DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 01–060–2]

Vector Tobacco; Availability of Determination of Nonregulated Status for Tobacco Genetically Engineered for Reduced Nicotine

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that the Vector Tobacco (USA) Ltd. tobacco designated as Vector 21–41, which has been genetically engineered for reduced nicotine, is no longer considered a regulated article under our regulations governing the introduction of certain genetically engineered organisms and products. Our determination is based on our evaluation of data submitted by Vector Tobacco (USA) Ltd. in its petition for a determination of nonregulated status, our analysis of other scientific data, and comments received from the public in response to a previous notice. This notice also announces the availability of our written determination document and our finding of no significant impact.

EFFECTIVE DATE: September 16, 2002.

ADDRESSES: You may read a copy of the determination, an environmental assessment and finding of no significant impact, the petition for a determination of nonregulated status submitted by Vector Tobacco (USA) Ltd., and all comments received on the petition and the environmental assessment in our reading room. The reading room is located in room 1141 of the USDA South Building, 14th Street and Independence Avenue SW., Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 690–2817 before coming.

APHIS documents published in the **Federal Register**, and related information, including the names of organizations and individuals who have commented on APHIS dockets, are available on the Internet at *http://www.aphis.usda.gov/ppd/rad/webrepor.html*.

FOR FURTHER INFORMATION CONTACT: Dr. Susan Koehler, Biotechnology Regulatory Services, Suite 5B05, 4700 River Road Unit 147, Riverdale, MD 20737–1236; (301) 734–4886. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734–4885; e-mail: *Kay.Peterson@aphis.usda.gov*.

SUPPLEMENTARY INFORMATION:

Background

On May 1, 2001, the Animal and Plant Health Inspection (APHIS) received a petition (APHIS Petition No. 01–121–01p) from Vector Tobacco (USA) Ltd. (Vector) of Durham, NC, requesting a determination of nonregulated status under 7 CFR part 340 for tobacco (*Nicotiana tabacum* L.) designated as Vector 21–41, which has been genetically engineered to produce a very low level of nicotine. The Vector petition states that the subject tobacco should not be regulated by APHIS because it does not present a plant pest risk.

On February 12, 2002, APHIS published a notice in the **Federal Register** (67 FR 6480–6481, Docket No. 01–060–1) announcing that the Vector petition was available for public review. In the notice, APHIS solicited comments from the public on whether this tobacco presents a plant pest risk. APHIS also made available for public comment an environmental assessment for the proposed determination of nonregulated status. APHIS received 45 comments on the petition and the environmental assessment during the 60-day comment period which ended April 15, 2002. The comments were received from tobacco farmers, tobacco companies, State

farmers' organizations, State department of agriculture officials, private individuals, tobacco growers' cooperatives, tobacco marketing organizations, university cooperative extension centers, members of the U.S. Congress, a foundation seed producer, an agronomic consultant, a county chamber of commerce, and a consumer organization. Twenty-three of the comments were in favor of a determination of nonregulated status for Vector 21–41 tobacco, and 22 comments either opposed deregulation or recommended no action on the petition until certain concerns are addressed about the effects of Vector 21–41 deregulation on traditional tobacco growers and markets. A majority of commenters in favor of deregulation stressed that Vector 21–41 did not present a plant pest risk, displayed disease and insect susceptibilities and agronomic characteristics similar to conventionally bred tobacco, and noted the benefits to local economies from growing Vector 21–41. Those commenters not expressing support for deregulation of the subject tobacco at this time generally expressed the following concerns about Vector 21–41: Blue mold and insect susceptibilities, the limited number of years of field testing, the potential for gene transfer to conventional tobacco, and the potential for commingling with conventional tobacco in the absence of growing and processing guidelines. One commenter found the environmental assessment inadequate, alleging that it failed to address the impacts of Vector 21–41 on organic farmers and certain human health effects, including the impacts of an antibiotic resistance gene. We have provided a response to the comments as an attachment to our finding of no significant impact, which is available from the person listed under FOR FURTHER INFORMATION CONTACT.

Analysis

Vector 21–41 tobacco has been genetically engineered to express a quinolinic acid phosphoribosyltransferase (QPTase) in the reverse, or antisense position, which disrupts the normal expression of QPTase, a key enzyme in the biosynthetic pathway leading to the production of nicotine and related alkaloids. The effect of this genetic change is to reduce the nicotine levels of nicotine, nor-nicotine, and total alkaloids in the leaves of Vector 21–41 tobacco. The subject tobacco also contains the *nptII* marker gene derived from the bacterium *Escherichia coli*. The *nptII* gene encodes the enzyme neomycin phosphotransferase type II (NPTII) and is used as a selectable marker in the initial laboratory stages of plant cell selection. Expression of the added genes is controlled in part by gene sequences from the plant pathogen *Agrobacterium tumefaciens*. The *A. tumefaciens* method was used to transfer the added genes into the parental recipient Burley 21–LA tobacco variety.

The subject tobacco has been considered a regulated article under the regulations in 7 CFR part 340 because it contains gene sequences from a plant pathogen. Vector 21–41 tobacco has been field tested since 1999 in the United States under APHIS notifications. In the process of reviewing the notifications for field trials of this tobacco, APHIS determined that the vectors and other elements were disarmed and that the trials, which were conducted under conditions of reproductive and physical containment or isolation, would not present a risk of plant pest introduction or dissemination.

Determination

Based on its analysis of the data submitted by Vector, a review of other scientific data, field tests of the subject tobacco, and comments submitted by the public, APHIS has determined that Vector 21–41 tobacco: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than tobacco developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed; (4) will not cause damage to raw or processed agricultural commodities; and (5) will not harm threatened or endangered species or organisms, such as bees, that are beneficial to agriculture. Therefore, APHIS has concluded that the subject tobacco and any progeny derived from hybrid crosses with other nontransformed tobacco varieties will be as safe to grow as tobacco in traditional breeding programs that is not subject to regulation under 7 CFR part 340.

The effect of this determination is that Vector's 21–41 tobacco is no longer considered a regulated article under APHIS" regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the subject tobacco or its progeny. However, importation of Vector 21–41 tobacco or seeds capable of propagation are still subject to the restrictions found in APHIS" foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment has been prepared to examine the potential environmental impacts associated with this determination. The environmental assessment was prepared in accordance with (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that environmental assessment, APHIS has reached a finding of no significant impact with regard to its determination that Vector 21–41 tobacco and lines developed from it are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the petition and the environmental assessment and finding of no significant impact are available upon request from the individual listed under the FOR FURTHER INFORMATION CONTACT section of this notice.

Done in Washington, DC, this 26th day of November 2002 .

Peter Fernandez,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 02–30518 Filed 12–2–02; 8:45 am]

BILLING CODE 3410–34–P

Approval of Vector Tobacco (USA) Ltd. Petition (01-121-01p) Seeking a Determination of Non-regulated Status for Reduced-nicotine Tobacco Line Vector 21-41

Environmental Assessment and Finding of No Significant Impact

September 2002

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA) has prepared an environmental assessment (EA) prior to approving a petition (APHIS number 01-121-01p) for a determination of nonregulated status received from Vector Tobacco (USA) Ltd. under APHIS regulations at 7 CFR Part 340. The subject of this petition, tobacco line Vector 21-41, is genetically engineered for reduced nicotine content by the insertion of an anti-sense version of the coding region of a tobacco gene encoding the enzyme quinolinic acid phosphoribosyltransferase, a key enzyme in the biosynthetic pathway for the synthesis of nicotine and related alkaloids. It is also genetically engineered to express a selectable marker, the enzyme neomycin phosphotransferase which confers antibiotic resistance. On February 12, 2002, APHIS published a notice in the Federal Register (67 FR 6480-6481, Docket no. 01-060-1) announcing the availability of the petition and EA for public review and comment. During the designated 60 day comment period, APHIS received 45 comments. APHIS' analysis of and response to these comments is included as an attachment to this finding. Based on the analysis in the EA and in our response to the comments, APHIS has reached a finding of no significant impact (FONSI) to the environment from its determination that tobacco line Vector 21-41, and progeny derived from it, shall no longer be considered regulated articles. This determination is attached to the EA as Appendix D.

Cindy Smith
Acting Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: SEP 16 2002

APHIS' Analysis and Response to Comments Received on Petition 01-121-01p and the EA.

APHIS received 45 comments on the petition and the EA during the 60-day comment period. The comments were received from tobacco farmers, tobacco companies, State farmers' organizations, State department of agriculture officials, private individuals, tobacco growers cooperatives, tobacco marketing organizations, university cooperative extension centers, members of the U.S. Congress, a foundation seed producer, an agronomic consultant, a county chamber of commerce, and a consumer organization. The comments in favor of a determination of nonregulated status for Vector 21-41 tobacco totaled 23, and 22 comments either opposed deregulation or recommended no action on the petition until certain concerns are addressed about the effects of Vector 21-41 deregulation on traditional tobacco growers and markets. A majority of commenters in favor of deregulation stressed that Vector 21-41 did not present a plant pest risk, that it displayed disease and insect susceptibilities and agronomic characteristics similarly to conventionally bred tobacco under typical management conditions, and that there were benefits to local economies from growing Vector 21-41. Those commenters not expressing support for deregulation of the subject tobacco at this time generally expressed concerns about its susceptibility to blue mold and insects, the limited number of years of field testing, the potential for gene transfer to and commingling with conventional tobacco and the subsequent effect on export markets. One commenter found the EA inadequate, stating that it failed to address the impacts of Vector 21-41 on human health, that it inadequately addressed the impact on organic farmers, and that an Environmental Impact Statement should have been prepared. Further characterization of and response to those comments in opposition are given below.

1. Susceptibility to blue mold and insects

Several commenters expressed concern over the susceptibility of Vector 21-41 to the blue mold fungal pathogen. Some indicated that this pathogen is highly adaptable and the potential for increased incidence of this pathogen in Vector 21-41 raises concerns regarding selection pressure on populations of this pathogen in the presence of genetic modifications in the plant. However, no rationale was provided for how the genetic modification in Vector 21-41 would enhance selection pressure for the blue-mold pathogen compared to many other tobacco varieties currently grown which are also susceptible to blue mold. It was further argued that it is irrelevant that the EA states that Vector 21-41 does not have increased susceptibility to diseases or insects when compared to the parental line Burley 21-LA since the parental line is known to exhibit increased susceptibility to blue mold and insect pests.

