Yonatan, Edwards, R.; Mavandad, M.; Hedrick, S. A.; Ribak, O.; Dixon, R. A. and Lamb, C. J. 1990. Abnormal plant development and down-regulation of phenylpropanoid biosynthesis in transgenic tobacco containing a heterologous phenylalanine ammonia-lyase gene. *Proc. Natl. Acad. Sci., USA.* **87**: 9057-9061
- Free, J. B. 1970. Insect Pollination of Crops. Academic Press, New York, New York. Pp. 355-356
- Litton, C. C. and Stokes, G.W. 1964. Outcrossing in Burley Tobacco. *Tobacco Science* **8**: 113-115.
- Way M. Heather; Kazan, K.; Mitter, N.; Goulter, K. C.; Birch, R.G. and Manners, J.M. 2002. Constitutive expression of a phenylalanine ammonia-lyase gene from *Stylosanthes humilis* in transgenic tobacco leads to enhanced disease resistance but impaired plant growth. *Physiological and Molecular Plant Pathology* **60**: 275-282.

Signed: _____/s/_____
Susan Koehler, Ph.D., Chief
Environmental and Ecological Analysis Branch
USDA/APHIS/BRS

Date: _____05/18/2005_____