

## CASE STUDY No. V

### BIOREMEDIATION USING POPLAR TREES

#### *Overview*

This case study examines a hybrid poplar that has been genetically engineered to detoxify a widespread industrial toxic chemical, trichloroethelene (TCE). The poplar, which was originally modified through the insertion of a human cytochrome gene, is still being tested in the laboratory using genes from other animals and plants, and has not yet been tested in the field. The federal agencies that will be involved in regulating the poplar will be the Animal and Plant Health Inspection Service (APHIS) and the Environmental Protection Agency (EPA).

#### *1. Description of proposed organism/product and its use (what, where, how much and when)*

#### **Introduction**

Scientists have been searching for inexpensive and safe ways to remove toxic chemicals from the soil and water on polluted sites. One approach being considered is the use of plants, including those that are genetically engineered. In a recent publication (Doty et al., 2000), scientists have shown that plants engineered with a gene from a mammal degrade the toxic chemical trichloroethylene (TCE). TCE is an environmental contaminant found throughout the industrialized world. It was commonly used as a metal degreasing agent and a dry cleaning solvent. Forty percent of all Superfund sites are contaminated with TCE. It is an EPA priority pollutant and a suspected human carcinogen. It is now known that exposure can result in central nervous system depression, hepatotoxicity, and nephrotoxicity (Costa, 1980, cited from Doty et al., 2000).

One plant species that has been engineered to detoxify TCE is a hybrid poplar, which is the subject of this case study. Poplar has been chosen because the nonengineered tree can grow in soils with low levels of TCE (Newman et al, 1997), it grows rapidly, and it can be vegetatively propagated (i.e., twigs can be clipped from the tree and rooted to establish additional, genetically identical plants). The latter two are key attributes if bioremediation is to succeed. In this case of phytoremediation (bioremediation using plants), a tree takes up a chemical from the soil or water and expresses an enzyme that modifies the chemical into less toxic or nontoxic substances that are translocated to the stems and leaves. There is no functional requirement for these plants to flower, since the desired chemical processes occur in the roots, stems and leaves. Therefore, outcrossing with wild relatives can be controlled in the field testing stage through cutting down the trees before they flower, or through other techniques of inducing sterility, without decreasing the effectiveness of bioremediation.

The organism in this case study is a hybrid between two cottonwood species, black cottonwood (*Populus trichocarpa*) and eastern cottonwood (*Populus deltoides*), both native to the U.S. Because these hybrids belong to the genus *Populus*, which includes poplars, aspen and cottonwoods, they tend to be generically known as “hybrid poplars,” and will be referred to as such for the rest of the case study. They are monoecious (spatially separate male and female reproductive features occur on each plant), wind-pollinated woody plants. Further information on the two

cottonwood species and their hybrids can be found in the United States Department of Agriculture Forest Service's Agriculture Handbook 654 Silvics of North America at the following website: “[http://willow.ncfes.umn.edu/silvics\\_manual/volume\\_2/](http://willow.ncfes.umn.edu/silvics_manual/volume_2/)”.

Because it is relatively easy to vegetatively propagate poplars and their hybrids, clones<sup>1</sup> (genetically identical cuttings taken originally from one individual) are generally used in experimentation and field production. Two such clones, 184-402 and H11-11, were used in the APHIS notification that is the basis for this case study. These clones were genetically engineered using a mammalian cytochrome gene to improve degradation of the compound TCE (Doty et al., 2000). The human cytochrome gene used, P450 2E1, has been intensively studied and the gene product oxidizes a range of compounds including TCE, ethylene dibromide (EDB), carbon tetrachloride, benzene, and styrene, chloroform and others. Details of the original construct are the same as in Doty et al., (2000), except that a different promoter (35s promoter from the cauliflower mosaic virus) was used. Due to the fact that the developers are currently experimenting with other cytochromes, this case study will not focus on the specifics of the original mammalian cytochrome construct. Nevertheless, if a real environmental assessment were to be developed by APHIS or EPA for such an organism, the specific information on the construct would be essential to adequately assess the risks.

