Finding of No Significant Impact

Animal and Plant Health Inspection Service
APHIS Permit 05-354-01r

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) received a permit application (APHIS number 05-354-01r) from Planet Biotechnology to conduct an environmental release with tobacco that is genetically engineered to produce an antibody. On March 27, 2007, APHIS published a notice in the Federal Register (72 FR 14259, Docket no. 2006-0038) announcing the availability of the draft environmental assessment (EA) for 30-day public comment period, ending April 26, 2007.

In the EA, APHIS considered three alternatives: Alternative A – Denial of the permit; Alternative B – Issue the permit as received; Alternative C – Issue the permit with Supplemental Permit Conditions. APHIS proposed Alternative C as its preferred alternative because after review of the processes and procedures to prevent the dissemination and establishment of plant pests as describe in the permit and the additional supplemental conditions, APHIS concluded that the permit conditions would be adequate to confine the field release and prevent the release of the regulated article.

Based upon analysis described in the revised, final EA and in APHIS's response to comments, APHIS has determined that the preferred alternative, to issue the permit with Supplemental Permit Conditions, will not have a significant impact on the quality of the human environment because:

1. The genetically engineered tobacco (*Nicotiana tabacum*) produces the antibody CaroRx™ that specifically binds to the bacterium *Streptococcus mutans*. In general, antibodies are non-toxic and clinical trials with CaroRx indicated no adverse effects on humans. Antibodies are ubiquitous in nature, so many insects and animals are routinely exposed to antibodies. The selectable marker gene for kanamycin resistance (*npt*II) is not toxic and is present in many plant lines previously deregulated. The NOS (nopaline synthase) protein is naturally produced by many plants and is not expected to have significant effects on nontarget organisms.

2. Tobacco is not considered a weed in Kentucky. The addition of the transgenes is not likely to render tobacco more weedy. None of the gene products are likely to increase the fitness, alter reproductive capacity, or affect other traits associated with weediness.

3. Tobacco is not likely to outcross to the surrounding tobacco because the common practice of topping tobacco cultivars means that receptive non-regulated tobacco flowers will not be near the environmental release. In addition, the flower buds will be removed from the regulated tobacco, reducing the chance of seed or pollen production. Furthermore, the regulated tobacco will be isolated from any other tobacco plants by a distance of at least ½-mile.

4. The regulated tobacco is not likely to outcross to other *Nicotiana* species because *Nicotiana* hybrids rarely produce fertile plants because, native or naturalized *Nicotiana* species with the same number of chromosomes do not occur in Kentucky and *Nicotiana* species are not frequently grown as ornamentals.

5. An analysis of critical habitat and threatened and endangered species allows APHIS to conclude that the release would have no effect on listed (or proposed) species.

6. The release site is on land that has been under agricultural cultivation for more than 10 years. The only past, present, and reasonably foreseeable actions associated with the location for the proposed release are those related to agricultural production. 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.

7. The field release is confined.

    a. Accidental transport of regulated articles from the site by humans is minimized by strict SOPs and permit conditions. All field plots will be tagged and GPS (Global Positioning System) coordinates recorded and communicated to APHIS. The field plot will be bordered on all four sides with 50 feet of perimeter fallow zone (not in production) to allow farm machinery to move around the site and yet still prevent physical mixing of the regulated plants with surrounding plants that may be used for food or feed.

    b. Tobacco seeds will be germinated in a greenhouse and plantlets will be transplanted out into the field. This reduces the possibility of the small seeds being released out into the environment. When the tobacco plants are sufficiently mature for flower production, the tobacco plants will be monitored for flower production 5 days/week and any flowers or flower buds detected will be removed.

    c. An isolation distance of at least ½-mile will be maintained between the regulated plots and non-regulated tobacco. This area will be monitored throughout the field test period. At least a 1-mile distance will be maintained between the field plots and any tobacco seed production. This area will be monitored throughout the field test period.

d. During the growing season the plants will be inspected for traits such as weediness, resistance/susceptibility to insects or disease, or unusual differences in plant growth or morphology.

e. All field equipment or vehicles entering the field, used for harvest, transport, and pest/weed control, will be cleaned prior to use and after use according to the APHIS approved Standard Operating Procedures (SOPs). Within 2 weeks following harvest and antibody extraction, the remaining plant material will be disked into the soil.

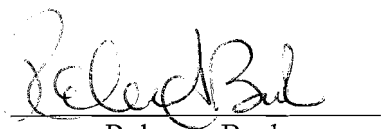
f. In Kentucky, tobacco does not grow without human intervention and does not survive freezing winter temperatures. Even if the tobacco did volunteer in fields in the following growing seasons, the release site and the 50-foot border area will be monitored for one year for volunteers.

g. 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 test and movement of the regulated article will be documented.

h. Dedicated facilities (locked or secured buildings, bins, or areas, posted as restricted to authorized personnel only) will be used for storage of equipment and regulated articles for the duration of the field test.

i. APHIS will inspect permittee records that cover multiple aspects of the field trial and inspect field trials timed to occur at critical steps in the production process.

Therefore, considering the organism and the trait introduced, the limited duration of the trial, and the manner in which the trial must be conducted, the size and location of the proposed field releases are unlikely to significantly affect the quality of the human environment. Because APHIS has reached a finding of no significant impact, no Environmental Impact Statement will be prepared regarding this decision.



Rebecca Bech
Acting Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: June 28, 2007

USDA/APHIS Final Environmental Assessment
June 28, 2007

In response to the Planet Biotechnology permit application 05-354-01r for an environmental release to produce antibodies in genetically engineered tobacco (*Nicotiana tabacum* L.)

U.S. Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture has prepared an environmental assessment (EA) in response to a permit application (05-354-01r) for the environmental release of genetically engineered tobacco plants. These genetically engineered tobacco plants produce an antimicrobial antibody that binds to a bacterium (*Streptococcus mutans*) associated with tooth decay in humans. Prior to submission of this permit application, the permittee, Planet Biotechnology, obtained APHIS permits for small-scale field testing of genetically engineered tobacco plants that produced antibodies. Pursuant to the conditions required by those permits, the permittee removed the flowers of the tobacco plants prior to pollen release to reduce the potential for outcrossing. In permit application 05-354-01r Planet Biotechnology seeks approval to grow tobacco plants genetically engineered to produce antibodies at a larger scale of production. As with previous field trials, mitigation practices for the proposed release also include the hand removal of flowers. However, because the proposed field release is of a scale where hand removal of flowers may not be 100 percent effective, it is possible that some flowers may remain and that some low level of pollen may be released into the environment. In response, APHIS has prepared an environmental assessment to identify and evaluate potential adverse environmental impacts associated with the proposed action.

APHIS has reviewed the information submitted by Planet Biotechnology and is considering whether to issue the permit as submitted (Alternative B), issue the permit with additional requirements (Alternative C), or deny the permit (Alternative A). This environmental assessment and the comments received from the public will serve to inform this decisionmaking process to allow or not to allow this environmental release.

The preferred alternative is "Alternative C," *Issue the Permit with Supplemental Conditions*. This decision is based upon the conclusion that the mitigation measures described in the environmental assessment are adequate to confine the regulated article to the release sites.

II. INTRODUCTION

A. Purpose of the Environmental Release

On December 21, 2006, APHIS received a permit application (05-354-01r) from Planet Biotechnology of Hayward, CA. The application requests permission to release a genetically engineered tobacco variety, H8-105, into the environment. The purpose of the environmental release is to grow the genetically engineered tobacco line, H8-105, which produces an antibody designated by Planet Biotechnology as CaroRx™. The environmental release, in Daviess County, Kentucky, is scheduled to begin in June 2007 and should be completed in the fall of 2007. Following harvest of the tobacco leaves, the permittee will extract and purify the CaroRx™ antibody. Application of CaroRx™ to human teeth is intended to prevent tooth decay (Ma 1998). In the United States, CaroRx™ is an Investigational New Drug (BB-IND # 7526) and in the European Union, it is a registered Medical Device.

APHIS has previously granted permits to Planet Biotechnology for small-scale field trials of transgenic tobacco, genetically engineered to produce CaroRx™ antibodies against *S. mutans* bacteria known to cause tooth decay, under permits 02-108-02r, 04-044-01r, 05-053-01r and 05-087-01r. APHIS would continue to grant permits for small-scale field trials to Planet Biotechnology, similar to those granted previously.

B. APHIS Regulatory Authority

APHIS' Biotechnology Regulatory Services (BRS) regulates the environmental release of genetically engineered organisms into the environment under the authority of the Plant Protection Act of 2000, 7 U.S.C. 7701-7772, and APHIS regulations under Title 7 of the Code of Federal Regulations Part 340 (7 CFR § 340), "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests." Genetically engineered organisms are considered to be regulated articles if the donor or recipient organism, the vector or vector agent used in engineering the organism belongs to a taxonomic group listed in the regulation and is also a plant pest, or if there is a reason to believe it is a plant pest, unless a determination of non-regulated status has been made by APHIS. The permit application submitted to APHIS by Planet Biotechnology requests approval for environmental release of transgenic tobacco line, H8-105, that contains regulatory genes from the cauliflower mosaic virus (CaMV). Because CaMV is listed as a plant pest under 7 CFR § 340.2, the organism is deemed a regulated article.

This EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.C § 4321 et seq.); (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR §§ 1500-1508); (3) USDA regulations and implementing NEPA (7 CFR § 1b); and (4) APHIS NEPA Implementing Procedures (7 CFR § 372).

Generally, the issuance of permits for environmental release of regulated articles is categorically excluded from the requirements for an EA under APHIS NEPA implementing procedures (7 CFR § 372.5(c)(3)(ii)). In certain cases, when APHIS determines that a confined field release of a genetically engineered organism has the potential to significantly affect the quality of the human environment as those terms are defined in 40 §§ CFR 1508.27 and 1509.14, an EA or environmental impact statement is prepared pursuant to 7 CFR § 372.5(d). Accordingly, this EA was prepared because the permit applicant intends to grow tobacco plants, genetically engineered to produce antibodies, at a large scale. The permittee designed the production practices for the proposed release, including the practice of flower removal to prevent pollen production, to confine the regulated article to the site of the field test. However, due to the large scale of the release, it is possible that some flowers will remain on the plants and release small amounts of pollen, which raises new issues. Consequently, APHIS has prepared this EA to identify and evaluate potential adverse environmental effects associated with the proposed release.

III. PURPOSE AND NEED FOR THE PROPOSED ACTION

A. Proposed Action

The proposed action is for APHIS, to issue a permit for the confined environmental release of tobacco line, H8-105, genetically engineered to express an antibody as well as marker gene neomycin phosphotransferase (NPTII) and nopaline synthase (NOS).

B. Need for This Action

Under APHIS regulations (7 CFR § 340.4(e)), the receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

> *"Administrative action on applications*. After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requested by APHIS, a permit shall be granted or denied."

IV. ALTERNATIVES TO THE PROPOSED ACTION

A. No Action

For the purposes of this EA, the "no action" alternative would be the denial of permit application 05-354-01r. This would be the preferred alternative if after review of the processes and procedures to prevent the dissemination and establishment of plant pests as described in the permit, APHIS concluded that the permit conditions would not be adequate to confine the regulated article.

B. Issue the Permit as Received

If APHIS issued this permit as received, the permittee would be allowed to proceed with the environmental release under the conditions designed and proposed by the permittee to confine the regulated material to the site, or sites, of release (see Section V. AFFECTED ENVIRONMENT , part C. Description of the Field Release). In addition, the permittee would also have to adhere to the standard permit conditions required by APHIS (see Appendix B: Standard Permit Conditions from 7 CFR § 340.4).

Under this alternative, the environmental release of the genetically engineered tobacco line, H8-105, would be authorized with no additional conditions implemented by APHIS-BRS.

This would be the preferred alternative if, after review of the processes and procedures to prevent the dissemination and establishment of plant pests described in the permit, APHIS concluded that the permit conditions would adequately confine the regulated article.

C. Issue the Permit with Supplemental Conditions

If APHIS made the decision to issue this permit with supplemental conditions, the permittee would be allowed to proceed with the environmental release in Daviess County, Kentucky, (see Section V. AFFECTED ENVIRONMENT , part C. Description

of the Field Release), but would have to adhere to supplemental permit conditions specified by APHIS. APHIS would base the supplemental conditions on APHIS' scientific analysis of the permit application, input from the state of Kentucky, and public comment on this EA (see Appendix C: Proposed Supplemental Permit Conditions). If warranted, APHIS would require mitigating measures, stipulated in the final supplemental permit conditions, to prevent dissemination of the organism outside the field production area.

Currently, APHIS proposes to include the following measures to promote a confined field release and to ensure no significant harm to the environment:

1. The permittee must document activities related to the field test and movement of the regulated article and make them available for APHIS inspections.
2. The permittee will report unintended releases according to timeframes and procedures provided in the supplemental conditions.
3. The permittee will provide reports to APHIS according to the guidance in the supplemental conditions.

This would be the preferred alternative if, after review of the processes and procedures to prevent the dissemination and establishment of plant pests as described in the permit and the additional supplemental conditions, APHIS concluded that the permit conditions would be adequate to confine the field trial and prevent the release of the regulated articles.

V. AFFECTED ENVIRONMENT

A. Description of the Regulated Article

Tobacco line, H8-105 is genetically engineered to produce the antibody CaroRx™ for use as a treatment to prevent tooth decay. CaroRx™ specifically binds to the bacterium, *S. mutans*, which has been identified as the major organism causing tooth decay (Loesche 1975). When the attachment of *S. mutans* to the tooth surface is blocked by an antibody, such as CaroRx™, which binds to the *S. mutans* SAI/II surface adhesion protein, the resulting infection and subsequent tooth decay may be prevented (Ma 1998).

An antibody is a protein that binds specifically to a particular substance, known as an antigen. While each antibody is unique in its ability to bind to its corresponding antigen, antibodies, in general, have the same overall structure. Typically, antibodies exist as one or more copies of a Y-shaped unit composed of four polypeptide chains called immunoglobulins (Ig). Each Y-shaped Ig contains two identical copies of a heavy chain, and two identical copies of a light chain (see Appendix A, Fig.1A). An antibody can also be fragmented via enzymatic digestion into the Fab, Fab2, and Fc fragments (see Appendix A, Fig. 1B). Secretory antibodies comprise the most abundant class of antibodies produced in humans (Ma 2005). They exist in the dimeric form with two Y-shaped Ig molecules bound to a J chain and to a secretory component (see Appendix A, Fig. 2). The J chain serves to dimerize the two Ig molecules and the secretory component serves to protect the immunoglobulin from proteases (Ma 1998). The Ig molecules in CaroRx™ were derived from Guy's 13 immunoglobulin G (IgG) monoclonal antibody

that specifically binds to *S. mutans* (Ma 1994). The CaroRx™ antibody differs from Guy's 13 antibody as the former is engineered to contain an Fc portion of the heavy chain from an immunoglobulin A (IgA). This chimeric IgA/IgG antibody binds to a J chain and further assembly with a secretory component (Ma 1995, Ma 1998). The secretory antibody CaroRx™ prevents bacterial colonization to protect humans against oral *S. mutans* infection (Ma 1998).

To generate the transformed tobacco line, H8-105, the constituent parts of the secretory CaroRx™ antibody (light chain from mouse, heavy chain from mouse, J chain from mouse, and secretory component from rabbit), which are all driven by the cauliflower mosaic virus (CaMV) promoter, were cloned and expressed in tobacco by independent transformation events. The events were then combined into a single line by classical breeding methods (Ma 1995). In line H8-105, two additional protein products are expressed under the control of a plant recognized NOS promoter (one of very few bacterial promoters known to be expressed in plants). These proteins are NPTII (from *E. coli*), an enzyme that confers resistance to kanamycin, which is used as selectable marker, and NOS (from *A. tumefaciens*), an enzyme that forms nopaline from the amino acid arginine and alpha-ketoglutaric acid. NOS, although present in the plasmid vector used in the production H8-105, was not utilized as a selectable marker in the construction of H8-105. Line H8-105 also contains *trf*A (from *E. coli*) that encodes for a DNA-binding protein important for plasmid DNA replication and *add*3 (from *E. coli*) that encodes for resistance to the antibiotic streptomycin/spectinomycin. However, because *trf*A and *add*3 are driven by bacterial promoters that are not recognized by plants, they are not expressed in H8-105. Additional non-coding sequences (sequences contained in the transformed plant, but not converted into protein products) present in H8-105 are ColEI and RK2 origin of replication sequences (from *E. coli*) and the *nos* terminator from *A. tumefaciens.*

In the transformed line H8-105, the expression level of CaroRx™ can be as high as 0.5 milligrams per kilogram of fresh weight in leaves (mg/kg FW), up to 0.4 mg/kg FW in stems, and up to 0.2 mg/kg FW in roots, but it is not detectable in pollen (data submitted by the applicant with the 05-354-01r permit).

B. Biology of Tobacco

The genus, *Nicotiana,* is composed of some 76 naturally occurring species (Chase 2003). Many *Nicotiana* species are native to South America with the remainder distributed throughout Central America, western North America, Australia, and various islands of the South Pacific. *Nicotiana tabacum* L. probably originated in Argentina by hybridization of *N. sylvestris* Speg. & Comes, and *N. tomentosiformis* Goodsp, where the progenitors are native. For the purposes of this EA "tobacco" refers to *N. tabacum*. Most other *Nicotiana* species are not cultivated, with the exception of Aztec tobacco, *N. rustica* (Chaplin 1979), and *N. alata, N. langsdorffi, N. sanderae*, and *N. sylvestris*, which are grown for ornamental purposes (Perry 2006, Smith 1979).

In Kentucky, tobacco does not grow without human intervention. In general, it does not escape cultivation and persist in the wild (Goodspeed 1954, Harlan 1992, Shew 1991), or even volunteer in fields in the following growing season (Dr. Robert Pearce, Tobacco

Extension Specialist, University of Kentucky, communicated to APHIS on 11/3/06). Current U.S. databases of native or naturalized plants indicate that *N. tabacum* is not naturalized in Kentucky (Kartesz 2005; USDA, NRCS 2006). Because tobacco does not survive freezing winter temperatures, it is cultivated as an annual in temperate climates, such as Kentucky (Poehlman 1959).