Relevance of the comparison of insect and disease susceptibility to Burley 21-LA

In response to these comments APHIS notes that the comparison of the reported disease and insect susceptibility of Vector 21-41 to the parental line Burley 21-LA, and Burley 21 (parent of Burley 21-LA) in Section V of the EA is relevant and appropriate to the determination of plant pest risk. The regulations at 7 CFR 340.6 (c) indicate that the required data and information to be submitted to support a determination of non-regulated status includes a description of known or potential differences from the unmodified recipient organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from

which it was derived, including disease and pest susceptibilities and agricultural or cultivation practices. The petitioner must also certify that the petition includes relevant data and information which are unfavorable to the petition. Because Burley 21-LA was not released as a commercial variety, APHIS included its parent, Burley 21 in the analysis. Burley 21 has not been grown for about 30 years, but is still used as a parent in breeding programs. APHIS also considered the availability and effectiveness of various cultural or chemical control methods to manage disease and insect pests to which Vector 21-41 tobacco is known or likely to be susceptible.

Characterization of the disease and insect susceptibility of Burley 21-LA and Burley 21

APHIS acknowledged in the EA that both Burley 21-LA and Burley 21 have low levels of disease resistance compared to newer Burley varieties and that Burley 21-LA is described as extremely susceptible to insect damage. In particular Burley 21-LA has been observed to be susceptible to blue mold as well as to two other fungal diseases: black shank (caused by *Phytophthora parasitica* var. *nicotinanae*), and black root rot (caused by *Thielaviopsis basicola).* It is slightly more susceptible to blue mold than Burley 21. Burley 21 has no resistance to black shank, low resistance to black root rot, and high resistance to TMV and wild fire disease (Gary Palmer, Extension Tobacco Specialist, University of Kentucky (personal communication to Susan Koehler, November and December 11, 2001). Most Burley tobacco varieties today have resistance to black root rot and a few have some degree of resistance to black shank and blue mold (Gary Palmer, personal communication to Susan Koehler, and see also http://www.uky.edu/Ag/TobaccoProd/pubs/2001GuidetoBurleyVarieties.pdf). Burley 21-LA is also described as "extremely susceptible to insect damage" (Legg, et al., 1970). In particular, it is highly susceptible to grasshoppers and other leaf-feeding insects (Glenn Collins, University of Kentucky, personal communication to Susan Koehler, November, 2001). As indicated in the EA, this is not unexpected, since nicotine and some other alkaloids are known to have insecticidal activity.

Observed insect and disease susceptibility of Vector 21-41, and the ability of current agricultural practices to effectively manage pests in Vector 21-41

APHIS' analysis documented the fact that in field trials in North Carolina in 2000, both Vector 21-41 and Burley 21-LA were shown to be extremely susceptible to insect pests in the absence of systemic insecticides, but no differences in disease or insect susceptibility or leaf yield were observed between Vector 21-41 or Burley 21-LA plants that received two applications of 1 lb/acre Orthene. This insecticide treatment is one of the options recommended for control of several of the major foliar-feeding insect pests of tobacco (North Carolina State University, 2000). Topping was also conducted 60 days after transplant, prior to flowering, and suckers (leaf buds which develop in the leaf axils after topping occurs) were chemically controlled by treatment with maleic hydrazide. Early topping and good sucker control are recommended for control of budworms and hornworms, since moths of these pests are strongly attracted to flowers to lay their eggs. This practice is also reported to speed the decline of aphids, especially under hot, dry conditions (NC State Univ., 2000). Other biological and cultural control options are described.

Field tests of Vector 21-41 in Pennsylvania in 2001 indicate that it is susceptible to blue mold, a serious disease of tobacco caused by the fungus *Peronospora tabacina.* The North American Blue Mold Warning and Forecast System, a cooperative network of state coordinators, plant pathologists, and meteorologists, alerts growers about the threat of blue mold through their forecasting and tracking activities so that preventative measures can be implemented. In the petitioner's response of October 19, 2001 to APHIS' deficiency letter dated September 26, 2001, they noted that this System reported that blue mold occurred on other tobacco varieties in most of the tobacco production regions of North America during the 2001 growing season (see http://www.ces.ncsu.edu/depts/pp/bluemold/fcst2001/exec2001.htm.). Furthermore, in over 600 farm field trials of Vector 21-41 in Pennsylvania, Illinois, Louisiana, Mississippi, and Iowa, which were extensively monitored for disease and insect pests by farm owners, workers, Vector Tobacco agronomists, and contracted consultants, no other exceptional disease or pest susceptibility was observed. They claimed that the specific rates of insecticide, fungicide, and herbicide application for any one field depended on traditional agronomic factors, the geographic location, climate, and time of transplanting, but were always within approved label amounts. APHIS considered the fact that disease control in tobacco involves an integrated program of numerous practices, including crop rotation, root and stalk destruction, nitrogen management, soil pH management, plant spacing, varieties, and pesticides (see The University of Georgia College of Agricultural & Environmental Sciences Cooperative Extension Service web site on *Tobacco Disease Control* by Paul Bertrand at http://www.griffin.peachnet.edu/caes/tobacco/handbook/disease98.html). At least four of these practices are applicable to control or management of black root rot, black shank and blue mold. Chemicals recommended along with other practices by the University of Kentucky or in the 2002 North Carolina Agricultural Chemicals Manual for the control of these diseases include at least one or more of the following: the fumigant, Chloropicrin, carbamate, methyl bromide, Ridomil Gold EC or WSP, Ultra Flourish 2EC or 2E, Dithane Rainshield, Actigard, or Acrobat MZ (see *Current Blue Mold Controls* by http://www.uky.edu/Agriculture/kpn/kyblue/kyblu01/controls.htm and *Tobacco Disease Control Planning Should Be a Priority* at http://www.uky.edu/Agriculture/kpn/kyblue/kyblu01/related/rtd0001.htm, both by W. Nesmith, Extension Professor, University of Kentucky, and _http://ipm.ncsu.edu/agchem/chptr6/605.pdf).

APHIS' analysis in the EA indicated that currently recommended and approved methods for controlling diseases and insects to which Vector 21-41 is susceptible should be effective and applicable for their control in Vector 21-41. These conclusions are supported by comments provided by growers of Vector 21-41 tobacco, and the tobacco company and crop consultant working with them to coordinate activities for planting, growing, and managing the crop.

2. Insufficient field data

Some of the same commenters also asserted there was insufficient field data to predict the long term impact under various and possible extreme weather conditions and wider range of individual farm production practices. APHIS notes that this concern appeared to be primarily aimed at the impacts from Vector 21-41 tobacco on traditional tobacco varieties as a result of out-crossing via pollen or commingling of seeds, plants, or leaf products and the impact on export markets. That concern is addressed below. APHIS notes that the field data or observations described above (and subsequently supported by field data reports received from the petitioner on June 5 and June 20, 2002 for field trials conducted in 2001 listed in Appendix A of the EA) regarding the disease and insect susceptibility of Vector 21-41 were made over 6 states, some representative of traditional tobacco growing areas, and others not. In addition, the petitioner noted in their letter of October 19, 2001 that no abnormal plant morphology or phenotypes due to the transgenes were observed. APHIS' analysis in the EA of data on cured-leaf nicotine levels from Vector 21-41 tobacco grown in four states indicated that it is consistently low as expected. Field data reports received June 5 and 20, 2002, which include analysis of additional samples and samples from two more states, indicate that the absolute average and median values for nicotine content from plants grown in 5 of the 6 states are still consistently lower than those reported for Burley 21 LA and normal wild-type tobacco varieties. Nicotine values from Vector 21-41 plants grown in a small (approximately 34 acre) experimental site in Hawaii, while higher than those from plants grown in the other states, were still below the 0.8% threshold required for the low-nicotine classification of Type 31-V or Type 73 tobacco defined by AMS, and were influenced by a combination of soil fertility, climatic conditions and cultural practices which are known to increase nicotine content. We believe that even though the field trials were conducted in only two years, the number and variety of locations should have presented a sufficiently broad range of weather conditions and individual production practices and pressures to predict with a reasonable degree of certainty that there will be no significant impacts to the environment.

3. Human health effects of tobacco products made with Vector 21-41

One commenter expressed concern that the commercialization of Vector 21-41 tobacco products would encourage smoking by giving a false sense of security. Along the same line, another commenter claimed that APHIS' EA was inadequate because it did not address human health effects (either direct; or later, indirect, reasonably-foreseeable effects) associated with the commercialization of a new tobacco product as required under NEPA and its implementing procedures. More specifically the commenter asserts that "the EA does not project human exposures rates of the tobacco, whether the products made with this tobacco will lead to increased smoking in the public, and many other health and disease impacts associated with smoking tobacco". The commenter also states that the EA does not address the human health impacts that may be caused when the public is exposed to the antibiotic resistance gene product through direct inhalation of smoke from this tobacco product.

In response to these comments APHIS notes that **addressing human health effects associated with the commerical use of the tobacco product is outside the scope of the EA.** The scope of the EA, as indicated in the Summary and under Alternative B, is to compare the environmental impacts of the introduction (importation, interstate movement and release) of Vector 21-41 tobacco and its progeny, without the safeguards afforded by 7 CFR Part 340, to impacts posed by the cultivation and distribution of tobacco not subject to these regulations. APHIS did consider the impacts of exposure to agricultural workers handling the tobacco plants.

Tobacco products, cigarettes, snuff etc., are made from tobacco leaves, which following detachment from the plant and curing, are no longer living. These products are therefore not subject to APHIS regulations at 7 CFR 340 because they do not meet the definitions provided therein of a regulated article or a plant pest. Impacts on human health from exposure to or use of these products, will therefore, not be determined by alternative actions identified in the EA. APHIS recognizes that use of some traditional tobacco products may have adverse consequences to human health, and did consider the potential human health effects from potential products made with Vector 21-41 tobacco, but this analysis was not included in the EA because it is believed to be outside the scope of impacts affected by the Agency's range of alternative actions. Vector 21-41 tobacco is a burley type of tobacco. Traditional burley tobacco is primarily used in cigarettes, but is also an important component of cigar, pipe, and chewing tobacco (Shew and Lucas, 1991). Tobacco is purchased and blended based on certain chemical criteria (including sugar and nicotine content) as well as appearance and smoke flavor. In addition, it is common practice to add sugars and other flavors to balance the smoke flavor, primarily by modifying the sensory impact of nicotine and other tobacco alkaloids (Leffingwell, 1999). It is difficult to predict what the composition of the final product might be, since as noted in the EA, data indicate that cured leaves of Vector 21-41 have significantly lower levels of nicotine and related alkaloids and higher levels of reducing sugars compared to a normal burley tobacco (Burley 21). The company is still working out the product formulation and currently, all the Vector 21-41 tobacco leaves harvested are still in the curing and aging process, which can typically last anywhere from 12 to 60 months (Scott Shore, Vector Tobacco (USA), Ltd., personal communication to Susan Koehler, May 3, 2002).