To date, no genetically engineered poplars have been field tested that detoxify TCE, although this is planned as soon as laboratory and greenhouse testing satisfies bioremediation goals of the developers. Unlike glufosinate-tolerant soybeans and Bt maize (described in other case studies), based on current understanding of this organism, APHIS believes that there are certain purposes, such as plants for bioremediation, in this case, or plants that produce pharmaceuticals, for which the plants will not be subject to deregulation. Plants for bioremediation will not be sold to the public. Trees grown at Superfund sites will require regulatory oversight by Federal, State and Tribal agencies whenever and wherever they are grown to ensure that workers and the environment are protected.

***Is there prior experience dealing with the same varieties not genetically engineered?***

Long, well-documented experience exists with regard to growing *Populus* species and hybrids for fiber and biomass production, and for riparian restoration. For eastern cottonwood in particular, growth of clonal plantations generated via rooted or unrooted cuttings has been conducted for decades, and management practices for these plantations are well developed (Zsuffa 1976, Cooper 1990). Hybrid cottonwoods (*Populus trichocarpa* x *P. deltoides*) have already been demonstrated to oxidize TCE to produce CO<sub>2</sub> and other metabolites (Strand et al. 1995, Newman et al. 1997, Newman et al. 1999).

***What are the projected location and extent of production, use and disposal?***

There are thousands of contaminated sites in the U.S. and abroad. Industrial sites and air force bases commonly have TCE contamination, and often there is free TCE existing in the aquifer. It is possible that if a clone or clones were developed that could grow in different environments, they could

---

<sup>1</sup> The term clone can be used to describe a transformation event, or a vegetatively propagated plant. In this case study, the term is used to mean a vegetatively propagated poplar. Whether the plant is genetically engineered or not should be clear from the context.

be widely used on these kinds of sites. On a typical site, stems would probably be harvested on short (5-7 year) rotations to preclude flowering, and the trees would continue to be cut down and allowed to resprout (coppiced) until TCE had been reduced to targeted levels.

Depending on the metabolites remaining in stem tissue, it is possible that the trees might be used for paper. It was hypothesized that the chemical reactions in pulping would break down any organochlorine compounds remaining in the tree. This was found to be the case in experiments by Newman and Gordon in the summer of 2000 (Gordon, personal communication). If this paper were to be produced and used, the Occupational Health and Safety Administration (OSHA) would regulate the safety of the manufacturing process. EPA would regulate air and water emissions.

***What types of adverse effects might occur by the GEOP throughout its life cycle, and where might they occur?***

Risks fall into four categories: (1) health and ecological risks from the products of the TCE breakdown process on the sites being remediated; (2) risk of escape of transgenes into the native eastern or black cottonwood populations, or other reproductively compatible *Populus* species; (3) risks of the transgenic trees themselves becoming weeds or otherwise invasive; and (4) health and ecological risks from materials and products derived from the trees. As stated above, characterizing the entire genetic construct would be essential in estimating risks.

With regard to health risks, trees and other engineered plants have the potential to release small amounts of TCE's into the air through transpiration. The levels of TCE transpired to air by non-engineered poplar plants are within the 4% to 7% range, as judged from field studies (Newman et al., 1997). This area of research is currently developing and undergoing continuing discussion via scientific workshops and publications. The actual amount of TCE transpired will vary depending on such factors as the analysis technique employed, availability of water, ambient air temperatures, and age of the trees. The half-life of TCE once in the air, and in the presence of sunlight, has been estimated to be approximately 9 hours. Metabolites in trees and resulting products are currently under study. The studies that have been performed to date with TCE and with the remediation of TCE by hybrid poplar indicate that the compound is completely destroyed within the poplar trees (Gordon, personal communication). There are very small quantities of intermediate metabolites--perhaps 1% of the original material is found as metabolites--but these are eventually also metabolized into CO<sub>2</sub> and chlorine ions (Newman et al., 1999). Nevertheless, organisms that encounter metabolites in trees or tree parts might be affected. Studies with insects have indicated that feeding herbaceous insects with residue of poplars treated with TCE has no effect on the growth rate or fecundity of the insects (Sorbet, 1998). Further research may be needed to study the effects on non-target species prior to field use of these trees.