Typical field production methods for the cultivars of tobacco that are grown in Kentucky include topping (removal of the flower bearing inflorescence and the uppermost leaves) and removal of suckers (lateral stems) to improve yield and quality (Purseglove 1968, Dr. Robert Pearce, Tobacco Extension Specialist, University of Kentucky, communicated to APHIS on 11/3/06). Tobacco grown for leaves is typically topped to prevent flowering. In contrast, tobacco grown for seed production is not topped and is allowed to flower openly.

N. tabacum is a highly self-pollinated species because fertilization generally occurs before the flower opens, resulting in inbreeding of greater than 95 percent (Shew 1991). Tobacco is pollinated by honey bees (*Apis mellifera* L.), sweat flies (*Didea fasciata* Macq.), bumblebees (*Bombus* species), hawkmoths (*Theretra tersa* L.), hummingbirds (*Archilochus colubris* L), and some bats (Hodges 1952, McMurtrey 1960, Nattero 2003, Poehlman 1959). Other factors, such as incompatibility, may influence crossing efficiencies (Purseglove 1968). Outcrossing rates have been reported for crossing up to a ½ mile to range from 0.3 to 19 percent, decreasing as distance between sexually compatible individuals increases (Free 1993, Litton 1964, McMurtry 1960). However, the amount of cross-pollination seldom exceeds 0.5 percent between untopped tobacco plants.

C. Description of the Field Release

The affected environment will be limited to the release site because the permit proposes a confined environmental release. The applicant will perform mitigation measures designed to confine the regulated material to the release site and maintain its separation from nonregulated material.

The applicant proposed the following measures in the submitted permit to confine the release to the field test site:

1. Dedicated facilities (locked or secured buildings, bins, or areas, posted as restricted to authorized personnel only) shall be used for storage of equipment and regulated articles for the duration of the field test.
2. All field plots shall be tagged and GPS (Global Positioning System) coordinates recorded and communicated to APHIS (helps to locate the field to monitor for volunteers).
3. The field plots shall be bordered on all four sides with 50 feet of perimeter fallow zone (not in production) to allow farm machinery to move around the site and will prevent physical mixing of the regulated plants with surrounding plants that may be used for food or feed.

4. An isolation distance of at least ½-mile shall be maintained between the regulated plots and non-regulated tobacco. This area shall be monitored throughout the field test period.
5. At least a 1-mile distance shall be maintained between the field plots and any tobacco seed production. This area shall be monitored throughout the field test period.
6. Seeds shall be germinated in a greenhouse and plantlets shall be transplanted out into the field. This reduces the possibility of the small seeds being released out into the environment.
7. When the tobacco plants are sufficiently mature for flower production, the tobacco plants shall be monitored for flower production 5 days/week and any flowers or flower buds detected shall be removed.
8. During the growing season the plants shall be inspected for traits such as weediness, resistance/susceptibility to insects or disease, or unusual differences in plant growth or morphology.
9. All field equipment or vehicles entering the field, used for harvest, transport, and pest/weed control, shall be cleaned prior to use and after use according to the APHIS approved Standard Operating Procedures (SOPs).
10. During the period of time when the regulated article is producing pollen, the surrounding land within a ½-mile radius shall be monitored to ensure that it is maintained free of reproductively compatible plants.
11. Within 2 weeks following harvest and antibody extraction, the remaining plant material shall be disked into the soil.
12. The field plot and 50-foot border area shall be monitored for volunteers for 12 months. Volunteers, if found, shall be uprooted by hand and destroyed by dismemberment and incorporation into the soil.
13. Personnel who handle the regulated material shall receive instruction in all the activities that they carry out involving the regulated material. This training shall be documented and the documentation shall be made available to APHIS inspectors. This training shall encompass conditions stipulated in the permit, the APHIS permit conditions, the APHIS supplemental permit conditions and the pertinent Federal regulations.
14. Activities related to the field test and movement of the regulated article shall be documented.

In addition, APHIS will perform the following activities to reduce the possibility of human error:

1) Inspection of records that cover multiple aspects of the field trial.
2) Multiple inspections of field trials timed to occur at critical steps in the production process.

VI. POTENTIAL ENVIRONMENTAL IMPACTS

The proposed action is to conduct a confined environmental release with a regulated article, a genetically engineered organism. The permit application describes the

procedures that the applicant has submitted to APHIS to confine the regulated organism to the release site according to the requirements under 7 CFR § 340. APHIS evaluated the proposed action based on the biology of the tobacco plant, any potential hazards associated with the transgenes and the likelihood that the transgenes could persist and potentially harm the environment.

A. Potential for Persistence of the Engineered Plants in the Environment

APHIS evaluated the potential for the tobacco line, H8-105, plants or their progeny to survive in the environment at the conclusion of the environmental release. Tobacco production starts in the greenhouse, where seeds are germinated under controlled conditions. Tobacco seedlings are then transplanted into the field. After the plants are in the field, the fields will be monitored to remove flowers. The field will be managed in a way to remove flowers on a regular basis (five days/week) and which will preclude most seed formation. Even if some seeds were formed and integrated into the soil, tobacco is not likely survive and persist at the field test site. In Kentucky, tobacco plants do not grow unless cultivated under specialized growing conditions (Goodspeed 1954, Harlan 1992, Shew 1991) and tobacco is not reported as naturalized in Kentucky (Kartesz 2005, USDA, NRCS 2006). Volunteer tobacco plants are not found when tobacco is grown under the typical cultivation procedures that includes topping (Dr. Robert Pearce, Tobacco Extension Specialist, University of Kentucky, 11/3/06). In addition, previous field tests that Planet Biotechnology conducted in Kentucky with tobacco, using production methods that include flower removal, resulted in no volunteer tobacco plants growing in the subsequent season (02-108-02r, 04-044-01r, 05-053-01r and 05-087-01r).

Because of the size of the environmental release (100 acres or less) and the number of plants, Planet Biotechnology acknowledges that some flowers may be not be removed prior to pollen release even though the field will be managed in a way to remove flowers on a regular basis.

APHIS evaluated the potential for the H8-105 to persist in the environment due to the possibility that the transgenic pollen would outcross to the surrounding non-transgenic tobacco. Tobacco plants are primarily self-pollinating such that natural outcrossing is reported to be infrequent, 4 percent or less (Litton 1964, McMurtrey 1960). This is caused in part by the shedding of pollen prior to flower opening, resulting in a predominance of self-fertilization. In addition, to reduce outcrossing, the transgenic tobacco will be separated from other tobacco plants by at least ½ mile. This distance is twice the Association of Official Seed Certifying Agencies' (AOSCA) standard required for 0.01 percent varietal purity. Another factor that will reduce the likelihood of outcrossing is that commercially-produced tobacco in Kentucky is managed to prevent flowering via topping and sucker control methods (Akehurst 1981, Dr. Robert Pearce, Extension Tobacco Specialist, University of Kentucky, Communicated with APHIS on 11/03/06). These production methods will reduce or eliminate the availability of receptive cultivated tobacco flowers for cross-hybridization. Even if some transgenic flowers were produced at the field site, the common practice of topping tobacco cultivars means that receptive non-regulated tobacco flowers will not be in the vicinity of the environmental release. Therefore, because flowering is limited in both regulated and commercial plants

due to topping, because the regulated plants will be separated from commercial plants by at least ½ mile, and because tobacco is largely self pollinating, APHIS believes that it is extremely unlikely for the regulated tobacco plants to outcross to the surrounding cultivated tobacco. As a redundant mitigation measure, the site of the environmental release will be monitored for tobacco plants in the following growing season.

Another concern that APHIS must consider is the proximity of tobacco seed production in the area of the environmental release. Such tobacco plants would not be topped and the seed would be saved for planting. The closest known tobacco seed production area is at least 10 miles from Daviess County, Kentucky (Dr. Robert Pearce, Extension Tobacco Specialist, University of Kentucky, 11/03/06). This distance is 20 times the distance specified by the AOSCA standard for 0.01 percent varietal purity (AOSCA 2004). APHIS concludes there is a negligible likelihood that the regulated tobacco plants will outcross to tobacco grown for seed production purposes.

APHIS evaluated the potential for tobacco line, H8-105, to outcross to other *Nicotiana* species. Hybridization between *Nicotiana* species rarely produce fertile plants that generate viable pollen and seed (Burk 1979, Nikova 1997). Even when seeds are produced under the best laboratory circumstances, the viability of such seeds is very low (Burk 1979). The greatest chance for successful production of *Nicotiana* species hybrids that will produce viable seed is between two *Nicotiana* species with the same chromosome number; *N. tabacum* contain 24 pairs of chromosomes. Native or naturalized *Nicotiana* species with the same number of chromosomes do not occur in Kentucky (USDA, NRCS, 2006). Ornamental *Nicotiana* species comprise approximately 1 percent of the ornamental plants grown in Kentucky (Robert G. Anderson**,** Extension Professor**,** Department of Horticulture, University of Kentucky**,** Lexington, KY, personal communication). Of the most common ornamental *Nicotiana* species hybrids: *N. alata, N. langsdorffi, N. sanderae* (a horticultural species hybrid between *N. forgetiana* and *N. alata*) and *N. sylvestris* (Perry 2006, Smith 1979), only *N. sylvestris* contains 24 chromosomes. However, *N. sylvestris* X *N. tabacum* hybrids do not produce viable seeds (Al-Almad, 2006). Therefore, it is extremely unlikely that outcrossing will with these *Nicotiana* species will result in the production of viable seed.