APHIS considered the information submitted by the petitioner regarding the content of nicotine and other alkaloids, total nitrogen, and reducing sugars in Vector 21-41 cured tobacco leaf, and TSNAs in smoke from cigarettes made from Vector 21-41 tobacco. We could find no reason why these differences would pose a greater human health threat than traditional burley tobacco if used in tobacco products, since the addictive component, nicotine, and carcinogenic TSNAs were reduced. The petition states that "Tobacco products made from Vector 21-41 deliver nicotine below the addictive levels suggested by Benowicz and Henningfield (1994)." Benowicz and Henningfield suggest that an absolute limit of 0.4 to 0.5 mg of nicotine per cigarette should be adequate to prevent or limit the development of addiction in most young people. This threshold

recognizes that nicotine uptake from smoke varies depending on how the cigarette is smoked and assumes a maximal bioavailability of 40%. As noted in the EA, the median value for nicotine content from samples taken from Vector 21-41 cured (oven-dried) leaves available at the time the EA was prepared was 0.05% (500 ppm or 0.5 mg/1000 mg), and assuming that 1000 mg of tobacco is used in the average cigarette, then it would be possible for cigarettes made from Vector 21-41 tobacco to meet this threshold. Additional nicotine data included in final field data reports submitted on June 5 and 20, 2002 do not change the conclusion that it would still be possible to meet this threshold. The effects of reduced nicotine in cigarette products on smoking behavior are debatable. But the stated intent of the petitioner is to develop a product which would help smokers reduce or control their smoking, and they indicate that trials are underway to confirm or quantify this. In as much as the product formulation is not finalized, any definitive conclusions regarding the human health effects of products made from Vector 21-41 tobacco would be speculative.

APHIS does not consider inhalation of the product of the antibiotic resistance gene in tobacco smoke to be an issue. The product of this gene is a protein, and proteins are broken down during curing, aging, and burning of the tobacco (Leffingwell, 1999). Breakdown products and derivatives of this protein would be similar to those derived from other proteins normally found in tobacco leaf.

Furthermore, APHIS identified within the EA the regulatory authority of other federal agencies or commissions relevant to the sale, use, packaging, labeling, and advertising of Vector 21-41 tobacco and its products. In particular, the FDA can assert jurisdiction over products made from Vector 21-41 tobacco if health claims are made regarding the product. The Federal Trade Commission oversees testing of "tar" and nicotine yields of cigarettes, which must be included in cigarette advertising and they would have the authority to address any unfair or deceptive cigarette advertising practices that might affect human health. APHIS' actions in no way prejudice or influence the regulatory authority or actions of these agencies with respect to Vector 21-41 tobacco products.

4) Impacts on organic farming were inadequately addressed in the EA.
One commenter stated that APHIS' EA contained an inadequate analysis of the potential impacts associated with the commercial use of genetically engineered tobacco on organic farming. They assert that we made an unsupported statement that nontransgenic tobacco seed would still be readily available for planting. APHIS checked several tobacco seed supplier websites, including domestic, international, private and public, and those marketing to organic growers (e.g. http://www.attra.org/attra-pub/tobacco.html), and there were plenty of traditional, non-genetically engineered tobacco varieties available, and there was no mention of these varieties not being available if genetically engineered tobacco seed was deregulated. There appears to be

an increasing interest among organic growers for indigenous and heirloom-variety types of tobacco and several possible sources of seed were listed.

The commenter further asserts that APHIS failed to analyze whether the marketplace and market-based standards will actually tolerate "adventitious presence" of genetically-engineered tobacco in organically produced tobacco products and the impact it will have on producers, processors and consumers of such products. APHIS has addressed the potential impacts of the Vector 21-41 tobacco on organic farmers in the EA. We made reference to the National Organic Program (NOP) administered by USDA's Agricultural Marketing Service, which considers that the presence of a detectable residue alone does not necessarily indicate use of a product of excluded methods that would constitute a violation of the standards. (Please refer to the preamble of the NOP final rule at residue testing, changes requested but not made, (3) Threshold for Genetic Contamination for a discussion of "adventitious presence" in relation to organic production at website: http://www.ams.usda.gov/nop/nop2000/Final%20Rule/preamble/pre-residues.htm.) Further, the NOP requires that organic production operations have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. The organic system plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. APHIS' analysis indicates that adventious presence of Vector 21-41 tobacco in organically-produced tobacco leaf of another type could only occur if the farmer planted seed that was commingled with or cross-pollinated by Vector 21-41 tobacco. Several reasons were provided in the EA for why this is extremely unlikely to occur.

Producers, processors, or consumers wishing to check for adventious presence of Vector 21-41 tobacco in their products could enlist the services of commerical laboratories (e.g. Genetic ID and Integrated Biomolecule Corporation) that offer testing for the detection of foreign genetic elements in commodities, including the nopaline synthase terminator used in both of the foreign gene constructs in Vector 21-41 tobacco. USDA's Agricultural Marketing Service and Grain Inspection, Packers and Stockyards Administration published an Advance Notice of Proposed Rulemaking on November 30, 2000 in the *Federal Register* (65 FR 71272- 71273) concerning the possible further development of additional testing and standardization for seeds and commodities designed to differentiate products such as non-biotechnology-derived commodities. Federal, State, private, and international groups involved in seed certification all allow for some level of accidental, incidental, or adventitious presence of off-types even in the purest seed categories, such as foundation and breeder seed.

5) APHIS should prepare an Environmental Impact Statement (EIS).

One commenter suggested that APHIS must prepare a complete environmental impact statement (EIS) to address the full range of impacts associated with the commercial planting of Vector 21-41 tobacco.

In response, APHIS notes that their NEPA Implementing Procedures (7 CFR 372) do not indicate that this type of action is of the class of actions normally requiring the preparing of an EIS. It is characteristic of the class of actions normally requiring an EA, but not necessarily an EIS. APHIS' analysis documented in the EA (and in the response to comments) does not indicate that a significant impact to the human environment is likely; therefore the preparation of an EIS is not necessary. This is the first petition for deregulation for a genetically engineered tobacco product that APHIS has received. APHIS does not believe a decision to deregulate Vector 21-41 tobacco, when combined with previous decisions to deregulate other genetically engineered crops with totally different traits, uses, and markets, will influence or cause a significant cumulative impact to the human environment which would warrant the preparation of an EIS.

6) Gene transfer to and commingling with conventional tobacco and market impacts.
Many commenters expressed concern that out-crossing or commingling of Vector 21-41 tobacco with conventional varieties could lead to a loss of export sales estimated at $250 million because purchasers of U.S. tobacco, particularly those that focus on the large export market, have imposed a "GMO" (genetically-modified organism) tolerance level of zero. They request that the Vector 21-41 tobacco continue to be regulated under 7 CFR 340 to prevent cross-pollination and commingling until the Vector 21-41 tobacco is produced in a male-sterile form and/or a system is in place to identify the locations of its production and provide production guidelines or mandated programs that will prevent outcrossing or commingling. A minor concern was raised that if contamination occurs, it could affect the nicotine levels of conventional tobacco and raise concerns about the quality of the tobacco related to this trait.

In response, APHIS believes that such impacts on marketing, which are neither directly or indirectly related to disease, damage or injury to plant products, are not germane to APHIS' decision as to whether Vector 21-41 poses a plant pest risk. Nonetheless, APHIS did address the potential for outcrossing and/or commingling in the EA under the sections on potential impacts from gene introgression and potential impacts on organic farming (section V. C. and E., respectively).

Furthermore, as noted in Section II. C. of the EA, production and marketing of tobacco is subject to regulation by other agencies within the USDA. In particular, APHIS noted that the USDA, Agricultural Marketing Service (AMS) has established a classification and certification system for certain low nicotine types of tobacco (less than 0.8% nicotine oven dry weight in the cured leaf). This includes Type 31-V air-cured Burley tobacco with low nicotine and Type 73 flue-cured tobacco with low nicotine, as defined under 7 CFR 30.38 and 7 CFR 30.42, respectively. Restrictions and controls relating to the production and marketing of Type 31-V tobacco as a prerequisite for certification are described under 7 CFR 30.38 (c). In summary, these restrictions require that the tobacco must be grown under contract filed yearly with the AMS on a specified number of acres, and that it must be separated from other types of tobacco during cultivation,

housing, and handling. It can not be offered for sale, or be sold, marketed or disposed of unless it is clearly represented and identified as being low nicotine tobacco. In addition, the grower can not transfer seed, seedlings or plants furnished under the contract, nor can the grower harvest or save seed produced under the contract. AMS samples tobacco from each grower and assays it to determine whether it meets the low nicotine criteria to meet the classification. Similar restrictions are in place under 7 CFR 30.42 for Type 73 tobacco. Vector 21-41 tobacco is the first tobacco to have ever received this certification.

According to Ken Wall, Senior Marketing Specialist, AMS Tobacco Programs, (personal communication to Susan Koehler, November 14, 2001) Vector 21-41 received certification under this program as both Type 31-V and Type 73 last year. Bill Hawks, Under Secretary for USDA's Marketing and Regulatory Programs indicated in a letter dated April 26, 2002 to Mr. Bill Dickerson, Director of Plant Industry Division, North Carolina Department of Agriculture and Consumer Services, that destination audits were conducted for the 2001-2002 marketing year to confirm that there was no commingling of this tobacco with other types of tobacco, and that more than 7 million pounds of Vector 21-41 tobacco have been certified under this program to date. The petitioner has indicated that contracts have already been filed with AMS to certify as low-nicotine types all of the Vector 21-41 tobacco being grown during the 2002 growing season for marketing, and they intend to continue growing under the certification program in future years. All the Vector 21-41 tobacco previously grown, or scheduled to be grown in 2002, in the United States with the intent of marketing has been in non-quota areas or states, including Mississippi, Pennsylvania, Iowa, Illinois, and Louisiana, (Scott Shore, personal communication, May 21, 2002). (Hawaii, another non-quota state, is also being evaluated by the petitioner as a viable production site.) In the vicinity of where Vector 21-41 has been grown in LA, MS, IL, and IA, there are no other types of tobacco being grown. The petitioner purposefully chose these locations to avoid any concerns regarding commingling, and the grower contracts stipulate that the tobacco must be topped and suckers controlled (Scott Shore, personal communication to Susan Koehler, May 21, 2002).