The breakdown products of TCE degradation in hybrid poplar are chloride in the soil and trichloroethanol and conjugates, and di- and tri-chloroacetic acid in the plant tissue, which are ultimately broken down within the plant into CO<sub>2</sub> and chlorine. It is expected that chloride in the soil would not be a concern, as it is not harmful to soil flora or water quality at the concentrations that would occur (Newman, personal communication).

Both eastern and black cottonwoods are perennial, undomesticated plants. Thus, not only

would transgenic plants persist for several years, they also could have wild relatives within pollination distance. Therefore, precautions will need to be taken to prevent escape of the transgenes to the wild population by either seeds or pollen. Greenhouse studies indicate that the phytoextraction processes will be performed very effectively by juvenile trees. Thus, stems can be cut and disposed of before the trees reach flowering and fruiting age. Since hybrid poplars can propagate itself vegetatively, using the practice of coppicing, the trees could be cut and allowed to resprout for several cycles, if necessary, without them ever reaching flowering age.

There are a number of methods currently under investigation in which the possible spread of genetically engineered material could be avoided without needing to cut down the trees before they flower. One method is the use of triploids where seeds or pollen would be approximately 99+% sterile, but not absolutely sterile, and research is ongoing on this and other mechanisms (Meilan, 1997). These sterility mechanisms would have to be tested in field trials over the relevant period of time (from the initiation of flowering age until when they would be cut down) in order to ensure that they would be effective prior to any operational use in trees for bioremediation.

Gene flow to related native species could have a variety of different impacts, depending on the gene involved. Due to the long-lived nature of trees, their importance as hosts to a variety of vertebrates and invertebrates, their well-developed capacity for pollen and seed dispersal and ability to colonize disturbed habitats, there are many environmental issues that related specifically to forest trees. Indeed, there is also a concern as to the integrity of native gene pools. It should be observed however, that some species of forest trees have been selected for two or three generations for timber or pulp production or disease resistance through traditional breeding. These activities have heretofore met with relatively little concern as to their impact on native gene pools. Nevertheless, the changes possible by genetic engineering can be different in kind and degree than traditional breeding, or marker assisted traditional breeding, due to the fact that genes can be transferred from other species, and it is not simply a matter of increasing the frequency of a gene already existing within a species or population. In addition, such traits as enhanced TCE degradation may lead to phenotypes that might be less fit in a forest environment, and the outcome is not clear without long term tests in a variety of environments. On the other hand, traits that have been the subject of traditional breeding efforts, such as height and diameter growth and disease resistance, are selection criteria that are generally known to contribute to long-term health and survival of trees in forest environments. Compared to crops, forestry production systems generally require more time on the landscape (10-100) years, and due to the long period of investments, forest landowners can afford less intervention with, for example, pesticides and fertilizers. Because of these long time periods, and the inherent risk of these long-term investments, foresters tend to be conservative with changes in plant material and practices, and tend to prefer proven robust and adapted genetic material.

Some have argued that forest gene pools are already “contaminated” due to the introduction of sympatric species from other parts of North America, Asia, and Europe. Like traditional breeding, however, these are *Populus* genes adapted to a *Populus* genetic background.

Dr. Steven Strauss, a prominent molecular geneticist, believes that there would be strong resistance of wild poplar stands to significant introgression from plantations for a variety of reasons (Ecological effects of pest resistance genes in managed ecosystems, available at

<http://www.isb.vt.edu/cfdocs/proceedings.cfm>). The experimental evidence for these concepts should be readily available if appropriate experiments were conducted. Other tree population geneticists have measured gene flow from a wide variety of wind-pollinated tree species and have generally found it to be substantial (e.g., Friedman and Adams, 1985).

While some individuals consider cottonwoods to be capable of growing where they are not planted in some environments, there is no reason to believe that the mammalian or other cytochrome gene would make them more so. On the other hand, there is no *a priori* reason to consider that they would not be, because the behavior of mammalian cytochrome genes in plants has not been characterized for these kinds of environments. Nevertheless, this could be tested experimentally prior to release into the environment during the confined field testing phase.