APHIS considered if the regulated plants or seeds could be moved from the environmental release site by animals. Animals that could move plant or plant parts do not frequent tobacco fields, except for skunks, which may forage on insects (Dr. Orlando Chambers, University of Kentucky, personal communication). In addition, seeds are not likely to be produced because the plants will be topped. Even if the tobacco plants or seeds were moved from the site of the release, it is not likely that the tobacco plants would persist because tobacco does not survive in Kentucky without human intervention. Therefore, APHIS concludes there is a negligible likelihood that tobacco would establish outside of the test site as a result of movement by animals.

APHIS also considered the likelihood that humans could inadvertently move the regulated article from the environmental release site. In a recent workshop hosted by APHIS dealing with gene confinement issues in genetically engineered crops (USDA-

APHIS 2004), one of the more likely mechanisms contributing to the breakdown of confinement and movement of seed was identified as human error, and the most reliable means of preventing this is to maintain and enforce stringent standard operating procedures. APHIS requests and reviews SOPs for equipment and processes related to the movement, planting, monitoring, and harvest of environmental release. Implementation of these procedures is verified during multiple inspections conducted by APHIS. In addition, the personnel who handle the regulated material are required to be trained according to APHIS-approved training processes to further ensure that the SOPs are carried out during the course of the environmental release. Therefore, APHIS believes that measures are in place to ensure that unauthorized movements resulting from human error are very unlikely.

In summary, APHIS took the following into account to make a determination about the likelihood that the regulated article would persist in the environment:

- tobacco does not survive without human intervention
- most of the flowers will be removed from the regulated tobacco plants
- tobacco plants are primarily self-pollinating
- non-regulated cultivated tobacco will not be present within 2640 feet of the environmental release
- non-regulated cultivated tobacco is topped (no flowers present)
- the distance to any untopped (flowers present) seed production site is over 10 miles
- seed set with an ornamental species is unlikely
- the field release will be conducted by trained personnel who will carry out and record the procedures and processes throughout the environmental release

APHIS believes that gene introgression from H8-105 into cultivated tobacco and ornamental *Nicotiana* species and persistence of the regulated article in the environment is extremely unlikely to occur as a result of the proposed environmental release.

B. Impacts from the presence of *npt*II, *nos, add*3, *trf*A and ColEI and RK2 origin of replication.

The selectable marker gene for kanamycin resistance (*nptII*) is expressed in H8-105. Because NPTII is not toxic, it shares no homology with proteins known to be toxic or allergenic (U.S. FDA 1998), and it is present in many plant lines previously deregulated by USDA, the expression of NPTII in tobacco plants is not expected to have deleterious effects or significant effects on nontarget organisms, including beneficial organisms and threatened and endangered species. The NOS protein has no known sequence homology to known toxins or allergens (Canadian Food Inspection Agency, 1998). In addition, many plant species, such as soybeans and cotton, naturally produce nopaline (Christou 1986). Similarly, the expression of NOS in tobacco plants is not expected to have deleterious effects or significant effects on nontarget organisms, including beneficial organisms and threatened and endangered species.

Other DNA sequences are present, but are not expected to be expressed in H8-105. Because the genes *trf*A (encodes for a DNA-binding protein that initiates replication from the RK2 origin of replication) and *add*3 (encodes for the resistance to streptomycin/spectinomycin) are under the control of bacterial promoters, they are unlikely to be expressed in the nuclear plant genome. Similarly ColEI and RK2, origin of replication sequences (from *E. coli*), and the *nos* terminator (from *A. tumefaciens*) are not expressed in H8-105. Therefore, APHIS has determined the presence of the NPTII and NOS proteins and the non-expressed DNA will have no significant environmental impacts.

C. Impact on animals

Animals, other than the occasional skunk foraging on insects, do not generally consume tobacco planted in an agricultural setting (Dr. Orlando Chambers, University of Kentucky, personal communication). There are reports that if pregnant pigs are allowed to consume tobacco, malformations of the fetus may occur (Crowe, 1969). Although animals are not known to consume tobacco leaves, even if animals did consume the H8-105 tobacco leaves, there is no reported toxicity of antibodies to vertebrate or invertebrate animals. In general, antibodies as a class are non-toxic. Antibodies are ubiquitous in nature, so insects and animals that consume eggs and milk are routinely exposed to antibodies (see Appendix A: Antibodies). Any antibody in plant debris that was produced via genetic engineering would have the same fate as antibodies in any decaying animal tissue or by-product, and would be quickly degraded and incorporated into the nitrogen cycle (see Appendix A: Antibodies for more detailed information on the degradation of antibodies in the environment). The genetically engineered CaroRx™ has been found to be non-toxic and non-allergenic. During clinical trials where CaroRx™ was administered to animals and human volunteers, no adverse effects were detected suggesting that plant preparations containing the purified antibodies did not induce an allergic response when given orally (Ma 1998, Ma 2005, Weintraub 2004). Therefore, APHIS believes that the genetically engineered tobacco presents no increased risk to animals compared to the non-GE tobacco.

D. Alteration in susceptibility to disease or insects

The presence of CaroRx™ in tobacco is not expected to alter the susceptibility of tobacco plants to diseases or to insects, as the antibody binds specifically to the *S. mutans* SAI/II surface adhesion protein in the bacterium *S. mutans*. *S. mutans* is an organism that causes tooth decay but has no reported effects on plants. In addition, previous field tests conducted with H8-105 tobacco in 2002 (02-108-02r), 2004 (04-044-01r), and 2005 (05-053-01r and 05-087-01r) did not reveal increased susceptibility to disease or insects. Therefore, APHIS believes that this environmental release will not increase tobacco disease or susceptibility of tobacco to insects.

E. Weediness

Tobacco is a highly specialized crop bred for intensive monoculture. Despite its ability to produce very large quantities of seeds, a trait associated with weedy plants, tobacco is not considered to be a weed in Kentucky (http://www.nationalplantboard.org/laws/noxious.html, Holm 1977 and 1979, Muenscher

1980, Reed 1977, Weed Science Society of America 1989). The addition of the transgenes is not likely to render tobacco more weedy. None of the gene products are likely increase the fitness, alter reproductive capacity, or affect other traits associated with weediness.

F. Impact on Existing Agricultural Practices

The transgenic tobacco plants engineered to produce antibodies will be cultivated using standard cultivation practices generally used for tobacco production in Kentucky. Additional measures will be taken to ensure that the regulated article is confined to the site of the environmental release. These measures are described in part V, section C, Affected Environment Description of the Field Release, and in Appendix C, Proposed Supplemental Permit Conditions, of this EA. Planet Biotechnology will monitor the fields throughout the growing season for deleterious effects on plants, non-target organisms, or the environment, and during the following year for volunteer plants. The use of these plants in the proposed environmental release should not affect current agricultural practices with tobacco.

G. Horizontal Gene Transfer to Other Organisms

Following harvest of the tobacco plants, some plant material will remain at the environmental release site and will be subject to natural degradation by soil-inhabiting microbes. APHIS has assessed the likelihood of whether DNA transfer could occur to soil-inhabiting microbes through a process known as horizontal transfer. Horizontal gene transfer of DNA from the tobacco plants to bacteria and expression in bacteria is unlikely to occur. First, many genomes have been sequenced from bacteria that are closely associated with plants, including *Agrobacterium* and *Rhizobium* (Kaneko 2000, Wood 2001, Kaneko 2002). There is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin 2001, Brown 2003). Third, FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes in genetically engineered plants and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms, in the gastrointestinal tract of humans or animals, or in the environment, is remote (http://vm.cfsan.fda.gov/~dms/opa-armg.html). Therefore, APHIS concludes that horizontal gene transfer is unlikely and even if it did occur would pose no significant environmental risk.

H. Impacts on Human Health

The tobacco plants in this environmental release will be used for the processing and extraction of antibodies, but will not be used directly for other purposes. After extraction of CaroRx™, the remaining tobacco plant material will be incorporated into the soil for natural decomposition. CaroRx™ has been the subject of clinical trials where its safety was demonstrated. No adverse effects to humans were reported when CaroRx™ antibodies were applied orally (Ma 1990). Because there may be some flowers produced and honeybees are known to pollinate tobacco, APHIS considered the possibility that the gene product would be present in honey. APHIS concludes that the CaroRx™ will not be detectable in honey because CaroRx™ is not expressed in pollen or nectar at levels that

can be measured with a sensitive antibody assay (data submitted with the 05-354-01r permit by Planet Biotechnology). No potential impact on people living in the area of the environmental release, or any other human population, can be identified. Therefore, APHIS concludes that the impact on human health poses no significant environmental risk.