Type 31-V and Type 73 tobacco, when classified and certified as such, are currently excluded from the quota system. They can be grown in non-quota areas and not be subject to penalties when marketed. If Vector 21-41 tobacco did <u>not</u> receive the Type 31-V or Type 73 certification, then it would just be considered Type 31 Burley (if air-cured) or Types 11-14 (if flue cured), which are subject to quotas. In that case a substantial penalty can be levied when it is marketed if it is grown in a non-quota area, and it could only be marketed without penalty in a quota area if an allotment is issued for the type. On May 15, 2002, the Farm Service Agency published a notice and request for public comment in the *Federal Register* (67 FR 34673) about whether to include Type 31-V or Type 73 in the definitions of tobaccos subject to quotas for the 2003 and subsequent crop years. The implications of a potential change in quota status of Type 31-V or

Type 73 on the potential for contamination of traditional types of tobacco via cross-pollination is discussed briefly in the notice.

Literature Cited (Includes only those references not already included in the EA.)

Leffingwell, J.C. 1999. Leaf Chemistry. Chapter 8 *in* Tobacco Production, Chemistry, and Technology, D.L. David and M.T. Nielsen eds. Blackwell Science Ltd., Oxford, England.

North Carolina State University. 2000. 2000 North Carolina Burley Tobacco Production Guide. Available at http://ipmwww.ncsu.edu/Production_Guides/Burley/chptr8.html) .

USDA/APHIS Decision on Vector Tobacco (USA) Ltd. Petition 01-121-01P Seeking a Determination of Nonregulated Status for Reduced-Nicotine Tobacco Line, Vector 21-41

Environmental Assessment

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APPENDICES

I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) prior to making its determination on the regulated status of tobacco (*Nicotiana tabacum*) line Vector 21-41 that has been genetically engineered (transformed) to have reduced nicotine. APHIS received a petition (designated 01-121-01P) from Vector Tobacco (USA) Ltd. (Durham, North Carolina) for a determination that Vector 21-41 reduced-nicotine tobacco does not present a plant pest risk, and therefore should no longer be treated as a regulated article under APHIS regulations found at 7 CFR Part 340. The petition contains information in support of such a determination.

The Vector 21-41 reduced-nicotine tobacco has been developed with the intent of producing cigarettes or other tobacco products that will help smokers to reduce or quit their smoking. Nicotine is the major alkaloid in tobacco leaves and is widely believed to be the addictive component of tobacco. Nicotine levels in the leaves were reduced by using recombinant DNA techniques to insert an anti-sense version of the coding region (cDNA) of a tobacco gene encoding the enzyme quinolinic acid phosphoribosyltransferase (QPTase) into the genome of a Burley tobacco variety. The anti-sense gene construct disrupts the normal expression of QPTase. QPTase is one of the key enzymes in the biosynthetic pathway leading to the production of nicotine and related alkaloids. Because these alkaloids are also the precursors for the production of tobacco-specific nitroasmines (TSNAs), which are potent mutagens and potential carcinogens, Vector 21-41 tobacco also has reduced levels of TSNAs. Vector 21-41 tobacco has also been transformed to express the *nptII* selectable marker gene derived from the enteric bacterium *Escherichia coli* transposon Tn5. The *nptII* gene encodes the enzyme neomycin phosphotransferase that confers resistance to the aminoglycoside antibiotic kanamycin. This gene was inserted only as a tool to allow selection of tobacco tissue transformed to contain the new gene constructs. Vector 21-41 tobacco is a regulated article under APHIS regulations at 7 CFR Part 340 because the plant pest *Agrobacterium tumefaciens* was used as a donor organism for some DNA sequences used to regulate the expression of the new gene constructs and also as a vector to introduce the new gene constructs into the tobacco genome.

As a regulated article, the importation, interstate movement, or cultivation in the United States of Vector 21-41 tobacco has been conducted under authorizations from APHIS that require conditions of physical and reproductive confinement to preclude the regulated article from becoming mixed with nonregulated plants or products or persisting in the environment outside the field site. This EA has been prepared prior to issuing a determination of nonregulated status for Vector 21-41 tobacco in order to specifically addresses the potential for impact to the human environment that might be incurred through the cultivation and use in agriculture of this tobacco without the restrictions imposed by 7 CFR Part 340.

II. BACKGROUND

A. Development of Vector 21-41 tobacco.

The Vector 21-41 reduced-nicotine tobacco was developed at the North Carolina State University, Department of Genetics under a research grant to Dr. Mark Conkling from a private company called Alternative Cigarettes. Vector Group, the parent company of Vector Tobacco (USA) Ltd.[1] and the Liggett Group cigarette company, has sublicensed the technology with the intent of producing cigarettes or other tobacco products that will help smokers to reduce or quit their smoking. Nicotine, is the major alkaloid found in *N. tabacum* (Tso, 1972, see Chapter 23, Alkaloids), and is widely believed to be the addictive component of tobacco (Benowitz and Henningfield 1994). It is synthesized in the root and transported to the leaves (Tso, 1972). Figure 1 of the petition shows the biosynthetic pathway for nicotine in tobacco. Nicotine is synthesized from two metabolic precursors, nicotinic acid and N-methylpyrroline, each of which is synthesized by two separate pathways. Quinolinic acid phosphoribosyl-transferase (QPTase) is the key rate-limiting, regulatory enzyme in the biosynthetic pathway that leads to nicotinic acid. It converts quinolinic acid to nicotinic acid mononucleotide, which is then converted directly or indirectly into nicotinic acid. To develop a reduced nicotine tobacco, the petitioner states that they started with a non-commerical tobacco variety developed through traditional breeding to have reduced levels of alkaloids, Burley 21 LA, and genetically engineered it by inserting the coding region (cDNA) of the tobacco gene *NtQPT1,* which encodes the QPTase enzyme, in the anti-sense orientation behind its own root-specific promoter. The anti-sense gene construct was designed to disrupt the normal expression of QPTase in the root, thereby lowering the pool of nicotinic acid available for the production of nicotine, nor-nicotine, and other alkaloids derived from the pyridine ring of nicotinic acid. Nicotine, nornicotine, and other minor tobacco alkaloids give rise to the production of tobacco-specific N-nitroasmines (TSNAs) during tobacco processing and during smoking (Bush et al., 1993). Some TSNAs been demonstrated to be potent mutagens and potential carcinogens, and are thought to contribute to the increased risk of cancer of the upper digestive tract in tobacco chewers and for the increased risk of lung cancer in smokers (Hoffman et al., 1994). The petition includes data that demonstrates that Vector 21-41 tobacco has reduced levels of nicotine, nor-nicotine, and total alkaloids in the leaves, and reduced levels of specific TSNAs in smoke from cigarettes derived from it.

Vector 21-41 tobacco has also been transformed to constitutively express the *nptII* selectable marker gene derived from the *Eschericia coli* bacterial transposon Tn5. This gene encodes the enzyme neomycin phosphotransferase (NPTII) that confers resistance to the antibiotic kanamycin and allowed tissue that was transformed to express the new genes to be selected on medium that contains kanamycin. Regulatory DNA sequences derived from the nopaline synthase (*nos*) gene from the plant pathogenic bacterium, *Agrobacterium tumefaciens*, were fused to both of these gene constructs to facilitate their transcription in the transgenic plant. *A. tumefaciens* was also

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[1]In January 2001, Dr. Conkling left the University to work for Vector Tobacco (USA) Ltd., and on November 14, 2001, Vector Tobacco (USA) Ltd. was changed to Vector Tobacco, Inc.

used as a vector to introduce the anti-sense *NtQPT1* and *nptII* gene constructs into the genome of the recipient tobacco variety to create the transgenic line Vector 21-41. Because this plant pathogen was used as a vector and donor organism in the production of the genetically engineered Vector 21-41 tobacco, this tobacco is considered to be a regulated article under APHIS regulations at 7 CFR Part 340, and authorization for its importation, interstate movement and field testing in the United States and its territories is required.

Vector 21-41 tobacco has been field tested in a wide variety of locations, in at least 7 States under notifications from APHIS issued since 1999 that are listed in Appendix A. It has also been field tested in Argentina. This field testing was conducted, in part, to confirm that Vector 21-41 tobacco exhibits the desired agronomic and quality characteristics and does not pose a greater plant pest risk than the unmodified recipient from which it was derived. Although these field tests were conducted in agricultural settings, APHIS performance standards for notifications (7 CFR 340.3 (c) 1-6) stipulate that the regulated article not be planted with nonregulated plant material that is not part of the release, that it be contained or devitalized when no longer in use, and that the regulated article and its offspring must not persist in the environment after completion of the test. Therefore, measures were employed to ensure physical and reproductive confinement from other sexually compatible plants and to manage volunteers. Reports for those field tests completed under APHIS authorization that were due at the time of the petition submission, and other information contained in the petition, have been submitted to APHIS to support a determination that Vector 21-41 tobacco does not pose a plant pest risk (see Appendix C for a list of supporting data and information).

B. APHIS Regulatory Authority.

APHIS regulations under 7 CFR Part 340, which are promulgated pursuant to authority granted by the Plant Protection Act (Title IV, Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. 7701-7772) regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. Vector 21-41 tobacco has been considered a regulated article because a plant pathogen was used as a vector agent and as a donor organism for some noncoding DNA regulatory sequences.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it is derived, the Agency can grant the petition in whole or in part. Therefore, APHIS permits or notifications would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

C. Regulatory Authority of Other Federal Agencies Relevant to the Production and Use of Vector 21-41 Reduced-Nicotine Tobacco and Its Products.