### ***What are the pathways for proliferation of those risks?***

If any TCEs remain, or if there are any remaining metabolites that pose risks, these could be passed on through dissemination of trees and their parts, including roots, leaves, stem material, seeds and pollen. These could be disseminated by wind, water, and terrestrial and aquatic animals that feed on or transfer material. For example, a bird might take a piece of branch or “cotton” surrounding the seed for its nest. Remaining TCE or TCE metabolites could also be disseminated through the use of trees and tree parts as firewood or in other products.

Another pathway for risk of proliferation is through gene flow from the transgenic trees to other sexually compatible species, whether native, ornamental or commercially grown. This could happen if trees are not carefully monitored for flowering or if they are not cut down before they flower, or if other sterility mechanisms are not effective. Once pollen or seeds are dispersed, the potential arises for the transgenes to enter the wild population. Also, because these trees have the ability to resprout, stumps and root systems would have to be killed when the task of remediation was finished. Depending on future land uses, this could be accomplished through stump treatments with herbicides, or by removing stumps and large roots with equipment. The latter is currently common practice in management of hybrid poplars for paper.

### ***What types of positive environmental impacts might occur because of this use?***

Hybrid poplars engineered to break down contaminants in soil and water would ultimately enable the ecological structure and function of contaminated sites to be restored, at least with respect to the contaminant that is removed by the poplars. Where necessary or desirable, these areas would be able to return to agriculture, forestry, residential or industrial uses. In addition, while the remediation process is occurring, the root systems of the transgenic trees will help to hold the soil in place and prevent erosion, thereby stabilizing the disturbed ecosystem. Trees also provide humus and microclimatic changes that can, compared to other kinds of plants, provide an environment more hospitable to a wide variety of other organisms.

### ***What is the rationale for using the GEOP, including its advantages vis-à-vis alternatives?***

TCE is one of the most widespread contaminants in the environment of the U.S. (Doty et al.,

2000). TCE is stable in groundwater and can persist for decades as dense nonaqueous-phase liquids. Difficulty in pinpointing the location of these pools of TCE can greatly hinder attempts at remediation. The fact that TCE is a suspected carcinogen (Newman et al. 1997, Gordon et al., 1997) has lent urgency to cleanup efforts. The principal methods for remediation of groundwater contaminated with TCE are pumping water from the aquifer and stripping the TCE by aeration or by charcoal absorption. These procedures can take years or decades and can be very expensive (Newman et al., 1999). Other techniques use bacteria to degrade TCE, sometimes through co-metabolism (which requires the use of inducers such as toluene or phenol, which themselves pose health and environmental risks, for degradation to occur) and sometimes, albeit more slowly, directly. Another option is monitored natural attenuation.

Hybrid poplars were chosen for a variety of reasons. Poplar has a wide geographical distribution and can be grown from southern Alaska into Central America, and therefore could, if successfully developed, serve to remediate diverse sites. It is not likely that one poplar clone would be environmentally adapted to such a diverse area, but if successful in one clone, the transformation could be applied to other poplar clones adapted to different environments. Members of the species can be easily crossed sexually. Propagation by cuttings is simple, yielding clones of a given individual, and poplars can be grown axenically (i.e., without bacteria or molds) in culture. Therefore it is relatively easy and inexpensive to propagate a genetically desirable individual, at least compared to other trees. Poplars have an enormous water absorption capacity from their roots' surface that can approach 300,000 km<sup>3</sup>/ha (Gordon et al. 1997). Trees are able to take up water from both soil and shallow aquifers, potentially remediating both. There are also procedures for purifying deep-lying ground water by pumping it up to the surface and using it to irrigate level stands of poplar trees, so that the number of sites that can be remediated using poplars is greatly expanded. Finally, poplars can be used to provide beneficial products such as wood and paper, and can help restore entire impacted ecosystems, while providing the specific environmental service of remediating toxic chemicals.

