NEPA Decision Summary for Permit #07-131-101r

Katrina Whelan of Kentucky Bioprocessing, LLC has requested a permit for a confined field release of 2.0 acres of genetically engineered Tobacco Mosaic Virus (TMV) that Tobacco (*Nicotiana excelciana)* will be inoculated with at a site in Daviess County, Kentucky.

Based on a review of Permit #07-131-101r, the following determinations were made:

1. The gene construct proposed for the confined field release is expected to result in tobacco that produces bovine lung aprotinin. The construct consists of a capsid protein subgenomic promoter from TMV (U1 strain). The gene is a modified cDNA bovine lung aprotinin consisting of 93-100 amino acids containing carboxy modification and a capsid protein 3' from TMV (U5 strain). The aprotinin gene is inserted downstream from the U1 capsid protein subgenomic promoter. This construct containing the aprotinin gene has been previously used under permits 04-309-02r, 04-044-02r, 04-040-01r, 03-147-01r, 01-187-01r and 01-023-03r. The gene construct contains non-coding regions derived from plant pest (Tobacco Mosaic Virus) that have been safely used to regulate the expression of transgenes within TMV. None of the genes encoding the desired traits have any inherent plant pest characteristics, and they are not likely to pose a plant pest risk. For more information on aprotinin, see the Environmental Assessment prepared by APHIS http://www.aphis.usda.gov/brs/aphisdocs/04_12101r_ea.pdf .

2. TMV is a highly researched virus and its epidemiology is very well understood. The virus enters the cell and replicates then moves from cell to cell via plasmodesmata. Plant symptoms from TMV usually take the form of molting, necrosis, stunting, leaf curling and yellowing of tissues. One of the key reasons why TMV is used in research is due to the fact it is only spread by mechanical transmission, and therefore, is not spread by wind or translocated by insect pollinators. Proper sanitation of field equipment will prevent the spread of TMV.

3. Genetically modified TMV is very efficient at producing a high level of the heterologous protein in an infected plant but only for a short predictable time. The inserted gene is recognized as nonessential for the TMV and therefore excised during replication. The nonessential inserted gene results in a virus with reduced vigor and competitions compared to the wild-type virus. During continual replication of the transgenic TMV, the inserted gene is lost with high frequency thereby reducing the likelihood that transgenic virus will be spread to other plants.

4. The aprotinin gene is incorporated into the viral genome. Tobacco plants used in the field trial are not transgenic.

5. Tobacco plants will be mechanically planted with a setter and they will be prepped for inoculation and sprayed with genetically engineered TMV. There will be 3 planting of the tobacco within 2 weeks of each harvest. The plants are only in the field from 10-28 days post-inoculation. The plants will be allowed to flower due to the fact that TMV is not seed-borne nor transmitted through pollen. The transgenic tobacco plants will be harvested by a dedicated mechanical harvester that is attached to a leak-proof sealed

wagon. The crop will be bagged and placed in a cooler for transport from the field site to the facility. All transport of transgenic material to and from the field will be performed under requirements of 7CFR 340.8.

6. The intent of this field release is to test the level of aprotinin expression in different *Nicotiana excelciana* lines, extract and purify aprotinin from Nicotiana plants, and to test the effects of agronomic management practices on the yield of TMV expressed aprotinin. The aprotinin protein is a natural serine proteinase inhibitor consisting of 58 amino acid residues in a single chain, cross-linked by 3 disulphide bridges with a total molecular weight of 6,152 daltons. Aprotinin is produced in bovine tissues and is consumed by humans and animals that eat meat. Aprotinin is not absorbed into the bloodstream when taken orally by mammals or birds. Insecticidal activity of aprotinin has been documented at higher concentrations than the aprotinin produced by the TMV infected plants. Amino acids of both the recombinant and native aprotinin were compared and confirmed identical in a number of activity and characterization assays. Allergy and toxicology screens were performed using the FARRP Allergen Database and NCBI BLAST search. The results indicated that the aprotinin protein and signal peptide showed 9 hits. None of the hits had significant cross reactivity with known allergens. Aprotinin has been studied in humans since 1960 and has a notable safety record. Thus, the protein produced during this pharmaceutical field trial is not considered toxic or allergenic.

7. The proposed field site is located in rural Kentucky in Daviess County. The site is surrounded on 3 sides by trees and on one side by corn that will be used for grain. Corn is not a host plant for TMV and therefore is not at risk of infection. The field site includes a fifty foot fallow zone to reduce physical contact and minimize vector transmission within the field site. There will be 3 plantings of the tobacco within 2 weeks of each harvest. The transgenic tobacco will be in the field for 10-28 days post-inoculation. The field site will be monitored 3 different times between inoculations and harvesting for weed hosts. Any weeds with TMV-like symptoms will be collected and analyzed. Upon completion of the field testing all field material will be disked under and monitored monthly for 12 months for volunteers. All volunteer tobacco plants found will be removed manually or by chemical treatment.

8. TMV is not insect transmitted and no plants susceptible to TMV will be grown within 100 feet of the field test site. TMV is not seed-borne or transmitted through pollen. TMV is only spread by physical contact. Employees entering and working in the field wear disposable gloves and protective clothing (boots). Protective wear that come into contact with the TMV such as gloves or boots are autoclaved and discarded or cleaned with bleach that inactivates the virus. Tools and equipment used in TMV fields are treated with bleach after each use. Vigorous weed control by herbicide treatment or hand roguing is used in the field test plot to eliminate any TMV compatible plants in the area.

9. There is a 50 foot fallow zone around the field test site. No food or feed will be harvested from the fallow zone and the plants within the fallow zone are not susceptible to TMV.

10. According to the Fish and Wildlife Service (http://ecos.fws.gov/tess_public/StateListingAndOccurrence.do?state=KY, accessed 06/07/07) there are 34 federally listed threatened and endangered animals and 8 threatened and endangered plant species in the state of Kentucky. Of the 34 listed animals, none are known to use tobacco as a food plant. None of the threatened and endangered species forage on tobacco pants. The only known animal that forages on tobacco is skunk. Furthermore, in the unlikely event of accidental consumption, the pharmaceutical protein produced during this field trial is non-toxic and is not expected to harm animals feeding on this plant. Therefore these field trials should have no effect on threatened or endangered species.

11. According to http://crithab.fws.gov/, accessed 06/07/07, there is no designated critical habitat or proposed designated critical habitat found in this county.

12. The gene products used in this field trial are not known to be toxic by oral or dermal exposure. Based on the above, these field trials should not harm or have adverse or other significant effects on threatened or endangered species either by direct or indirect exposure.

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].

For the above reasons, and those documented on the NEPA/ESA decision document, APHIS has determined that permit application 07-131-101r involves contained movement and confined field trails of 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, Plant Pests and Protectants
Biotechnology Regulatory Services

Date:___8/9/2007_____
AMH_/s/_