I. Impacts on Threatened and Endangered Species

APHIS evaluated the potential for impacts on Threatened and Endangered Species (TES) proposed and listed with the U.S. Fish and Wildlife Service (FWS) using the U.S. Fish and Wildlife database http://www.fws.gov/endangered/wildlife.html and NatureServe database: http://www.natureserve.org/explorer/. Analysis of published data and studies supplied by the applicant support the applicant's conclusion that the confined release of H8-105 would not harm any Federally listed (or proposed) TES (see Appendix E: The Threatened and Endangered Species worksheet that was prepared by the permit applicant). The analyses found that none of the listed (or proposed) TES are associated with tobacco fields in Kentucky. Even if any of the listed (or proposed) TES frequented the environmental releases, none of the species would likely be exposed to the engineered products because they do not consume tobacco plants and the engineered products are not detectable in pollen. If the listed species were exposed to the engineered products (antibody, NOS, NPTII), the risk would be negligible because there is no reported toxicity of the products and, therefore, the tobacco would not be any more hazardous to these organisms.

APHIS has reached a determination that the proposed environmental release will have no effect on federally listed threatened or endangered species or species proposed for listing, and no effect on designated critical habitat or habitat proposed for designation in the action area. Consequently, consultation under Section 7 of the Endangered Species Act with the United States Fish and Wildlife Service is not required for the action described in the preferred alternative of this EA.

J. Cumulative Environmental Effects

Concurrent commercial growth of tobacco for leaf and seed purposes has already been discussed above. There are no other past, present, or reasonably foreseeable actions that could, in aggregation with the environmental release of H8-105, cause cumulative impacts on the environment.

K. Special Considerations: Other Environmental Statutes and Considerations

Executive Order (EO) 12898, "Federal Actions To Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and significant human health or environmental effects. Each alternative was analyzed in its ability to affect minority and low-income populations.

None of the alternatives were found to pose disproportionately high or significant human health or environmental effects to any specific minority or low-income group.

EO 13045, “Protection of Children from Environmental Health Risks and Safety Risks,” acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with APHIS’s mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. None of the alternatives are expected to have disproportionately high or significant human health or environmental effects to children.

EO 13112, “Invasive Species,” states that Federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. Tobacco is not invasive and is widely cultivated in the United States. Based on the data submitted by the applicant and reviewed by APHIS, the engineered plant is not significantly different in any fitness characteristics from its parent that might increase its invasive potential.

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APPENDIX A: ANTIBODIES

Introduction
The following section describes the structure and function of antibodies as well as their uses in research and disease treatment.

The Immune System
Healthy humans are born with an innate (or natural) immunity, a type of immunity that occurs naturally as a result of a person's genetic constitution or physiology and does not arise from a previous infection or vaccination. Innate immunity also includes the external barriers of the body, like the skin and mucous membranes (like those that line the nose, throat, and gastrointestinal tract). There are many germs that affect other species but do not harm humans and *vice versa*. There is also a second kind of protection called adaptive (or active) immunity. Adaptive immunity evolves as a person or animal is exposed to diseases or immunized against diseases through vaccination and generally produces long-term immunity. Passive immunity is the transfer of antibodies from another individual, as through injection or placental transfer to a fetus; it essentially is "borrowed" from another source and it lasts for a short time.

The immune system (both active and innate) is the body's defense against infectious organisms and other invaders. It is made up of a network of cells, tissues, and organs that work together to protect the body. The cells that are part of the adaptive defense system are white blood cells, or leukocytes. One type of leukocyte is called a lymphocyte, which allows the body to remember and recognize previous invaders. There are two kinds of lymphocytes: B lymphocytes (B cells) and T lymphocytes (T cells). Lymphocytes start out in the bone marrow and either stay there and mature into B cells, or they leave for the thymus gland, where they mature into T cells. One of the main jobs of B cells is the production of antibodies. The part of the immune system that involves antibodies secreted by B cells is called humoral immunity. A substance introduced into the body that stimulates the production of an antibody is called an antigen. Antigens include toxins, bacteria, foreign blood cells, and the cells of transplanted organs. When an organism detects an antigen, several types of cells work together to recognize and respond to it. These cells trigger the B cells to produce antibodies. Antibodies are specialized proteins that lock onto specific antigens. Although antibodies can recognize an antigen and lock onto it, they are not capable of destroying it without the help of T cells. T cells are part of the system that destroys antigens that have been tagged by antibodies or cells that have been infected or somehow changed. Antibodies can also neutralize toxins (poisonous or damaging substances) produced by different organisms.

Antibodies
An antibody is a protein that binds specifically to a particular substance (an antigen). While each antibody is unique in its ability to bind to its corresponding antigen, antibodies in general have the same overall structure and are referred to collectively as immunoglobulins. Antibodies exist as one or more copies of a Y-shaped unit, composed of four polypeptide chains. Each Y contains two identical copies of a heavy chain, and two identical copies of a light chain, named as such by their relative molecular weights (Fig.1A). An antibody can also be broken into two pieces (via enzymatic digestion) called the Fab, Fab2, and Fc fragments. The Fab fragment is the portion of the immunoglobulin where the relevant antigen binds and the Fc fragment is the other section of

the immunoglobulin (Fig 1B). Antibodies can be divided into five classes: IgG (IgY in birds), IgM, IgA, IgD and IgE, based on the number of Y units and the type of heavy chain. Antibodies are produced by plasma cells (B cells) in response to infection or immunization. By binding to an antigen (or pathogen), the antibody either neutralizes or prepares the antigen (or pathogen) for uptake and destruction by phagocytes (Janeway 2001).

Polyclonal versus Monoclonal Antibodies

Antibodies produced by the immune system are, by definition, polyclonal; meaning many different B cells produced the antibodies in response to antigen stimulation. A monoclonal antibody is produced by a single clone of a B cell. Large amounts of monoclonal antibodies are produced by a hybridoma; a cell line that is made by fusing a B cell with a myeloma cell (Köhler 1975). Monoclonal antibodies can also be produced in genetically transformed microbial cells. Recombinant antibodies have been successfully made in microbial expression systems and mammalian cell cultures for over two decades. The estimated cost to produce a monoclonal antibody using hybridoma technology or microbial expression systems is US $5000/gram (Institute for Laboratory Animal Research and Council 1999). The cost to produce recombinant proteins (such as antibodies) is reduced 80-98 percent in plants as compared to traditional microbial and mammalian cell systems (Institute for Laboratory Animal Research and Council 1999, Giddings 2001), with much of the costs focused on downstream purification systems.

Importance of Monoclonal Antibody Production

Some of the early applications of monoclonal antibodies were blood-group typing, pregnancy testing, and identifying viruses, cancers, blood clots, and heart disease. Today, along with multiple diagnostic test uses, monoclonal antibodies are part of many cancer treatments, as well as treatments for arthritis, a variety of viruses, diabetes, and multiple sclerosis (http://users.path.ox.ac.uk/~scobbold/tig/new1/mabth.html). The FDA has approved many monoclonal antibodies for use in cancer therapy (www.cancer.org):

Trade Name (Generic Name)	Cancer Treated	Approved
Rituxan (Rituximab)	Non-Hodgkin lymphoma	1997
Herceptin (Trastuzumab)	Breast cancer	1998
Mylotarg (Gemtuzumab ozogamicin)	Acute myelogenous leukemia (AML)	2000
Campath (Alemtuzumab)	Chronic lymphocytic leukemia (CLL)	2001
Zevalin (Ibritumomab tiuxetan)	Non-Hodgkin lymphoma	2002
Bexxar (Tositumomab)	Non-Hodgkin lymphoma	2003
Erbitux (Cetuximab)	Colorectal cancer	2004

Avastin (Bevacizumab)	Colorectal cancer	2004

Another important therapeutic use of antibodies is the post-exposure rabies treatment to prevent disease outbreak in a bitten human (considered category III exposure by WHO, http://www.who.int). The administration of antibody to an unimmunized individual is called passive immunization. One of the first monoclonal antibodies to be marketed for passive immunization was palivizumab, which is used to prevent serious lower respiratory tract infections caused by respiratory syncytial virus (RSV) in infants. Passive immunization of *Campylobacter jejuni*, infected chickens with oral monoclonal antibodies, was found to be successful as both prophylaxis and therapy (Tsubokura 1997). With rising medical costs and increasing use of monoclonal antibodies in the medical and industrial field, researchers have found ways to produce monoclonal antibodies at a reduced cost to the consumer by creating plants containing the monoclonal antibody gene construct.

Ubiquitous Presence of Antibodies in Nature

Humans consume 50-100g of protein per day as part of their normal dietary intake (USDA and HHS 2005). Foods that contain naturally present polyclonal antibodies include eggs, meat, milk, and milk products. Egg yolks contain approximately 100-150 mg of total IgY antibody per yolk, while the egg whites contain trace amounts of IgM and IgA (Polson 1990). Polyclonal antibodies are a normal component of animal blood (Tizard 2000); and therefore are present in all meat. Cow's milk (not colostrum, whose antibody content is considerably higher) contains 50-100 mg IgA/dl, 5-10 mg IgM/ dl, and 20-50 mg IgG/dl (Tizard 2000).

