NEPA Decision Summary for Permit #12-032-107r

Kentucky Bioprocessing, LLC (KBP) has requested a permit for a small-scale confined field release (less than 10 acres with no more than 5 releases) of genetically engineered tobacco mosaic virus (TMV) that will be used to inoculate *Nicotiana excelsiana* at one site in Daviess County, Kentucky.

Based on a review of Permit #12-032-107r, the following determinations were made:

1. The gene construct is expected to result in the production of bovine aprotinin in tobacco using a TMV expression vector. This construct containing the aprotinin gene has been previously released under permits 11-041-107r , 10-011-101r , 09-090-101r, 08-051-101r and 07-131-101r. The gene construct contains sequences derived from a plant pest (TMV). The gene encoding the recombinant protein, aprotinin, does not have any inherent plant pest characteristics and is not likely to pose a plant pest risk.

2. The intent of this field release is to test the level of recombinant aprotinin expression in *N. excelsiana* plants that were inoculated with recombinant TMV. Aprotinin will be purified from the plants to test the effects of agronomic management practices on the yield of TMV-produced aprotinin in tobacco plants. An Environmental Assessment was prepared by APHIS for the field release of corn expressing aprotinin, http://www.aphis.usda.gov/brs/aphisdocs/04_12101r_ea.pdf. Aprotinin is a naturally occurring Kunitz-type serine protease inhibitor. The amino acid sequence of recombinant aprotinin is biochemically equivalent to naturally occurring aprotinin according to a number of activity and characterization assays. Because it is present in bovine tissue, most individuals who consume meat have been exposed to this protein due to oral consumption of beef. According to criteria established by the WHO, recombinant aprotinin is not considered cross-reactive with any known or putative allergen. Given that the recombinant aprotinin has the identical amino acid sequence as the native aprotinin and lack of similarity to known proteins and allergens, it is unlikely that the recombinant aprotinin would display either toxic or allergenic properties.

3. During the field release, the engineered TMV will be inoculated onto tobacco plants that are not transgenic. TMV is used for the expression of recombinant proteins because it is one of the most studied viruses, only spread by mechanical transmission and not transmitted by insect vectors. APHIS has previously prepared four EAs (91-007-08r, 94081-01r, 95041-01r, 96-051-01r) for the environmental release of transgenic TMV.

4. The engineered trait is expected to be confined to the release site due to the following:

    - The gene inserted into the virus not been shown to be involved in plant pathogenicity, would not result in broadening the host range of TMV and does not provide the virus with any apparent selective advantage over the nonengineered virus to become established in the environment.

    - Inserted sequences are rapidly deleted during viral multiplication in host plants resulting in a virus that is virtually identical to wild-type (naturally occurring) parental TMV. The engineered TMV is less competitive and stable when compared to its nonengineered parental strain. If the engineered virus did escape and infect another susceptible plant, the engineered virus would be at a competitive disadvantage to endemic TMV.

    - Tobacco seedlings will be transplanted to the field and sprayed with genetically engineered TMV. As the virus multiplies in the plant, the newly acquired gene will be synthesized and encoded protein produced. Once the plants are inoculated, they will remain in the field for 10-28 days. On average, the plants will be harvested within two weeks of inoculation. TMV does not persist in soil except when infected tissue is present and is degraded when the host tissue decays (Gooding 1986).

- The spread of the engineered virus will be minimized by physical methods, decontamination of farm implements, and the absence of susceptible host plants near the field site. The test site is isolated by 20 foot fallow zone to reduce physical contact and minimize unintended transmission of the virus. The field site will be monitored at between inoculation and harvest at each planting for potential weed hosts; any plants showing TMV-like symptoms will be destroyed.

- Tools and equipment used in the release site will be treated with a bleach solution to inactivate the recombinant TMV, and rinsed with fresh water at the field site after each use before storage or transport. Employees entering and working in the field after inoculation and before harvest will wear disposable gloves that will be autoclaved and discarded; and footware (boots) will be cleaned with bleach to inactivate the virus.

- Infected plant material will be harvested using a dedicated mechanical harvester and then transported to KBP extraction facility either a leak-proof sealed wagon or sealed in a plastic bag, and placed in a cooler.

- On completion of the field testing all plant material will be chopped up and root systems destroyed with a tractor-mounted disk harrow. The test plots will be redisked to ensure destruction of all TMV material.

- The size of the release site is less than 10 acres.

5. The gene product used in this field trial is not known to be toxic to humans by oral or dermal exposure and has been used previously in clinical trials (see "Aprotinin Biology Information" attached to the permit file). Also, there is no significant absorption of aprotinin in the blood stream of vertebrates. Insecticidal activity of aprotinin toward European corn borer (*Ostrinia nubilalis*) and corn rootworm larvae (*Diabrotica undecimpunctata howardii*) has been documented at higher concentrations of aprotinin than is present recombinant TMV-infected plants. In addition, European corn borer and corn rootworm larvae are non-TES organisms and not known to feed on *Nicotiana* species. During the 2010 and 2011 field trial, the field site was monitored for honey bee mortality and no honey bees were ever found in in the field.

6. There is no designated critical habitat or proposed designated critical habitat found in Daviess County, KY (http://crithab.fws.gov/, accessed 2/10/12). According to the Fish and Wildlife Service (http://ecos.fws.gov/tess_public/countySearch!speciesByCountyReport.action?fips=21059 ; accessed on 02/14/12) there is one listed or proposed federally listed threatened and endangered animal in Daviess County, Kentucky (Indiana bat (*Myotis sodalist*). The only known animal that forages on tobacco is skunk. Indiana bats hibernate during winter in caves or, occasionally, in abandoned mines. During summer they roost under the peeling bark of dead and dying trees. Indiana bats eat a variety of flying insects found along rivers or lakes and in uplands. In the unlikely event of accidental consumption, the pharmaceutical protein produced during this field trial is non-toxic to mammals and is not expected to harm animals feeding on this plant. Therefore, this field trial should have no effect on threatened or endangered species.

7. 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 12-032-107r involves confined field trials 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.

Gooding, G.V. (1986) Tobacco mosaic virus: Epidemiology and control. In: The Plant Viruses, Vol.2. M.H.V. van Regenmortel & H. Fraenkel-Conrat (eds.) pp. 133-152.