Production and Marketing of Tobacco: Production and marketing of tobacco, including Vector 21-41 reduced-nicotine tobacco, is subject to regulation by other agencies within the USDA. The Agricultural Stabilization and Conservation Service and the Commodity Credit Corporation set production quotas and price levels for tobacco leaf under the federal tobacco support program ("No Net Cost Tobacco Program"). Under the quota system, limits are placed on the amount of different types of tobacco that can be grown. Under 7 CFR Part 30, the USDA's Agricultural Marketing Service (AMS) classifies tobacco based on distinctive characteristics determined by the variety, growth conditions, and methods of cultivation, harvesting, and curing. Two types of low nicotine tobacco (less than 0.8% nicotine oven dry weight in the cured leaf) are excluded from the quota system, when classified and certified as such by the AMS under their regulations at 7 CFR 30.38 and 7 CFR 30.42. Special restrictions and controls relating to the production and marketing of these types of tobacco are described under these regulations. Vector 21-41 is the first tobacco variety to have received this certification (Kenneth Wall, Senior Marketing Specialist, AMS Tobacco Programs and Mark Conkling, personal communication to Susan Koehler, 11/14/01).

Product Sale and Use: Currently, tobacco is not generally considered a food, feed, drug, or dietary supplement by the U.S. Food and Drug Administration. (FDA). But the FDA has asserted jurisdiction over cigarettes as a "drug" when health claims were made by vendors or manufacturers, and the courts have sustained the agency's assertions of jurisdiction. Should health claims be made on cigarettes made with Vector 21-41 tobacco, the FDA would have jurisdiction over them as a drug. In 1996 the FDA asserted jurisdiction over tobacco products under the Food, Drug, and Cosmetic Act (FFDCA) to regulate tobacco advertising and promotional campaigns as well as labeling and purchasing restrictions. But the legal authority of the FDA to regulate tobacco products in the absence of health claims was challenged by the tobacco industry and in June 2000, the United States Supreme Court ruled that Congress had not expressly given the FDA legal authority to regulate the tobacco industry.

Packaging, Labeling and Advertising of Tobacco Products: The Bureau of Alcohol, Tobacco and Firearms regulations cover packaging and require the disclosure of certain information on every tobacco product carton or package and prohibit certain promotional practices. The Federal Cigarette Labeling and Advertising Act bans cigarette advertising on television, radio, and other electronic media, and requires cigarette packages and advertising to carry specified health warnings, such as the Surgeon General's warning. The Department of Justice, the Federal Communications Commission and the Federal Trade Commission (FTC) ensure compliance with this Act. The FTC oversees testing of "tar" and nicotine yields of cigarettes, which must be included in cigarette advertising, and they have the authority to address unfair or deceptive cigarette advertising practices under the Federal Trade Commission Act.

III. PURPOSE AND NEED

In compliance with the National Environmental Policy Act (NEPA) of 1969 (42 U.S.C. 4321 *et seq.*) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372), APHIS has prepared this EA before making a determination on the status of Vector 21-41 tobacco as a regulated article under APHIS regulations. Vector Tobacco (USA) Ltd., (Durham, North Carolina) submitted a petition requesting a determination from APHIS that Vector 21-41 reduced-nicotine tobacco no longer be considered a regulated article under 7 CFR Part 340.

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the "no action" alternative, APHIS would come to a determination that Vector 21-41 tobacco and its progeny should continue to be regulated under 7 CFR Part 340. Permits or acknowledgment of notifications from APHIS would still be required for their introduction. APHIS would choose this alternative if there were insufficient evidence to demonstrate lack of plant pest risk from the cultivation or movement of Vector 21-41 and its progeny without the containment restrictions that would otherwise be imposed under 7 CFR Part 340.

B. Proposed Action: Determination of Nonregulated Status

Under this alternative, Vector 21-41 tobacco and its progeny would no longer be considered regulated articles under 7 CFR Part 340. Permits or notifications to APHIS would no longer be required for introductions in the United States and its territories of Vector 21-41 tobacco or its progeny. A basis for this determination would be established, which would result in a "Finding of No Significant Impact (FONSI) under NEPA. Unrestricted cultivation and movement of this tobacco would be permitted by APHIS. Such a determination, however, does not preclude any restriction which might be placed on the cultivation, distribution, or sale of this tobacco by other regulatory agencies also having authority over this tobacco, e.g. those restriction imposed by the USDA, AMS as described in section II, C above. Importation of Vector 21-41 tobacco seeds and other propagative material would still continue to be regulated and inspected for pests and noxious weeds under APHIS regulations at 7 CFR 319.37 and under the Federal Seed Act at 7 CFR 201.

C. Determination of Nonregulated Status, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (i) state that APHIS may "approve the petition in whole or in part." There are two ways in which a petition might be approved in part:

Approval of only some of the lines requested in the petition. Some petitions request de-regulation of lines derived from more that one independent transformation event. In those cases, supporting data must be supplied for each line; and APHIS could approve certain lines requested in the petition, but not others.

Approval of the petition with geographic restrictions. APHIS might determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In this case, APHIS may choose to approve the petition with a geographic limitation stipulating that the approved lines could only be grown in certain geographic areas.

V. POTENTIAL ENVIRONMENTAL IMPACTS

The potential environmental impact of each of the alternatives cited in IV. A.- C. will be presented.

Alternative A.

In a decision to choose alternative A., no action, these plants would still require APHIS authorization to be planted, imported, or moved interstate. Such introductions could continue under APHIS authorized notifications. In this case the applicant must certify that they will meet the performance standards that are designed to ensure physical and reproductive confinement of Vector 21-41 tobacco and any progeny derived from it. Thus far, no adverse effects to the environment or human health have been reported as a result of field tests of Vector 21-41 tobacco grown under APHIS authorization.

Growers could choose to grow other tobacco varieties if they choose not to grow Vector 21-41 under contract with the developer under APHIS authorization. However, there are currently no other tobacco varieties that have been commercialized that meet the AMS definition of "low nicotine" tobacco (Kenneth Wall, AMS and Dr. Gary Palmer, Extension Tobacco Specialist, University of Kentucky, personal communication to Susan Koehler, November 2001).

Alternative B.

A decision to choose alternative B, deregulation of Vector 21-41 reduced-nicotine tobacco, is addressed below. The environmental impacts of the introduction of Vector 21-41 tobacco and its progeny into the United States and it territories without the safeguards afforded by 7 CFR Part 340 are compared to any impacts posed by the cultivation and distribution of tobacco not subject to these regulations.

A. Plant pathogenic properties

APHIS considered the potential for the transformation vector or process, the introduced DNA sequences, or their expression products to cause or aggravate disease symptoms in Vector 21-41 tobacco or in other plants, or to cause the production of plant pathogens. We also considered whether data indicate that unanticipated plant pest effects would arise from cultivation of Vector 21-41 tobacco.

The petition states that a disarmed binary vector system based on the tumor-inducing Ti-plasmid of *Agrobacterium tumefaciens* was used to introduce the new genes into Burley 21 LA tobacco (see Petition, pg. 16). The system is considered "disarmed" because (1) the plant hormone genes necessary for the formation of crown gall tumors, which are normally produced upon infection of plants with *A. tumefaciens*, are removed from the T-DNA, that portion of the Ti-plasmid that is normally transferred to plant cells upon *A. tumefaciens* infection; and (2) the virulence functions are provided on a separate Ti-plasmid that lacks the T-DNA. Therefore, the transformation process should not lead to crown gall disease in Vector 21-41 tobacco.

APHIS analyzed molecular genetic and inheritance data provided in the petition that characterize the new genetic material in the transgenic line selected from a single transformation event (T_0 #41) and designated as Vector 21-41(see Appendix C). Data demonstrate that Vector 21-41 tobacco contains within a single locus: (1) two complete copies of the T-DNA insert from the disarmed Ti-plasmid pYTY32 in reverse orientation with respect to each other, each of which contains (a) the *nptII* gene encoding neomycin phosphotransferase, originally derived from *E. coli* Tn5, whose transcription is initiated and terminated by the promoter and termination/polyadenylation sequences, respectively, derived from the nopaline synthase (*nos*) gene from *A. tumefaciens*, and (b) the anti-sense quinolinic acid phosphoribosyl-transferase (QPTase) *Nt-QPT1* cDNA derived from tobacco whose transcription is initiated by its own promoter and is terminated by the *nos* terminator as described above; (2) a small portion of the backbone sequence located outside of the left T-DNA border in pYTY32 that includes truncated versions of the *Pseudomonas aeruginosa* derived *tetA* gene and the *E. coli* plasmid pRK2 derived *trfA* gene; and (3) the T-DNA left and right border sequences from *A. tumefaciens* bordering each T-DNA insert.

Data provided in the petition indicate that both the *nptII* and anti-sense *Nt-QPTI* genetic constructs are expressed in Vector 21-41 and produce the expected traits. Full length copies of the bacterial *tetA* and *trfa* genes would encode proteins that confer resistance to the antibiotic tetracycline and plasmid DNA transfer and conjugations functions respectively, as expressed in their donor organisms. However since only half of these genes are present in Vector 21-41, and they lack regulatory sequences necessary to initiate expression in plants, they would not be expressed in Vector 21-41 tobacco. Molecular genetic data were provided that indicate that no other complete protein coding sequences present outside of the T-DNA border in pYTY32 were inserted into Vector 21-41.

Although some of the donor organisms of the introduced sequences are described as plant or human pathogens, the introduced sequences are not known to cause or aggravate diseases in plants, nor in animals or humans. *A. tumefaciens* is a plant pathogen, but the non-coding regions of the *nos* gene derived from it are commonly used to regulate transgene expression in plants. Neither these sequences nor the T-DNA borders will cause or aggravate disease symptoms in plants. *E. coli* is not a plant pathogen. It is a coliform bacterium found commonly in the guts of humans and animals, and is not generally considered to be a human, animal, or plant pathogen (American Biological Safety Association, 1998; CAB Intl., 2001) . The *nptII* gene has a safe history of use as a selectable marker for plant transformations. *P. aeruginosa* is not a plant pathogen, but is listed as a human pathogen (ABSA, 1998) and as a natural enemy of certain insects (CAB, Intl., 2001). Regardless, of its status as a pathogen, the truncated *tetA* gene derived from it will not be expressed in Vector 21-41 tobacco.

