NEPA Decision Summary for Permit #08-051-101r

Kentucky Bioprocessing, LLC has requested a permit for a confined field release of genetically engineered Tobacco Mosaic Virus (TMV) that will be used to inoculate [2.0 acres] of tobacco plants (*Nicotiana excelciana*) at a site in Daviess County, Kentucky.

Based on a review of Permit #08-051-101r, 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). This construct containing the aprotinin gene has been previously used under permits 07-131-101r, 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 a plant pest (tobacco mosaic virus) that have been safely used to regulate the expression of transgenes within a TMV vector. 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 has been the subject of extensive research 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, it is not spread by seed or translocated by insect vectors. 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 inserted heterologous protein in an infected plant, but only for a short predictable time. The inserted gene is recognized as nonessential by the TMV. During replication of the transgenic TMV the inserted gene (aprotinin) is excised with high frequency; TMV only preserves the sequences needed for optimal replication and movement. Furthermore, transgenic TMV has a lower replicative capability then the wild type TMV virus. In a comparative challenge study on tobacco, between recombinant and wild-type TMV, the results indicated that the wild-type was more competitive, vigorous and pathogenic than the recombinant virus. Therefore, lower replicative capability of the transgenic TMV, along with the high frequency of excision of the inserted gene, reduces the likelihood that the 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. [
] The plants will be allowed to flower; because, TMV is not seed-borne or transmitted through pollen, and there is no potential for dissemination of the virus. 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 trial to the on-site KBP 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 test the effects of agronomic management practices on the yield of TMV expressed aprotinin. [ ] Given that the recombinant aprotinin has the identical amino acid sequence as the native aprotinin, as well as its lack of similarity to known proteins and allergens, it is unlikely that the recombinant aprotinin would display either toxic or allergenic properties.

7. The proposed field site is located in rural Kentucky in Daviess County. The site is surrounded on 3 sides by corn and on the fourth side by a road, across which is soybean or wheat grown for agricultural purposes. Neither corn, soybean, nor wheat are considered host plants for TMV and therefore are not at risk of infection nor would be able to express the aprotinin. The field site includes a fifty-foot fallow zone to reduce physical contact and minimize mechanical transmission within the field site. All weeds will be monitored and removed from the field site at least 3 times [ ]. Any weeds with TMV-like symptoms, along with random samplings of weeds near the tobacco, will be analyzed for TMV and the inserted gene. 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. Employees entering and working in the field will wear disposable gloves and protective clothing (boots). Protective wear that come into contact with the TMV such as gloves or boots will be autoclaved and discarded, or cleaned with bleach to inactivate the virus. Tools and equipment used in TMV fields will be treated with bleach after each use. Vigorous weed control by herbicide treatment or hand rouging is used in the field test plot to eliminate any TMV compatible weeds in the area.

9. According to the Fish and Wildlife Service (http://ecos.fws.gov/tess_public/StateListingAndOccurrence.do?state=KY, accessed 05/09/08) there are 33 federally listed threatened and endangered animals and 8 threatened and endangered plant species in the state of Kentucky. Of the 33 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, according to Natureserve (http://www.natureserve.org/explorer/servlet/NatureServe?loadTemplate=tabular_report.wmt&paging=home&save=all&sourceTemplate=reviewMiddle.wmt, accessed on 5/09/08) only two animals and no plants are listed in Daviess county, Kentucky as threatened and endangered, the animals include: *Nerodia erythrogaster neglecta* (copper belly water snake) and *Myotis sodalis* (Indiana bat). Neither of these animals feed on tobacco nor have habitats in agricultural fields. 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.

10. According to http://crithab.fws.gov/, accessed 05/09/08, there is no designated critical habitat or proposed designated critical habitat found in this county.

11. 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.

12. 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 08-051-101r 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
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Date:___5/09/2008_____
AMH_/s/_