Poplars are capable of breaking down TCEs without genetic engineering. The addition of a cytochrome gene would enable faster metabolism of TCE, thereby decreasing the amount of time until sites are restored, and thus the time organisms are exposed to TCE. The investigators at University of Washington found as much as a 600-fold enhancement in TCE metabolism in tobacco using the mammalian cytochrome (Doty et al., 2000). As of summer 2000, when this case study was written, the investigators found only a 4-fold increase in metabolism of TCE in transgenic poplars compared to non-transgenic poplars in greenhouse experiments. Due to public concerns with genetically engineered organisms, the investigators have chosen to use rabbit or plant cytochrome genes instead of human genes, and also to experiment in the greenhouse until they achieve a much more significant improvement compared to transgenic poplars in the greenhouse before conducting field testing.(Gordon and Newman, personal communication).

## **2. Relevant regulatory agencies, regulatory authority and legal measures**

The overall APHIS process for authorizing introductions of transgenic organisms is described in the regulatory section more fully in the Herbicide-Tolerant Soybean case study. Under authority granted by the Federal Plant Pest Act (FPPA), 7 U.S.C. §§ 150aa-150jj as amended, and the Plant Quarantine Act (PQA), 7 U.S.C. §§ 151-164a, §§ 166-167 as amended, APHIS regulates the

introduction (i.e., importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. APHIS believes that is highly unlikely that trees (or any plant) intended for bioremediation would ever be granted deregulated status as has been done for glufosinate-tolerant soybeans and Bt maize (as described in the case studies about those organisms). Thus, a permit would still be required from APHIS for actual bioremediation applications.

The FPPA and PQA, together with several other statutes, were consolidated in August 2000 in the Plant Protection Act (PPA), 7 U.S.C. §§ 7701-7772. The regulations issued pursuant to FPPA and PQA will continue in effect until APHIS issues new regulations under the PPA.

The hybrid poplar qualifies for introduction under APHIS' notification process. The applicant provided the kind of information in the letter in Appendix 1, including certifying that the engineered tree will be introduced in accordance with the eligibility criteria and the performance standards set forth in 7 CFR 340.3. The three major steps APHIS took in this case are the standard ones: (1) evaluate relevant information (both that submitted by the permit applicant and that gathered by APHIS from other primary and secondary sources); (2) notify and consult with regulatory officials in States where the applicant proposes to field test; and (3) reach a decision as to whether to acknowledge or deny the notification. Additional information on USDA regulations pertaining to plants can be found at the USDA web site, <http://www.aphis.usda.gov/bbep/bp/>. The letter was received on 10/22/99, and on 11/21/99 a notification was acknowledged. The notification will remain in force until 8/01/03. Another notification would be required if any factors are amended. As described above, because the trees did not exhibit the desired levels of TCE metabolism in the greenhouse, field testing has not been initiated. If the field testing were successful, and it was desired to plant these trees for bioremediation on various contaminated sites, then a permit would be necessary. Permits are described further in the Herbicide Tolerant Soybean case study.

## **EPA**

EPA has established regulations under section 5 of the Toxic Substances Control Act, applicable to intergeneric microorganisms for uses such as the clean-up of wastes. (40 C.F.R. Part 725). While EPA has not issued similar regulations for transgenic plants, EPA's authority under TSCA section 5 is also applicable to transgenic plants. Section 5 of TSCA establishes requirements for EPA review of new chemicals and significant new uses of existing chemicals. Although EPA's regulations are currently focused on microbial products and on the requirements of TSCA section 5 (see Bioremediation and Biosensing Using Bacteria Case Study), the breadth of EPA's jurisdiction under TSCA is much broader. EPA has publicly asserted jurisdiction over other living organisms, such as certain plants intended for the clean-up of wastes. In the preamble of the proposed rule establishing Part 725, EPA expressly stated that it was reserving authority under TSCA to screen transgenic plants and animals, as needed to protect the environment and human health. (59 Fed. Reg. 45526, 45527 (September 1, 1994)). The preamble to the proposed rule states:

Plants and animals could also be chemical substances under TSCA. Nevertheless, as a matter of policy, EPA has limited this rulemaking to microorganisms, e.g., microalgae of the plant kingdom....Traditional chemicals extracted from a plant or animal may also be subject to TSCA, as are other chemical substances. EPA is reserving authority under TSCA to screen

transgenic plants and animals in the future as needed.(59 Fed. Reg. at 45527).