Molecular genetic and inheritance data provided in the petition indicate that the new *nptII* and anti-sense *NtQPT1* genetic constructs are expressed and inherited as dominant, tightly linked traits, consistent with insertion of both transgenes at a single locus. Early generations of transformant #T_0-41 from which Vector 21-41 was selected all exhibit kanamycin resistance, and Vector 21-41 plants were shown to express NPTII protein in the leaves, roots, and stems. These plants also had nicotine levels in their leaves that were consistently lower than those determined for progeny of the original transformant #T_0-41 that lost the transgenes through segregation and for those reported for "wild-type" Burley 21-LA and "promoter control" plants that were grown in North Carolina in 2000. ("Promoter control" plants were reported to be Burley 21-LA transformed to carry only the *NtQPT1* promoter fused to a marker gene.) In the North Carolina experiment, Vector 21-41 leaves also had significantly lower levels of nor-nicotine and total alkaloids compared to both the "wild-type" and "promoter control" Burley 21-LA plants. APHIS notes, however, that the nicotine and total alkaloid levels of leaves derived from both the "promoter control" and the "wild-type" Burley 21-LA plants in the 2000 North Carolina field experiment and the nicotine content derived from the leaves taken from null segregants in the greenhouse are all higher than those reported previously for Burley 21-LA (Legg et al., 1970; and Paul Legg and Lowell Bush, personal communication to Susan Koehler, December 6, 2001). The source of this discrepancy remains unresolved, but could possibly be due to cultural or environmental effects which are known to effect nicotine content (Mark Conkling, letter to Susan Koehler, January 11, 2002.). The nicotine levels measured by AMS from at least 830 leaf samples from Vector 21-41 tobacco grown during the 2001 growing season in over 100 locations from four states (Louisiana, Mississippi, Illinois, and Pennsylvania) did vary slightly between states, with both the average and median values ranging from approximately 0.04 to 0.10 % oven dry weight (400 - 1000 ppm). The average and median values for all samples combined were 0.079% and 0.05%, respectively, and the maximum value reported was 0.40%. The average nicotine content reported for Burley 21-LA and Burley 21 (the normal alkaloid progenitor of Burley 21-LA) is 0.2 to 0.25% and 3.5%, respectively (Legg et al., 1969 and 1970). Therefore the data indicate that the average nicotine content for cured Vector 21-41 tobacco leaves grown over a variety of locations is approximately 2.5- and 44- fold lower, respectively, than the

average previously reported for Burley 21-LA and Burley 21, and only 0.02% of Vector 21-41 samples exceeded the average reported for Burley 21-LA.

Data also indicate that specific TSNAs, which are derived from nicotine and other alkaloids, were 15- to 39-fold lower in smoke from cigarettes made from Vector 21-41 tobacco when compared to standard, full-flavor generic cigarettes. In particular, the levels of N-nitrosonornicotine (NNN) and N-nitrosonicotine keytone (NNK) from Vector 21-41 cigarette smoke are approximately 15- and 18-fold lower, respectively, than those derived from the standard cigarette, and are approximately 12- and 2.7-fold lower, respectively, than those reported from cured Burley 21-LA tobacco leaf (Lowell Bush, Professor of Agronomy, University of Kentucky, personal communication to Susan Koehler, December 6, 2001). Both of these TSNAs are known carcinogens (Hoffman et al., 1994). This data taken together indicate that the anti-sense *NtQPT1* gene construct in Vector 21-41 is functioning as expected to lower the pool of nicotinic acid available in the root for the synthesis of nicotine, nor-nicotine and other alkaloids derived from the pyridine ring of nicotinic acid. However, in addition to reduced levels of nicotine and other alkaloids, Vector 21-41 was also found to have levels of reducing sugars in the leaves that were about twice those of the "wild-type" and "promoter control" Burley 21-LA plants, but which are well within the range of reducing sugars observed among different tobacco varieties. APHIS knows of no information which would lead one to believe that this would cause a plant pest risk.

APHIS analysis of agronomic data and observations provided on field tests conducted in 2000 in Clayton, North Carolina, and in 2001 in Pennsylvania, Illinois, Louisiana, Mississippi, and Iowa using a typical insect and disease management program indicate that Vector 21-41 does not have increased susceptibility to diseases or insects when compared to the parental line, or to that reported previously for Burley 21-LA (Legg et al., 1970) or Burley 21 (Gary Palmer, Extension Tobacco Specialist, and Glenn Collins, University of Kentucky, personal communication to Susan Koehler, November and December, 2001). Both Burley 21 and Burley 21-LA have low levels of disease resistance compared to newer Burley varieties and Burley 21-LA is described as extremely susceptible to insect damage. The petitioner notes that a field experiment was conducted in Clayton, North Carolina in 2000 to compare Vector 21-41 tobacco with the "wild-type" and "promoter control" plants grown in the absence of systemic insecticides, and all plants were destroyed by insects, primarily tobacco horn worms and tobacco bud worms. No difference in insect predation was observed between Vector 21-41 and the controls.

B. Potential impacts based on the relative weediness of Vector 21-41 tobacco compared to currently cultivated tobacco varieties.

APHIS evaluated whether Vector 21-41 tobacco is any more likely to become a weed than the parent tobacco line, or other tobacco currently cultivated. The assessment considers the weediness status and any weediness characteristics of tobacco and the unique characteristics of

Vector 21-41 tobacco. APHIS also evaluated whether Vector 21-41 tobacco is any more likely to transmit weedy characteristics to other cultivated tobacco.

Tobacco (*N. tabacum*) has been grown for commercial purposes in at least 97 countries around the world (Shew and Lucas, 1991). In a search of the major weed references (Holm et al., 1977 and 1979; Muenscher, 1980; Reed, 1977, Weed Science Society of America, 1989), it is listed as a common weed in Hawaii by Holm et al.(1979), but it is not listed as a weed anywhere else in the world, nor is it present on the lists of noxious weed species distributed by the Federal Government (7 CFR Part 360) or on the list of weeds regulated by the states (see http://www.aphis.usda.gov/npb/statenw.html). Its status as a weed in Hawaii is questionable, as the only *Nicotiana* species listed in a recent reference of Hawaiian weeds is *N. glauca* Graham, commonly referred to as tree tobacco (Haselwood and Motter, 1983). APHIS' analysis of the characteristics of tobacco reveals that it possesses few of the characteristics described by Baker (1965) that are thought to contribute to the weediness potential of plants that are notably successful weeds. The only ones identified are self-compatibility and very high seed output in favorable conditions (up to 3,000 seeds per flower) (Shew and Lucas, 1991). One characteristic of weeds not mentioned by Baker, but which is in part responsible for the classification of some Solanaceous plants as weeds, including *N. glauca* and *N. suaveolens*, is the production of alkaloids, including nicotine, which can cause adverse health effects in humans and livestock (Holm et al., 1979; James et al., 1992). Cultivated tobacco is unlikely to become a weed under most agricultural situations because the common practice of topping prevents seed formation. Even if seed were produced, the seeds are very small (0.5 mm in diameter) and usually require germination in a carefully prepared sterile seedbed or in a greenhouse to avoid devastating seedling pathogens, most important of which is blue mold (Shew and Lucas, 1991). Tobacco volunteers are not common, but if found, can be easily controlled by herbicides or mechanical means.

Vector 21-41 tobacco exhibits no characteristics that would cause it to be more weedy than the non-transgenic parent tobacco line or other cultivated tobacco, and therefore is unlikely to transmit weedy characteristics to other tobacco. In particular, as noted above, Vector 21-41 is significantly lower in nicotine and other alkaloids than Burley 21-LA and Burley 21; it is susceptible to blue mold, and is equally susceptible to insects as Burley 21-LA. In addition, Burley tobacco requires higher rates of nitrogen fertilizer than other types of tobacco (Shew and Lucas, 1991). APHIS evaluated data collected from the 2000 field trial in Clayton, North Carolina that show that no significant differences were observed between Vector 21-41 and the non-transgenic parent tobacco line in other agronomic characteristics which might influence the plant's ability to compete as a weed. These characteristics include days from transplant to flowering, height at flowering, leaf number at flowering, leaf size (width and length) and yield. In addition, data were provided that demonstrate that the average and range in the number of seeds produced per pod from greenhouse grown plants of Vector 21-41 tobacco is similar to that

of Burley 21-LA and Tn90, a currently cultivated Burley type of tobacco, and is slightly less than that for two other flue-cured tobacco varieties, Ti121 and Ti57.

C. Potential impacts from gene introgression from Vector 21-41 tobacco into its sexually-compatible relatives.

APHIS evaluated the potential for genes from Vector 21-41 tobacco to introgress into sexually compatible wild relatives and considered whether such introgression would result in increased weediness or other adverse environmental effects. Only six *Nicotiana* species are described as native to the United States, and none of these are listed as threatened or endangered by the U.S. Fish and Wildlife Service. The potential for genes from *N. tabacum* to naturally introgress into these species is extremely low for several reasons (see Appendix B for details). First, all but one of these do not occur in proximity to areas of cultivation or seed production of tobacco in the United States. Second, the species are either not cross compatible with *N. tabacum* or their progeny exhibit sterility or genetic instability. Vector 21-41 is not reported to have flowering characteristics different from those observed for other *N. tabacum,* and there is no reason to believe that it would have a greater ability to produce fertile hybrids with these species.

Several other reasons exist for why gene introgression from Vector 21-41 is extremely unlikely to occur in these indigenous species, or other species of *Nicotiana* that either occur as naturalized weeds in the United States or that are used for ornamental, medicinal or commercial purposes, e.g. *N. alata, N. glauca, N. forgetiana, N. langsdorffii, N. longiflora, N. rustica, N. sauveolens, N. sylvestris, N. sanderae,* and *N. tomentosa* (Shew and Lucas, 1991; see taxonomic information for *Nicotiana* species in the GRIN database at http://www.ars-grin.gov/cgi-bin/npgs/html). (1) As stated in the petition (pg. 4), Vector Tobacco intends to produce all seed in greenhouses that will house only Vector 21-41 plants; and the vents of these greenhouses will be screened and managed in such a way as to prevent the entry or exit of pollen-carrying insects. (2) Restrictions placed on the cultivation of low-nicotine varieties by AMS regulations preclude the harvesting or transfer of seed produced from these plants under contract. (3) Topping and sucker control methods, which are common practice for production of commercial tobacco and Vector 21-41, will reduce or eliminate the availability of Vector 21-41 pollen for cross-hybridization. (4) Even if some flowers were produced, tobacco is primarily self-pollinating (95%) and fertilization generally occurs before the flower opens. It is not wind pollinated and any outcrossing relies on insect pollinators. McMurtrey et al., (1960) reported that outcrossing rates of adjacent plantings of untopped tobacco (including a Burley variety) measured over 3 years in Maryland ranged from 2-11 percent, and outcrossing rates declined with distance, and were usually less than 0.3% at 1/4 mile (1,320 feet, the isolation distance for certified seed of tobacco of different varieties). Since Vector 21-41 tobacco does not exhibit characteristics that might be expected to cause it to be any more competitive or weedy than other cultivated tobacco, or to adversely effect nontarget organisms (see Section D), any impact from gene introgression from Vector 21-41 into wild relatives is not expected to be greatly different from that of other varieties of cultivated tobacco.