Antibody Degradation in the Environment

The growth of all organisms depends on the availability of nitrogen, which is required in large amounts as an essential component of proteins, nucleic acids, and other cellular constituents. Nitrogen is often the limiting factor for growth and biomass production in all environments where there is suitable climate and availability of water to support life. Along with nitrogen fixation (the conversion of atmospheric nitrogen to ammonium or nitrate ions), microbes degrade organic material and debris in the soil, releasing fixed nitrogen for reuse by other organisms (http://soil.gsfc.nasa.gov/NFTG/nitrocyc.htm). Microbes are known to degrade many different types of complex organic and inorganic molecules such as TNT, Dioxins, and polychlorobiphenyls (PCBs) (Tiedje 1993, Wittich 1998, Lewis 2004). Antibodies are relatively simple molecules (see Figure 1) consisting of a chain of amino acids that fold into a three-dimensional shape due to amino acid interactions and disulfide bonds. Any antibody in plant debris that was produced via genetic engineering would have the same fate as antibodies in any decaying animal tissue or by-product, and would be quickly degraded and incorporated into the nitrogen cycle.

Antibody Degradation in the Digestive Tract

Without degradation into smaller peptides, proteins (such as antibodies) are too large to pass intact through the intestinal wall. They need to be broken down into amino acids or small peptides before they can be absorbed. The breakdown of protein begins in the stomach where hydrochloric acid (HCl) denatures the protein and facilitates the action of pepsin, the major gastric enzyme that splits the peptide bonds. Other proteolytic enzymes (enzymes that break

down protein bonds) involved in the gastric process are trypsin, chymotrypsin, carboxypeptidase, and elastase. Most monoclonal antibody immunizations and therapies are given intravenously and not orally due to the instability of the antibody during digestion (Zeitlin 1999).

Orally administered antibodies break into F(ab)2, Fab, and Fc fragments in the digestive system (Fig. 1B). It has been found that 19 percent of these fragments retain their neutralizing activity in the ileum of healthy adults (Roos 1995). Another study (Bogstedt 1997) analyzed the amount of neutralizing antibody activity in the fecal material of healthy adults orally administered antibodies and only found minute amounts present (<0.01percent of the ingested antibodies), suggesting the majority of the peptides are absorbed in the small intestine. When the time of passage through the gastrointestinal tract is short (as with patients who have diarrhea) antibodies can retain more activity in the lower gastrointestinal tract and be more effective in a therapeutic setting (Hammarström 1994).

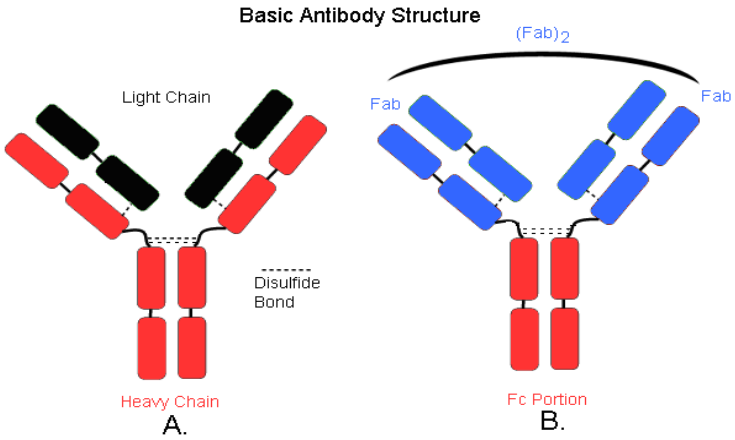

Figure 1. Basic Antibody Structure. A. Typical Y structure with two light chains (in black) and two heavy chains (in red) bound by disulfide bonds (dotted lines); **B.** Location of the Fab and Fc fragments on the antibody molecule. The two Fab antibody regions are typically indicated as $(Fab)_2$.

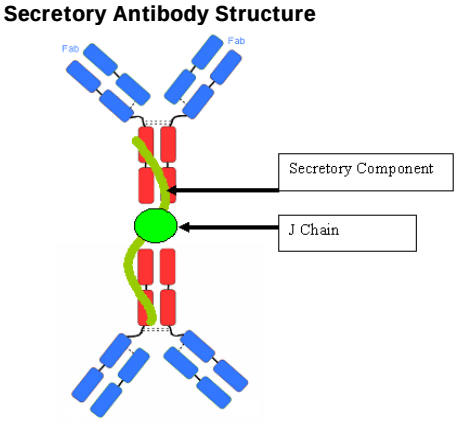

Figure 2. Secretory Antibody Structure. Complex antibody where two Y shaped antibodies are joined by a J Chain to a secretory component.

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APPENDIX B: STANDARD PERMIT CONDITIONS FROM 7 CFR 340.4

(f) *Permit conditions*. A person who is issued a permit and his/her employees or agents shall comply with the following conditions, and any supplemental conditions which shall be listed on the permit, as deemed by the Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packing material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner so as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit;

(4) The regulated article shall be maintained only in areas and premises specified in the permit;

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article;

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article;

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article;

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the Administrator to be necessary to prevent the spread of plant pests;

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, non-target organisms, or the environment;

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i)Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms);

(11) A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only at a port of entry that is designated by an asterisk in 7 CFR 319.37-14(b);

(ii) Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial

invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 340.5 of this part.

APPENDIX C: SUPPLEMENTAL PERMIT CONDITIONS

I. Compliance with Regulations

1. Any regulated article introduced not in compliance with the requirements of 7 Code of Federal Regulation Part 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. The responsible party may be subject to fines or penalties as authorized by the Plant Protection Act (7 U.S.C. §§ 7701-7772).

2. This Permit (APHIS form 2000) does not eliminate the permittee's legal responsibility to obtain all necessary Federal and State approvals, including: (A) for the use of any non-genetically engineered plant pest or pathogens as challenge inoculum; (B) plants, plant parts or seeds which are under existing Federal or State quarantine or restricted use; (C) experimental use of unregistered chemicals; and (D) food, feed, pharmacological, biologic, or industrial use of regulated articles or their products and co-mingled plant material. In the latter case, depending on the use, reviews by APHIS, the U.S. Food and Drug Administration, or the U.S. Environmental Protection Agency may be necessary.

3. The procedures, processes, and safeguards used to prevent escape, dissemination, and persistence of the regulated article as described in the permit application, in APHIS-approved Standard Operating Procedures (SOPs) and, in the supplemental permit conditions must be strictly followed. The permittee must maintain records sufficient to verify compliance with these procedures, including information regarding who performed the activity. Persons performing such activities shall have received training as described in a training program submitted to and approved by APHIS. These records are subject to examination by APHIS. APHIS, BRS must be notified of any proposed changes to the protocol referenced in the permit application.

II. Reporting Unauthorized Releases and Unintended Effects

1. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

    - For immediate oral notification, contact APHIS/BRS Compliance Staff at (301) 734-5690 and ask to speak to a Compliance and Inspection staff member.
    - In the event of an emergency and you are unable to reach the BRS Compliance Staff at the above number, you may call:

The APHIS/BRS Regional Biotechnology Coordinator assigned to the state, where the field test occurs

For Western Region, contact Ralph Stoaks by phone at (970) 494-7573 or e-mail Ralph.D.Stoaks@aphis.usda.gov

For Eastern Region, contact Ashima SenGupta by phone at (919) 855-7622 or e-mail Ashima.SenGupta@aphis.usda.gov

Or

The APHIS/PPQ Regional Biotechnology Coordinator assigned to the state where the field test occurs

For Western Region, contact Stacy Scott by phone at 970-494-7577 or e-mail Stacy.E.Scott@aphis.usda.gov

For Eastern Region, contact Susan Dublinski by phone at (919) 855-7324 or e-mail Susan.G.Dublinski@aphis.usda.gov

Or

The APHIS State Plant Health Director for the state where the field test occurs. The list of APHIS State Plant Health Director is available at http://ceris.purdue.edu/napis/names/sphdXstate.html

KY	Mike Madryga, Prospect	(502) 228-8224	(502) 228-6306	michael.b.madryga@aphis.usda.gov

2. According to 7 CFR § 340.4(f)(10)(ii), APHIS shall be notified in writing as soon as possible but within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the permit application or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

3. Written notification should be sent by one of the following means:

By e-mail:

BRSCompliance@aphis.usda.gov

By mail:

Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
USDA/APHIS
4700 River Rd. Unit 147
Riverdale, MD 20737

III. Perimeter Fallow Zone

1. To ensure that transgenic plants are not inadvertently commingled with plants to be used for food or feed, a perimeter fallow zone of at least 50 ft. must be maintained around the transgenic test site in which no crops are grown to be harvested or used for food or feed.

2. The permitted border rows of non-transgenic plants that are the same as, or sexually-compatible with, the regulated article are considered part of the field test. The perimeter fallow zone shall start outside the border rows.

3. The perimeter fallow zone shall be managed in a way that allows detection and destruction of volunteer plants that are the same as, or sexually compatible with, the transgenic plants.

IV. Required Isolation Distances

1. An isolation distance of at least ½-mile will be maintained between the regulated plots and non-regulated tobacco. This area will be monitored throughout the field test period. At least a 1-mile distance will be maintained between the field plots and any tobacco seed production. This area will be monitored throughout the field test period.

V. Dedicated Planting and Harvesting

1. To ensure that the regulated article is not inadvertently removed from the site, harvesting equipment must be dedicated for use in the permitted test site(s) or used on non-regulated research tobacco that are not used for food or feed (BRS Variance 05-001) from the time of planting through the end of harvesting.

2. After harvest, you will not be required to obtain APHIS authorization to use this equipment on APHIS -permitted sites (same sites or different sites) planted with same transgenic crop, with the target protein(s) authorized under this permit, in subsequent growing seasons under an extension of this permit or a different permit.