In addition, EPA's authority under TSCA is not restricted to the requirements of section 5. EPA is also authorized to regulate "existing" chemical substances under TSCA section 4 (data generation), sections 6 and 7 (impose restrictions to prevent unreasonable risks of injury to human health or the environment), and section 8 (information collection). Further information on TSCA regulations and biotechnology products can be found in this report in the Bioremediation and Biosensing using Bacteria case study and the EPA website <http://www.epa.gov/opptintr/batik>.

The introduction of TCE-remediating trees to waste sites on a commercial scale will likely require regulatory coordination with other legal mandates. For example, on a Superfund site, there is generally an EPA Remedial Project Manager (RPM) in charge of the selection of which remedy, or remediation technology, to clean up a site. The RPM has nine criteria, as laid out under Superfund laws, used to direct the remedy selection process. One of the criteria to consider is the extent to which the remedy provides overall protection of human health and the environment. For more on the Superfund risk assessment process, see Rock and Sayre (1999). The information in the TSCA risk assessment and consent order could be passed on for consideration by RPMs. Similar coordination with site managers could occur for non-Superfund sites, where RCRA and/or State or Tribal considerations dominate site decisions.

### **Interagency Coordination**

In 1998, APHIS and EPA's TSCA Biotechnology Program held a series of meetings to evaluate the status of regulation of plants that might currently have issues related to or were in the pipeline for use in bioremediation. The agencies concluded that the current process adequately addressed the issues that were identified and assessed. This meeting and discussion focused on field testing and disposal.

## **3. Hazard Identification and Risk Assessment**

### **APHIS**

Field testing of these plants can be divided into two phases. The first is "proof of concept" and the second is successful bioremediation of contaminated soils. For example, before field tests are begun, scientists perform preliminary experiments in greenhouses or growth chambers to determine if the plant detoxifies the TCE. However, decades of experience have shown that only a few percent of plants that perform well in greenhouse tests will perform well in the field. Therefore, scientists generate hundreds or thousands of plants in the greenhouse to test in the field to identify and select the handful that perform as intended. This selection process takes several years. With respect to hybrid poplars, the plants are likely to be planted for testing at sites that are not contaminated with TCE, in specially designed containers with controlled concentrations of TCE (designed with the help of the State Department of Ecology for Washington State). In the first five years before flowering, trees can be selected for both good growth and metabolism of TCE. When a clone has been identified that detoxifies TCE and has acceptable growth characteristics, the proof of concept phase is over.



Before bioremediation can begin on a large scale, the number of trees would have to be increased. This would be accomplished by taking cuttings from the selected clones and vegetatively propagating them, taking cuttings from those cuttings when they are large enough and so on. This could take several years. During this time, APHIS envisions that coordination between other Agencies (EPA, INT, NIH, FS and others as appropriate), States, and Tribal governments, as appropriate, would take place. At this time, sites would be identified and site-specific environmental issues identified and addressed through site specific Environmental Assessments (EAs) or Environmental Impact Statements (EISs) or other relevant EPA decision processes.

The assessment criteria that needed to be, and were, met for this notification are:

1. The introduced genetic material is "stably integrated" in the plant genome.
2. The function of the introduced genetic material is known and its expression in the regulated article does not result in plant disease.
3. The introduced genetic material does not: (i) cause the production of an infectious entity, (ii) encode substances that are known or likely to be toxic to nontarget organisms known or likely to feed or live on the plant species, or (iii) encode products intended for pharmaceutical use.
4. To ensure the introduced genetic sequences do not pose a significant risk of the creation of any new plant virus, they must be: (i) noncoding regulatory sequences of known function, or (ii) sense or antisense genetic constructs derived from viral genes from plant viruses that are prevalent and endemic in the area where the introduction will occur and that infect plants of the same host species.
5. The plant has not been modified to contain the following genetic material from animal or human pathogens: (i) any nucleic acid sequence derived from an animal or human virus, or (ii) coding sequences whose products are known or likely causal agents of disease in animals or humans.