D. Potential impact on nontarget organisms, including beneficial organisms and threatened or endangered species.

APHIS evaluated the potential for Vector 21-41 tobacco and its products to have damaging or toxic effects on nontarget organisms representative of the exposed agricultural environment, including those that are recognized as beneficial to agriculture or as threatened or endangered in the United States.

The expression of NPTII in tobacco plants is not expected to have deleterious effects or significant impacts on nontarget organisms, including beneficial organisms and threatened and endangered species, based on information provided in the petition (pg. 18) and APHIS analyses of previously deregulated transgenic plants that express NPTII. The protein is not toxic and it shares no homology with proteins known to be toxic or allergenic (U.S. FDA 1998).

The reduced levels of nicotine and total alkaloids in Vector 21-41 are expected to make this tobacco less toxic to insects or other animals that might feed on tobacco. In general, tobacco is not used as a feed plant because of its vertebrate toxicity. Teratogenic problems resulting from tobacco alkaloids have occurred in the United States when pregnant swine were allowed to forage on tobacco stalks (Crowe, 1969). Nicotine is a naturally occurring insecticide and feeding deterrent in *Nicotiana* species, in particular *N. tabacum* and *N. rustica* (Bush and Crowe, 1989). However as noted earlier, many pests of tobacco have adapted to the alkaloids in tobacco or have ways to avoid it (Tso, 1972, Chapter 23, Alkaloids). APHIS' evaluation of the many insects that feed on tobacco indicate that none of these insects feed exclusively on tobacco or other *Nicotiana* species, and therefore they are unlikely to have a specific requirement for nicotine or its metabolites. Reducing sugars, which were reported to be about two fold higher in Vector 21-41 leaves compared to non-transgenic parent line, are not included among the types of defense compounds produced by plants that have toxic or anti-feeding properties.

APHIS can not identify any threatened or endangered species that specifically feed or live on tobacco, and no *Nicotiana* species are listed as threatened or endangered. Vector 21-41 tobacco does not exhibit traits that would expand or change the range in habitats in which tobacco is normally grown, so its cultivation is not expected to displace any plant species. For these reasons, no effect on species recognized or proposed as threatened or endangered in the United States by the U.S. Fish and Wildlife Service is expected.

E. Potential impacts on agricultural and cultivation practices

APHIS considered potential impacts associated with the cultivation of Vector 21-41 tobacco on current agricultural practices, including potential impacts on organic farming. Potential impacts on minorities, low income populations, and children were also considered.

Potential impacts of Vector 21-41 on pest control and other agricultural practices

As noted in Section V.A. above, Vector 21-41 does not have increased susceptibility to diseases or insects when compared to the parent line or to that reported previously for Burley 21-LA or Burley 21 when using a typical disease and insect management program. Various cultural and chemical control methods have been described by agricultural extension services for the management of disease and insect pests of tobacco to which Vector 21-41 is known or likely to be susceptible. There is no reason to believe that these methods would not also be implemented for Vector 21-41 tobacco. The petitioner notes that "The experience from the over 600 farm field trials conducted in five states this year [2001] indicate that existing approved amounts of pesticides labeled for use on tobacco can control insect, disease, and weed problems.". Pesticide use on U.S. tobacco has been restricted and monitored for many years by the USDA under The Food Security Act of 1985. Growers must certify to the Farm Service Agency that any pesticides used in tobacco production have been approved by the EPA for use on tobacco and were applied in accordance with labeled directions. Failure to comply with requirements can result in loss of price support, fines, or imprisonment (USDA, ERS, 2001).

Potential impacts on organic farming

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic tobacco leaf, will be significantly impacted if a determination for non-regulated status were to be granted for Vector 21-41 tobacco for the following reasons. (1) Nontransgenic tobacco will still be sold and will be readily available to those who wish to plant it. (2) USDA's National Organic Program requires that organic farmers plant certified (nonengineered) seed (http://www.ams.usda.gov/nop/nop2000/Final%20Rule/nopfinal.pdf), and if a grower can verify that this was the case, then any adventitious presence of transgenic material in organically grown products will not keep that product from being certified as organic. (3) According to AMS regulations, the reduced-nicotine Vector 21-41 tobacco would need to be grown under contract with the company, therefore growers will know it is transgenic. (4) Vector Tobacco intends to take several steps to ensure identity preservation of its seed stock of Vector 21-41 tobacco (see Petition, pg. 4.). (5) Cross pollination of other tobacco in the field by Vector 21-41 tobacco is highly unlikely (for the reasons described in Section V. Alternative B. Paragraph C. above). (6) Even if cross-pollination of non-transgenic plants were to occur, only an extremely small percentage of transgenic seed would likely be produced, but the leaves harvested from the cross-pollinated plants to be marketed would not be transgenic.

Potential impacts on humans, including minorities, low income populations, and children

Under Executive Order 13045, we attempted to identify and assess environmental health or safety risks that might disproportionately affect children. We also considered any possible adverse impacts on minorities and low income populations as specified under Executive Order 12898. As noted above, APHIS does not anticipate that there will be a significant change in pest control or cultural practices if Vector 21-41 were deregulated, and pesticide use in tobacco is

closely monitored by the USDA. Because Vector 21-41 tobacco has significantly reduced levels of nicotine compared to traditional tobacco, APHIS does expect there to be some benefit to farm workers or their children who might come into contact with tobacco in the field, particularly during topping of the tobacco plants. A green tobacco syndrome is caused by adsorption of nicotine from skin contact in workers who handle wet tobacco plants. Symptoms, including headache, dizziness, and vomiting, have been reported in up to 50% of tobacco handlers. Fortunately they usually resolve within a few hours after tobacco plant contact ceases and contaminated areas of the body are washed (see *Risks From Chemical Use on the Farm* by Frederick W. Oehme DVM, PhD, Comparative Toxicology Laboratories, Kansas State University, at http://www.vet.ksu.edu/links/agromed/oehmemorning.htm.).

F. Potential impacts on raw or processed agricultural commodities.

APHIS analysis of information and data provided in the petition regarding: (1) the disease and insect susceptibility of Vector 21-41 tobacco; (2) the analysis of air-cured leaves for percent nicotine, nor-nicotine, total alkaloids, total nitrogen and reducing sugars; and (3) the analysis of smoke from cigarettes derived from Vector 21-41 tobacco leaves for TSNAs, reveal no differences between either the reported non-transgenic recipient Burley 21-LA or other tobacco that could have a direct or indirect plant pest effect on any raw or processed plant commodity.

G. Potential environmental impacts outside the United States associated with a determination of nonregulated status as requested by Vector Tobacco (USA) Ltd.

APHIS has also considered potential environmental impacts outside the United States and its territories associated with a determination of nonregulated status for Vector 21-41 tobacco. Any international trade in tobacco subject to this determination would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that are signatory to the Convention (116 countries as of June, 2001). In addition, issues that may relate to commercialization and transboundary movement of particular agricultural biotechnology products are being addressed in IPPC and other international fora. APHIS also continues to play a role in working toward harmonization of biosafety and biotechnology guidelines and regulations included within our regional plant protection organization, the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection. APHIS also participates regularly in biotechnology policy discussions at fora sponsored by the European Union and the Organization for Economic Cooperation and Development. APHIS periodically holds bilateral or quadrilateral discussion on biotechnology regulatory issues with other countries, most often with Canada, Mexico, Japan, and Argentina. We also have participated in numerous conferences intended to enhance international cooperation on safety in biotechnology and sponsored several workshops on safeguards for

planned introductions of transgenic crops (crucifers, maize, wheat, potatoes, rice, tomatoes) most of which have included consideration of international biosafety issues. Of particular relevance to crops with their centers of origin in South and Central America, we sponsored a Latin-American Biosafety workshop on field testing and commercialization of transgenic plants. Mexico and many of the countries in South America where most of the wild relatives of tobacco exist (e.g. Brazil, Argentina, and Uruguay) have procedures in place that require a full evaluation of transgenic plants before they can be introduced into their environment.

Alternative C, Approval of the Petition in Part.

Approval of only some of the lines requested in the petition. The petition received from Vector Tobacco (USA) Ltd. requested a determination of nonregulated status for only a single line, Vector 21-41. Data demonstrate that this line was derived from a single transformation event, transformant #41. Therefore, APHIS can consider only that one line for approval.

Approval of the petition with geographic restrictions. APHIS could identify no impacts to the human environment that are specific to a particular geographical region of the United States or its territories. Even if Vector 21-41 tobacco were grown in regions of the United States where it's wild relatives occur, there are several factors described in Section V, Alternative B, Paragraph C, that make gene introgression unlikely, and any impact from gene introgression into wild relatives is expected to be no worse than that from other cultivated tobacco. APHIS could not identify any risks to non-target organisms or to threatened and endangered species. Therefore, APHIS finds no reason to place geographic restrictions on the cultivation or movement of Vector 21-41 tobacco.

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Appendix A

USDA-APHIS approved field tests of Vector 21-41 tobacco.

APHIS Notification Number	States	Effective Date[1]	Acreage Requested
99-180–05N[2]	NC	7/29/99	5.0
01-024-26N	PA	2/23/01	1838.0
01-061-01N	PA	4/01/01	1061.5
01-081-03N	TX	NOT PLANTED	
01-094-07N	MS	5/04/01	100.0
01-102-01N	MS	5/12/01	210.0
01-102-03N	LA	5/12/01	367.0
01-102-04N	IL	5/12/01	1030.0
01-102-05N	PA	5/12/01	424.0
01-114-02N	LA	5/24/01	81.0
01-116-01N	MS	5/26/01	21.0
01-117-03N	IA	5/27/01	6.0
01-122-01N	PA	6/01/01	5.0
01-143-07N	HI	6/22/01	40.0
01-158-01N	IL	7/07/01	20.0

[1]Plantings must take place within one year of the effective date.

[2]Field data report submitted, and included in the petition. Field data reports are due within 6 months of termination of the field test.