3. Authorization is required from APHIS before harvesting equipment used during this field test can be used on sites planted to crops not included under this permit. The permittee must notify APHIS/BRS and the State Regulatory Official at least 21 calendar days in advance of cleaning this equipment for this purpose so that APHIS may schedule an inspection to ensure that the equipment has been cleaned appropriately.

VI. Cleaning of Equipment

1. To minimize the risk of seed movement and commingling, equipment used for planting and harvesting, as well as other field equipment (e.g. tractors and tillage attachments,

such as disks, plows, harrows, and subsoilers) used at any time from the time of planting through the post-harvest monitoring period must be cleaned in accordance with procedures submitted to and approved by APHIS before they are moved off of the environmental release site.

2. Equipment used to transport seeds or harvested material must be cleaned prior to loading and after transportation to the authorized site in accordance with procedures submitted to and approved by APHIS.

3. Seed cleaning and drying must be performed in accordance with the procedures submitted to and approved by APHIS to confine the plant material and minimize the risk of seed loss, spillage, or commingling.

VII. Use of Dedicated Storage Facilities

1. Dedicated facilities (locked or secured buildings, bins, or areas, posted as restricted to authorized personnel only) must be used for storage of equipment and regulated articles for the duration of the field test.

2. Before returning these facilities to general use, they must be cleaned in accordance with procedures submitted to and approved by APHIS. **The permittee must notify** APHIS/BRS and the State Regulatory Official at least 21 calendar days in advance to allow for APHIS to schedule an inspection to ensure that the facilities have been cleaned appropriately. APHIS authorization must be received before facilities are returned to general use.

VIII. Post Harvest Monitoring

1. The field test site including the perimeter fallow zone must be monitored for the presence of volunteer **tobacco** plants for **one year** after termination of the field test. Viable plant material should not remain at the test site following termination. Volunteers, if found, will be uprooted by hand and destroyed by dismemberment and incorporation into the soil.
2. Fields must be checked for volunteers once every 2 weeks, over a period of 4 weeks, immediately post harvest. Then, before the Fall and Winter months arrive, (the first frost) the fields will be checked once every 6 weeks. During the Fall and Winter months the fields will be checked once every 8 weeks. During Spring and the following Summer the fields will be checked once every three weeks.

IX. Post Harvest Land Use Restrictions

1. Production of food and feed crops at the field test site and the perimeter fallow zone is restricted during the growing season that follows harvest or termination of the field test.

2. Permission must be obtained from APHIS/BRS prior to planting any food or feed crop at the field test site and perimeter fallow zone during the post-harvest monitoring period. Requests for such permission are not encouraged and will not be granted in cases where there is a reasonable potential for plant material derived from, or originating from, the regulated articles to become mixed with the proposed food or feed crop during harvesting.

X. Inspections

1. APHIS Biotechnology Regulatory Services (BRS) and/or an APHIS/PPQ Regional Biotechnologist, APHIS/BRS Regional Biotechnology Coordinator or APHIS State Plant Health Director may conduct inspections of the test site, facilities, and/or records at any time.

2. APHIS may invite the FDA or State Regulatory Officials to participate in these inspections.

3. Inspections will likely correspond to the beginning of the field test, mid-season or during flowering, at and/or following harvest, and during the post-harvest monitoring period.

4. Inspections will include examination of records that verify compliance with regulations and SOPs.

XI. Reports and Notices

Send notices and all reports (CBI and CBI-deleted or non-CBI copies) to BRS by e-mail, mail, or fax.

BRS E-mail:
BRSCompliance@aphis.usda.gov

BRS Mail:
Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
USDA/APHIS
4700 River Rd. Unit 147
Riverdale, MD 20737

BRS Fax:
Compliance and Inspection Branch
(301) 734-8669

In addition, fax the CBI deleted or non CBI version of the pre-planting and pre-harvest (termination) notices to the State Regulatory Official:

State Plant Regulatory Official	John Obrycki, State Entomologist
Mailing Address	Department of Entomology S-225 Ag. Science Center North University of Kentucky Lexington, KY 40546-0091
Phone	859-257-5838
Fax	859-257-3807
Email	john.obrycki@uky.edu
Web Site	http://www.KyStateEnt.org

Contact information for State Officials
http://www.nationalplantboard.org/member/index.html

1. **Pre-Planting Notice**
   At least 7 calendar days before planting, submit a Pre-Planting notice that includes the following information for each field test site:
   i. Provide APHIS with the contact information for each field test site.
   ii. Indicate if planting and harvesting equipment will be moved between authorized field test sites.
   iii. A map that clearly identifies the site location to facilitate any inspections by USDA personnel.
   iv. The planned number of acres for each gene construct.
   v. The planned planting date

2. **Planting Report**
   Within 28 calendar days after planting, submit a planting report that includes the following information for each field test site:
   i. A map of the site, with sufficient information to locate it, that includes: the state, county, address, GPS coordinates for each corner of the plot (inclusive of the border rows of any sexually compatible plants);
   ii. The location and the approximate number and/or acres of transgenic plants which were actually planted at the test site for each of the target proteins;
   iii. The total acreage of the test plot (exclude border rows, if any);
   iv. The distance from the genetically engineered plants to the nearest plants of the same crop, which will be used for food, feed, or seed production. A survey should be done within the distance specified in the permit.
   v. A list of the specific confinement option(s) selected at each site if your permit allows different confinement options (e.g. bagging flowers, border rows, or isolation distance.).
   vi. The actual planting date.

3. **Pre-Harvest/ Termination Notice**

At least 21 calendar days prior to the anticipated harvest or termination, submit a Notice indicating the planned date of harvest **or** termination and the contact information for each field test site. For multiple harvests, submit the notice prior to the initial harvest.

4. Field Test Report

Within 6 months after the end of the field test (final harvest or crop destruct), the permittee is required to submit a field test report. Field test reports shall include:

i. APHIS reference number
ii. Methods of observation.
iii. Resulting data.
iv. Analysis of all deleterious effects on plants, non-target organisms, or the environment.
v. A list of the lines planted at each site
vi. Disposition table

The disposition table should contain the following information: site name (or GPS), crop, gene, harvest date, and disposition of harvested material.

The disposition table is a formal record of how the regulated material was removed from the environment. An accounting of the harvested material should be provided with regards to what material is harvested, how much material is harvested per site, what is done to devitalize residual and harvested material at the site, where the harvested material is transported, stored and further processed up to the time it is taken to a contained facility.

5. Monitoring Report

Within 3 months after the end of the monitoring period, submit a volunteer monitoring report. The report must include:

i. Dates when the field site and perimeter fallow zone were inspected for volunteers.
ii. Number of volunteers observed.
iii. Any actions taken to remove or destroy volunteers.

XII. Flower Removal

The field will be surveyed to remove flowers prior to pollen release five days a week. It is possible that on occasion a very small amount of mature pollen will be produced. However, the applicant will apply due diligence to remove flowers prior to pollen release. A log book needs to be maintained to demonstrate this activity is being performed.

APPENDIX D: APPLICANT SUPPLIED TES WORKSHEET

12-15-05

TES WORKSHEET Page 1 CBI DELETED COPY

PLANTS EXPRESSING CARORX AND THE STATE OF KENTUCKY

RECIPIENT ORGANISM:

Transgenic tobacco.

PRODUCT:

CaroRx, a monoclonal, chimeric secretory immunoglobulin A antibody is assembled, *in planta*, from four subunits derived from mouse and rabbit gene sequences. CaroRx binds to SA I/II, a protein located on the surface of the oral, cariogenic, bacteria *Streptococcus mutans*. Using this surface protein, *S. mutans* adheres to teeth, a necessary step in the formation of cavities. The binding of CaroRx to these bacteria can prevent them from adhering to teeth and may be used to reduce, or eliminate, the infection of teeth by these cariogenic bacteria (Ma, et al., 1998; Lehner, et al., 1985; Lehner, et al., 1975; Lehner, et al., 1986; Ma, et al., 1989; Ma and Lehner, 1990; Ma, et al., 1987). CaroRx is intended for use in humans to reduce or eliminate the colonization or infection of teeth by *S. mutans*.

In the United States, CaroRx is an Investigational New Drug (BB-IND # 7526) and CaroRx is a registered Medical Device in the European Union.

LOCATION OF FIELD RELEASE:

Daviess County, KY.

LEVELS OF CARORX PRODUCED IN VARIOUS TISSUES:

Fully assembled, partially assembled and degraded forms of CaroRx are present in extracts prepared from plant tissues. [.] Functional CaroRx includes the canonically equivalent SIgA/G, dIgA/G, IgA/G and F(ab')$_2$ forms of the plantibody. CaroRx is not an enzyme and CaroRx's only function is to bind its target. CaroRx does not prevent its target organism from growing once it has bound to the target.. The free or degraded subunits are present in smaller amounts which have not been quantified in any tissues. For the forgoing reasons concerning the assembled antibody, these entities are also not expected to present any danger of toxicity to mammals, birds or to the environment as a whole.

CBI Deleted

ASSESSMENT OF CARORX PLANTS

Importantly, there were no unusual occurrences during field trials of any CaroRx-expressing plants, including no observations of any deleterious effects on the environment or on non-target organisms, as reported in the on February 25, 2003 for permit number 02-108-02r, the report submitted, in early 2005, for permit number 04-044-01r and the reports submitted, in late 2005, for permit numbers 05-053-01r and 05-087-01r.