APHIS decided that the above conditions were met for this notification. In addition, confinement would be ensured by following the protocols previously derived for other genetically engineered poplars. These are described in Section 5.

If these trees were to be used operationally for bioremediation in the future, APHIS would coordinate the development of an environmental assessment with the other regulatory agencies consistent with NEPA implementing regulations of all Agencies. To reiterate, APHIS is not planning to deregulate plants used for this application. For an example of an APHIS EA for a poplar, see those for the permits numbered 9303902r, 8910903r and 9503101r on the APHIS website.

### **3. Information and data (what, why and how is data and information collected and generated)**

At any time, APHIS may request from applicants any additional information necessary to ensure that the performance standards are being met. This can include information on containment and potential impacts on nontarget organisms.

For a discussion of how EPA approaches information in the context of applying TSCA to microorganisms, see the Bioremediation and Biosensing Using Bacteria Case Study.

#### **4. Mitigation and management considerations: approvals and conditions on research, development, production, distribution, marketing, use and disposal**

The APHIS document on confinement considerations for *Populus* species can be found at <http://www.aphis.usda.gov/bbep/bp/sec6j.html>. In transgenic poplars, genes can easily escape by windborne pollen and seed, or by vegetative means. Because of this, and the wide distribution of sexually compatible species within the U.S., steps must be taken to prevent gene flow and persistence of plants in the environment by these methods in order to perform field trials under notification. A wide range of poplar nursery practices for pollination, fertilization, and cloning have been developed over decades. After trees reach reproductive maturity, some means to either prevent flowering, remove flowers, or prohibit pollen shedding must be made to preclude gene escape to other sexually compatible poplars in the general area. Some means must also be taken to prevent seed set or to restrict shedding of the seeds. Finally, steps must be taken to prevent movement of vegetative parts out of the site, and to destroy any vegetative parts, both above ground and below ground, which may remain after the test is complete. For example, transgenic poplars could be cut down and the vegetative material killed by a broad spectrum herbicide prior to flowering. The remaining stumps and root systems could be treated with an appropriate herbicide. The dead tree stumps could be removed from the test site and along with any plant stems, roots, and suckers removed from the site for analysis, these could be killed by high temperature treatment (autoclaving, oven baking, or incineration). Following removal of transgenic plant material, the test site could be treated with an appropriate herbicide and cultivated to control any volunteers prior to flowering in subsequent seasons.

Use of transgenic poplars beyond the field testing stage is likely to require additional information about the behavior of the new varieties and their interactions with wild and/or weedy relatives. For certain experiments designed to investigate these parameters, it may be appropriate to perform field trials under permit.

In addition, once field tests occurred on sites with TCEs, specific mitigation considerations would be developed at each site in accordance with CERCLA, RCRA or TSCA. An approach to disposal within the context of TSCA and bacteria can be found in Bioremediation and Biosensing Using Bacteria Case Study.

#### **5. Monitoring and consideration of new information**

Monitoring of the field tests under APHIS would be expected to be similar to other tree field tests. Monitoring is authorized under the Plant Protection Act for obtaining information on plant pest risks. Since APHIS would not deregulate trees used in bioremediation, it would still be possible to change the performance standards of the permit as new information was acquired on the basis of plant

pest risk.

## **6. Enforcement and compliance**

APHIS has qualified personnel in every State that can inspect field sites for compliance to the performance standards for all field testing. In addition, headquarters staff will be inspecting plant derived biologics applicants on a yearly basis and will inspect field tests performed under notification on a case-by-case basis.

