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__/s/_
Michael Watson
Branch Chief, Plant Pest and Protectants
Biotechnology Regulatory Programs
Animal Plant and Health Inspection Service AFH_/s/_

NEPA Decision Summary for Permit #06-088-01r

Edenspace has requested a permit for a confined field release of genetically modified tobacco plants at a site in Jackson County in the state of Arkansas. Based on a review of Permit #06-088-01r, the following determinations were made:

- 1. A single gene construct is proposed for confined field release in Jackson County, Arkansas, containing the genes endo-1,4-beta-glucanase (E1) from *Acidothermus cellulolyticus* and flowering locus C (FLC) from *Arabidopsis thaliana*. The selectable marker genes are kanamycin resistance and the bar gene for resistance to glufosinate (donors claimed as CBI). The gene constructs contain non-coding regulatory regions derived from plant pests (claimed as CBI) that have been safely used to regulate the expression of transgenes in plants. None of the genes encoding the desired traits or the selectable marker, nor the regulatory elements controlling their expression, have any inherent plant pest characteristics, and they are not likely to pose a plant pest risk.
- 2. Based on disarmed *Agrobacterium tumefaciens* transformation, only the genetic constructs that are designed to be expressed in genetically engineered tobacco are expected to be efficiently inserted into the tobacco genome. No plant pest vectors are associated with the transformed tobacco lines as a result of the transformation process.
- 3. The Allergen Database for Food Safety (<u>allergen.nihs.go.jp</u>) was searched by BLAST analysis for homologues to the E1 protein sequence. The E1 protein does not share any protein sequence homology of at least six consecutive amino acids with any of the 1286 registered allergens or more than 35% identity in a segment of 80 or more amino acids. This result was confirmed when the Food Allergy Research and Resource Program (FARRP) database (<u>www.allergenonline.com</u>) containing 1537 allergens was analyzed using the same criteria.
- 4. The *E1* gene is an endoglucanase found in microbes that break down plant lignocellulosic material, so while it is conceivable that the protein product has been consumed inadvertently with the plant, the E1 protein is new to the plant. However, there are a number of proteins identified in both *Arabidopsis thaliana* and rice (*Oryza sativa*) that are high homologues to E1. Among the rice homologues are a cellulase (#CAH67677) and a glucan 1,3-β–glucosidase precursor (#NP_921098) protein. In addition, a 1,4-β-endoglucanase protein produced from *Trichoderma reesei*, which is very similar to E1, has been determined by the Joint FAO/WHO Expert Committee on Food Additives (http://www.inchem.org/documents/jecfa/jecmono/v22je06.htm) to be non-toxic.
- 5. The *FLC* gene inserted into the E1/FLC tobacco has homologues in many plant genomes so far analyzed, including rice (Genbank accession #AAQ01161), *Brassica rapa* (Genbank accession #AY273165), cabbage (#AY273161), radish

- (#AY273160) and salt cress (Genbank accession #AY957537), and may be considered to be a substance commonly found in food and feed.
- 6. All plants will be topped prior to flowering to assure that no seed is produced. Because tobacco plants will not be allowed to flower, the isolation distance will be 1320 ft (the AOSCA standard for production of foundation tobacco seed when flowers are not bagged or removed) from any flowering tobacco. Any tobacco found between the 1320 and 2640 ft distance will be topped and not used for seed production. Tobacco is not commercially grown in Arkansas. There will be no tobacco grown on the 1500 acres that make up the field site. There is no known cultivated tobacco grown within 50 miles of the field site.
- 7. After harvest, remaining vegetative material will be disked/plowed under. The gene products have no known or foreseeable toxic effects, so this method of disposal should have no negative impacts on the environment.
- 8. Because tobacco plants will not be allowed to flower, no seed will be produced, vastly reducing the potential for germinating volunteers after harvest. Additionally, tobacco plants do not overwinter, and any volunteers that germinate after harvest in October will die after the first frost. On volunteer monitoring session will occur 4 weeks after harvest. Subsequent monitoring for volunteers will resume in April. Starting the first week in April, and commencing every four weeks after that until the end of the growing season in October, the field plot will be monitored for the presence of tobacco volunteers. If any tobacco volunteers are found they will be devitalized by either an herbicide (i.e. Roundup) treatment or removed and incinerated.
- 9. During the following growing season, the field plot and fallow zone will either remain fallow or used to grow E1/FLC tobacco again under permit.
- 10. The proposed field trial is less than 10 acres. Trials of such size are easily monitored. Conditions specified in the permit application and in the standard and supplemental permit conditions provide that field trials are confined to permitted areas.
- 11. Preliminary studies have found the transgenic tobacco to have similar morphological properties as non-transgenic tobacco. Thus, preliminary data indicated that the transgenic tobacco plants pose no greater plant pest risk than non-transgenic tobacco.
- 12. Tobacco is not observed to be capable of establishment in wild environments: it is reliant on continuous human intervention for its survival. In previous field tests and applications, seed dormancy in tobacco has not been observed.
- 13. There are 31 animals and 6 plants listed as threatened or endangered in Arkansas (http://ecos.fws.gov/servlet/TESS webpage for the state of Arkansas accessed

April 7, 2006). Based on literature review, no animal species, except with the possibility of skunks and plant pests, feed on tobacco (http://www.aphis.usda.gov/brs/aphisdocs/05_05301r_ndd.pdf). Tobacco is mainly insect pollinated so insects could potentially be exposed to transgenic pollen, however the plants will be regularly topped so flowers and pollen are not expected to develop. Tobacco is not sexually compatible with any threatened and endangered species. Because there is no identifiable direct effect of a field test with E1/FLC transformed tobacco on any wild plant and animal species, there should be no adverse effects on any endangered or threatened species.

- 14. Over 250 field trials have been performed with transgenic tobacco plants under APHIS authority, and APHIS is familiar with tobacco biology and methods to manage confined tobacco field trials.
- 15. 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, APHIS has determined that (1) pursuant to 7 CFR 372, the field trials proposed under Permit number **06-088-01r** will not significantly affect the physical environment and (2) there are no applicable, extraordinary, or other reasonably foreseeable circumstances under which significant environmental effects could occur despite the protective and ameliorative measures specified above. Therefore, this field test is deemed confined within the meaning of 7 CFR 372.5.