Appendix B. Potential for introgression from *Nicotiana tabacum* to its sexually compatible relatives.

N. tabacum has a somatic chromosome number of 2N = 48 (i.e., 24 pairs of chromosomes) and originated in South America as an amphidiploid species resulting from a hybridization of two progenitor species believed to be *N. sylvestris* (2N=24) and *N. tomentosiformis* (2N=24) (Smith, 1979). The USDA, Agricultural Research Service, Germplasm Resources Information Network (GRIN) database recognizes 68 different taxa at the species level within the genus *Nicotiana,* which is subdivided into three subgenera and 14 sections (Smith 1979). Of these species, 45 species are native to North and/or South America, 17 are native to Australia, one is native to Polynesia, Tonga, and S. Melanesia, and one is native to Africa. Others are listed as cultivars. Only six *Nicotiana* species are listed as native to the United States. Their distribution within the United States (as referenced in the GRIN database and references therein), their somatic chromosome number (Smith, 1979), and their reported ability to produce viable, fertile offspring with *N. tabacum* (as described in the petition, pg. 2; and in Chaplan and Burk, 1979; and confirmed in discussion with Dr. David Zaitlin, tobacco breeder, University of Kentucky, Tobacco and Health Research Instititue, personal communication to Susan Koehler, 12/9/01) are summarized in the table below. The potential for genes from *N. tabacum* to naturally introgress into its sexually compatible relatives in the United States is extremely low for several reasons. First, with the exception of *N. plumbaginofolia*, none of these occur in proximity to major areas of cultivation or seed production of tobacco. In the United States, tobacco is typically grown in the midwest and eastern states. Statistics show that for the years 1997-1999, that the largest acreage occurred in the following states in descending order: North Carolina, Kentucky, Tennessee, South Carolina, Virginia, Georgia, Ohio, Indiana, Pennsylvania, Maryland, Florida, Missouri, Connecticut, Wisconsin, West Virginia, and Massachusettes (USDA, NASS, 2000). Most farmers do not produce their own tobacco seed, but use certified seed produced under conditions of reproduction isolation as specified under 7 CFR 201.76, most of which is grown in the same areas where commerical production of tobacco occurs. Second, the species are either not reported to be cross compatible with *N. tabacum* or their progeny exhibit sterility or genetic instability which would further reduce the potential for gene introgression back into the wild population.

Naturally occurring intergeneric crosses with tobacco as one parent (e.g., tobacco x tomato) have not been reported. Intergeneric hybrid plants using tobacco as one parent have been produced under laboratory conditions by fusion of protoplasts (cells lacking cell walls) and subsequent regeneration of whole plants. Most of these intergeneric hybrids are either sterile or produce nonviable seeds (Steward and Krikorian 1979, and Glen Collins, personal communication to Susan Koehler, Nov. 2001).

Nicotiana species native to the United States	Somatic chromosome number	Distribution within the U.S.	Cross-compatible with *N. tabacum*	Fertility of hybrids
N. attenuata Torr. ex S. Watson	2N=24	Western U.S., Mexico to Canada	No	
N. obtusifolia M. Martens & Galeotti (Syn = *N. palmeri* A. Gray and *N. trigonophylla* Dunal)	2N=24	Texas, New Mexico, Arizona, California, Nevada, Utah	Only in laboratory experiments	
N. repanda Willd. ex Lehm.	2N=48	Texas (gulf coast)	Yes	Male sterile
N. quadrivalvis Pursh (Syn = *N. bigelovii* (Torr.) S. Watson)	2N=48	California, Nevada, Oregon	Yes	Male sterile
N. clevelandii A. Gray	2N=48	California, Arizona, New Mexico	No	
N. plumbaginifolia Viv.	2N=20	Florida	Only under controlled conditions.	Male and female sterile: genetic instability, loss of *N. plumb.* genome

References:
See Literature Cited, Section VI.

Appendix C. Data submitted with the petition or in the October 19, 2001 response to the deficiency letter dated September 21, 2001 (RTDL) in support of nonregulated status for Vector 21-41 Tobacco.

Molecular Genetic Characterization and Inheritance Data
NtQPT1 gene is expressed in the roots: Petition Fig. 2., pg. 11. In situ localization of *NtQPT1* mRNA in parraffin sections of tobacco roots.
NtQPT1 promoter is capable of directing transgene expression in a root-specific manner: Petition Fig. 3, pg. 11. Histochemical localization of GUS activity in trangenic roots; Petition Fig. 4., pg. 12. GUS activities in roots, leaves, and stems of transgenic tobacco plants carrying the CaMV 35S promoter, the promoterless GUS, and 5' nested deletions of the *NtQPT1* promoter fused to GUS.
NtQPT1 gene encodes a functional QPTase: Petition Fig. 5., pg. 13. Complementation of *E. coli* Strain TH266 (*nadC*) by expression of the *NtQPT1* cDNA.
Vector 21-41 contains two copies of the *nptII* gene: Petition Fig. 9., pg. 23. Genomic DNA Blot of Vector 21-41 (probed for *nptII*).
Vector 21-41 contains full length copies of the *nptII* gene and *NtQPT1* promoter in the expected orientation: Petition Fig.10., pg. 24. PCR Analysis of the Transgene in Vector 21-41.
Vector 21-41 contains the full length copies of the anti-sense *NtQPT1* cDNA with its *NtQPT1* promoter and *nos* terminator in the correct orientation: Petition Fig. 11., pg. 26. PCR Analysis of the Transgene in Vector 21-41.
Vector 21-41 contains partial *tetA* and *trfA* sequences flanking the left T-DNA border located between the left T-DNA border of the two inverted repeat copies of the T-DNA inserts. Petition Fig. 12., pg. 28. PCR Analysis of the Transgene in Vector 21-41 and Petition Fig. 13., pg. 30. Amplification Between the Two Tandem Inverted T-DNA Repeats.
Vector 21-41 contains no complete protein coding sequences present outside of the T-DNA border in pYTY32: RTDL, Fig. 1, pp. 6-8.
Progeny from transformant T_0 #41 segregated 3:1 for kanamycin resistance: Petition Table 2., pg. 19. T_0 Segregation Analysis of Tranformant #41.
T_2 seeds from plant T_1-1 derived from T_0 #41 are homozygous for kanamycin resistance: Petition Table 3., pg. 20. Segregation Analysis of T_1 Generation of Transformant #41 (for kan^r).
T_1-1, T_2-1, and later generations (T_3 and T_4) designated Vector 21-41 have low nicotine levels in their leaves compared to null segregants. Petition. Table 4. Pg. 21. Nicotine levels in Generations of Vector 21-41 Tobacco.

Phenotypic Characterization: Intended and Unintended Effects
NPTII expression in Vector 21-41 T_4 plants in leaves, roots, and stems as measured by enzyme-linked immunosorbent assays (ELISAs). Petition pg. 31 (See also Table 2 mentioned above for kanamycin resistance.)
Agronomic traits and chemical traits (nicotine, nor-nicotine, total alkaloids, nitrogen and reducing sugars in cured leaves) of Vector 21-41 tobacco grown in Clayton, NC in 2000 compared to promoter control and wild type (Burley 21-LA) plants. Petition Table. 5., pg. 32. (See also Table 4 mentioned above for nicotine content in NC and other locations, and also RTDL, pg. 3, and pg. 13 for additional agronomic data for 53 field trials in PA in 2001.) (AMS also provided nicotine data to APHIS for Vector 21-41 tobacco grown in 2001 at the request of the petitioner.)
Seed production per pod from greenhouse grown plants of Vector 21-41 tobacco compared to Burley 21-LA and four other tobacco varieties. RTDL, Table 1, pg. 12. (Note: Number of pods in that Table 1 refers to the number of pods from which the data was gathered. The number of pods was limited to 50/plant, M. Conkling personal communication 10/24/01.)
Disease and insect susceptibility of Vector 21-41 tobacco. Petition, pg. 31, & RTDL, pp 10-11.
Yields of Tobacco-Specific Nitrosamines in Mainstream Tobacco Smoke: "Standard" Conditions. (Vector 21-41 vs. a "full-flavor" cigarette) Petition Table 6, pg. 34

Appendix D. Determination of non-regulated status for Vector 21-41 tobacco.

In response to a petition (designated 01-121-01p) from Vector Tobacco (USA) Ltd., APHIS has determined that genetically engineered tobacco line Vector 21-41 and progeny derived from it will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits or acknowledged notifications that were previously required for environmental release, importation, or interstate movement under those regulations will no longer be required for Vector 21-41 tobacco and its progeny. Importation of seeds and other propagative material would still be subject to APHIS foreign quarantine notices at 7 CFR Part 319 and the Federal Seed Act regulations at 7 CFR Part 201. This determination is based on APHIS' analysis of field, greenhouse, and laboratory data and references provided in the petition and other relevant information as described in this environmental assessment that indicate that Vector 21-41 will not pose a plant pest risk for the following reasons. (1) It exhibits no plant pathogenic properties - although a plant pathogen was used in the development of this tobacco, these plants are not infected by this organism, nor do they contain genetic material from this pathogen that can cause plant disease. (2) It exhibits no characteristics that would cause it to be more weedy than the non-transgenic parent tobacco line or other cultivated tobacco. (3) Gene introgression from Vector 21-41 tobacco to native, introduced, or naturalized species of *Nicotiana* in the United States is extremely unlikely, and it is not likely to increase the weediness potential of any resulting progeny nor adversely effect genetic diversity any more than would introgression from other cultivated tobacco. (4) Given current tobacco pest management practices and grading and certification systems for tobacco leaf, the disease and insect susceptibility of Vector 21-41 tobacco and the quality characteristics of its cured leaves should not cause a direct or indirect plant pest effect on raw or processed plant commodities, nor should it reduce the ability to control pests in tobacco or other crops, compared to the non-transgenic parent or other tobacco. (5) It has no potential to have a greater damaging, harmful, or toxic effect on organisms beneficial to agriculture than does other cultivated tobacco. In addition to our finding of no plant pest risk, there will be no affect on threatened or endangered species resulting from a determination of non-regulated status for Vector 21-41 tobacco and its progeny.

APHIS also has concluded that there may be new varieties bred from Vector 21-41 tobacco; however, they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for tobacco descended from Vector 21-41 tobacco, or those observed for other tobacco varieties not considered regulated articles under 7 CFR Part 340.

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Biotechnology Regulatory Services
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Date: SEP 16 2002