Based on literature review and discussions with tobacco scientists, we can identify no organisms, except plant pests and possible skunks, that consume tobacco tissues (personal communication from Dr. Orlando Chambers and Mr. Richard Mundell). Earthworms are negatively impacted by nicotine in the soil.

CaroRx, or its constituent subunits, have no known toxicities.

Based on the literature, and on safety data from human clinical trials, CaroRx is not known to be toxic when applied orally and subsequently ingested (BB-IND # 7526 and Ma, et al., 1998; Weintraub et al., 2005). It is relevant to note that CaroRx is non-toxic even when applied to its target, *Streptococcus mutans* (Ma, et al., 1990 and report for permit number 04-044-01r).

Tobacco is not sexually compatible with any TES plant listed for Kentucky. Any unexpected effects from a field test of CaroRx plants would be minimized by the confinement of the plants to the test site. After harvest the plants will be destroyed in such a way as to maximize the capture the CaroRx and to minimize the possibility of the escape of any viable transgenic tissue in solid or liquid waste streams.

CONCLUSION:

A previous field trial of the CaroRx plants in Kentucky reported no unusual occurrences, and, in particular, reported no observations of any effects on the environment or on non-target organisms.

Since there is no identifiable direct effect of this field test on any plant or animal species, there will be no anticipated adverse effects on any threatened or endangered species.

REFERENCES:

Lehner T, Caldwell J, Smith R. (1985) Local passive immunization by monoclonal antibodies against streptococcal antigen I/II in the prevention of dental caries. *Infection and Immunity*. 50: 796-799.

Lehner T, Challacombe SJ and Caldwell J. (1975) Immunological and bacteriological basis for vaccination against dental caries in rhesus monkeys. *Nature*, 254:517-520.

Lehner T, Ma J, Grant K, and Smith R. (1986) Passive dental immunization by monoclonal antibodies against *Streptococcus mutans* antigen in the prevention of dental caries, IN: Lehner T and Cimasoni G (ed.)., Borderland between caries and periodontal disease, vol. III *Editions Médicine et Hygiéne*, Genéva, p. 347-355.

Ma JK-C, Hiatt A, Hein M, Vine ND, Wang F, Stabila P, van Dolleweerd C, Mostov K, and Lehner T. (1995) Generation and assembly of secretory antibodies in plants. *Science*, 268:716-719.

Ma J, Hikmat B, Wycoff K, Vine N, Chargelegue D, Yu L, Hein M, Lehner T (1998) Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat. Med. 4: 601-606.

Ma JK-C, Hunjan M, Smith R, Lehner T. (1989) Specificity of monoclonal antibodies in local passive immunization against *Streptococcus mutans*. *Clin. Exp. Immunol.*, 77:331-337.

Ma JK-C, and Lehner T. (1990) Prevention of colonization of *Streptococcus mutans* by topical application of monoclonal antibodies in human subjects. *Archs. Oral Biol.*, 35:115S-122S.

Ma JK-C, Smith R, and Lehner, T. (1987) Use of monoclonal antibodies in local passive immunization to prevent colonization of human teeth by *Streptococcus mutans*. *Infection and Immunity*, 55:1274-1278.

Weintraub, JA, Hilton, JF, White, JM, Hoover, CI, Wycoff, KL, Yu, L, Larrick, JW and Featherstone, JD (2005) Clinical trial of a plant-derived antibody on recolonization of mutans Streptococci. Caries Res 39(3):241-250.

TES Listing by State accessed at:
http://ecos.fws.gov/tess_public/TESSWebpageUsaLists?state=KY
Accessed on: December 15, 2005

• Kentucky -- 51 listings

Animals -- 43

Status	Listing
E	Bat, gray (*Myotis grisescens*)
E	Bat, Indiana (*Myotis sodalis*)
E	Bat, Virginia big-eared (*Corynorhinus (=Plecotus) townsendii virginianus*)
XN	Bean, Cumberland (pearlymussel) AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (*Villosa trabalis*)
E	Bean, Cumberland (pearlymussel) Entire Range; Except where listed as Experimental Populations (*Villosa trabalis*)
T	Bear, grizzly lower 48 States, except where listed as an experimental population or the Yellowstone population (*Ursus arctos horribilis*)
XN	Blossom, tubercled (pearlymussel) AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (*Epioblasma torulosa torulosa*)
E	Blossom, tubercled (pearlymussel) Entire Range; Except where listed as Experimental Populations (*Epioblasma torulosa torulosa*)
XN	Catspaw (=purple cat's paw pearlymussel) AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (*Epioblasma obliquata obliquata*)
E	Catspaw (=purple cat's paw pearlymussel) Entire Range; Except where listed as Experimental Populations (*Epioblasma obliquata obliquata*)
E	Clubshell Entire Range; Except where listed as Experimental Populations (*Pleurobema clava*)
XN	Combshell, Cumberlandian AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (*Epioblasma brevidens*)
E	Combshell, Cumberlandian Entire Range; Except where listed as Experimental Populations (*Epioblasma brevidens*)
XN	Crane, whooping U.S.A.(AL, AR, GA, IL, IN, IA, KY, LA, MI, MN, MS, MO, NC, OH, SC, TN, VA, WI, WV) (*Grus americana*)
T	Dace, blackside (*Phoxinus cumberlandensis*)
E	Darter, duskytail Entire (*Etheostoma percnurum*)
E	Darter, relict (*Etheostoma chienense*)
T	Eagle, bald lower 48 States (*Haliaeetus leucocephalus*)
E	Elktoe, Cumberland (*Alasmidonta atropurpurea*)
E	Fanshell (*Cyprogenia stegaria*)
T	Lynx, Canada lower 48 States DPS (*Lynx canadensis*)
E	Mapleleaf, winged Entire; except where listed as experimental populations (*Quadrula fragosa*)
E	Mucket, pink (pearlymussel) (*Lampsilis abrupta*)
XN	Mussel, oyster AL; Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, AL (*Epioblasma capsaeformis*)
E	Mussel, oyster Entire Range; Except where listed as Experimental Populations (*Epioblasma capsaeformis*)
E	Pearlymussel, cracking Entire Range; Except where listed as Experimental Populations (*Hemistena lata*)
E	Pearlymussel, dromedary Entire Range; Except where listed as Experimental Populations (*Dromus dromas*)
E	Pearlymussel, littlewing (*Pegias fabula*)
E	Pigtoe, rough (*Pleurobema plenum*)

E	Pimpleback, orangefoot (pearlymussel) (*Plethobasus cooperianus*)
T	Plover, piping except Great Lakes watershed (*Charadrius melodus*)
E	Pocketbook, fat (*Potamilus capax*)
E	Puma (=cougar), eastern (*Puma (=Felis) concolor couguar*)
E	Riffleshell, northern (*Epioblasma torulosa rangiana*)
E	Riffleshell, tan (*Epioblasma florentina walkeri (=E. walkeri)*)
E	Ring pink (mussel) (*Obovaria retusa*)
E	Shiner, palezone (*Notropis albizonatus*)
E	Shrimp, Kentucky cave (*Palaemonias ganteri*)
E	Sturgeon, pallid (*Scaphirhynchus albus*)
E	Tern, least interior pop. (*Sterna antillarum*)
T	Trout, bull U.S.A., conterminous, lower 48 states (*Salvelinus confluentus*)
E	Wartyback, white (pearlymussel) (*Plethobasus cicatricosus*)
E	Wolf, gray lower 48 States, except MN and where XN; Mexico (*Canis lupus*)

Plants -- 8

Status	Listing
E	Clover, running buffalo (*Trifolium stoloniferum*)
E	Goldenrod, Short's (*Solidago shortii*)
T	Goldenrod, white-haired (*Solidago albopilosa*)
T	Potato-bean, Price's (*Apios priceana*)
E	Rock-cress, Braun's (*Arabis perstellata*)
T	Rosemary, Cumberland (*Conradina verticillata*)
E	Sandwort, Cumberland (*Arenaria cumberlandensis*)
T	Spiraea, Virginia (*Spiraea virginiana*)

Appendix E. Attachment to Finding of No Significant Impact and Decision Notice Response to Comments
APHIS No. 05-354-01r

APHIS received 2 comments from private citizens by the close of the comment period. The two comments were opposed to genetic engineering in general. With respect to these comments, USDA believes that all methods of agricultural production (conventional, organic, or the use of genetically engineered varieties) can provide benefits to the environment, consumers, and the agricultural economy. The role of Biotechnology Regulatory Services in APHIS is to provide regulatory oversight that allows for the safe development and use of genetically engineered organisms. The regulation in 7 CFR 340.4 describes the process that APHIS uses to issue permits for the confined release of regulated genetically engineered organisms. APHIS considers scientific data provided by the applicant, published in scientific journals, and provided by interested parties during the public comment period. The determination is based on whether the regulated article will be confined to the field test site in a manner that is not likely to have significant adverse effects on the environment. APHIS has found that the information submitted by Planet Biotechnology meets the requirements of 7 CFR 340.4 and is sufficient to allow the issuance of the permit according to the procedures and processes outlined above and described in more detail in the permit and the EA.