USDA ensures that the conditions described in the notification, e.g., not flowering or isolation strips, would be maintained by reviewing the design protocol to ensure that it will meet performance standards, and inspections by both USDA and State Department of Agriculture officials on site. Failure to comply with performance standards under notification or permit conditions can result in the owner being ordered to take remedial action (7 U.S.C. § 7714(b)(1)) if necessary to prevent the spread of plant pests (7 CFR 340.4 d 7). If the owner fails to take such action, the Department can take the action and recover the cost of the action from the owner (7 U.S.C. § 7714(b)(2)). The owner can also be assessed a criminal or civil penalty for failing to comply with the regulations (7 U.S.C. § 7734). For example, some remedial actions might involve removing the plants by burning, spraying herbicide, hoeing or discing. Since these trees have not yet been outplanted, either under confinement under notification, nor under permitting, there has been no opportunity to observe compliance.

If the trees were used for bioremediation (compared to field tests under notification) the same kind of audit procedure would ensure that conditions of the permit were met (7 USC §§ 7714, 7734).

In terms of TSCA, if issues related to TSCA were seen, then monitoring and termination procedures would be put into place. See Bioremediation and Biosensing Using Bacteria Case Study for further information.

## **7. Public involvement and transparency**

A description of the overall APHIS approach to public involvement is described further in the Herbicide Tolerant Soybean Case Study. This involves posting all notifications and permits on the website. Comments are received from the public on them. Also, people may ask for information on permits and notifications from their State Departments of Agriculture, and if the material is not confidential business information, the State can make that available and also take comments from the public. In this case, the information is not confidential, so these two methods can be used to give input on the decision.

Notifications do not have an environmental assessment prepared in accordance with APHIS' NEPA implementation regulations (7 CFR 372). The rationale is that these are not exposed to the environment due to the performance standards that ensure confinement (see the Ornamental Plant (Bentgrass) sidebar for example of performance standards). Due to the changes in the regulations regarding notification in 1993 and 1997, species currently under notification may have had EAs prepared in the past, when a permit was required for that species, and the permit required an EA. For this reason there are EAs for other genetically engineered poplars (engineered for traits other than bioremediation) available on the

APHIS website (9303902r, 8910903r and 9503101r).

If an application for deregulation were submitted, the standard process would be as described in the Herbicide-tolerant Soybean case study. However, as noted above, APHIS believes that this application will not be deregulated. During the permitting process, which would occur prior to operational use, the application for the permit would be made public and comments received on it. APHIS would then prepare an environmental assessment (if the biotechnology staff determine that there are significant new issues compared to previous poplar EAs) under NEPA and then either the Agency would issue a FONSI (finding of no significant impact) or an EIA would be developed. Since 1999, APHIS has been making its draft EAs available for a 30 day comment period, so that would occur for any permitting of a poplar for bioremediation. To the extent that the Comprehensive Emergency Response, Compensation, and Liability Act (CERCLA), 42 U.S.C. §§ 9601-9675, would be involved in the field sites for bioremediation, the criteria for the decision on the cleanup technique includes community acceptance determined by the result of obtaining public notice and comment (EPA Rules of Thumb, EPA 540-R-97-013).

## REFERENCES

- Cooper, D.T. 1990. *Populus deltoides*, Eastern cottonwood. In *Silvics of North America, Vol 2. Hardwoods* (R.M. Burns and B.H. Honkala, Eds.). USDA Forest Service Agriculture Handbook 654, Washington, DC. pp. 530-537.
- Costa, A.K., I.D. Katz and K. M.Ivanetich. 1980. *Biochemical Pharmacology* 29:433-439. Cited from Doty et al., 2000.
- Dix, M.E., N.B. Klopfenstein, J.-W. Zhang, S.W. Workman and M.-S. Kim. 1997. Potential use of *Populus* for phytoremediation of environmental pollution in riparian zones. In *Micropropagation, Genetic Engineering, and Molecular Biology of Populus* (N.B. Klopfenstein, Y.W. Chun, M.-S. Kim, and M.R. Ahuja, Eds.). USDA Forest Service Gen. Tech. Rep. RM GTR-297, Fort Collins, CO. pp. 206-211.
- Doty, S.L., T.Q. Shang, A. M. Wilson, J. Tangen, A. D. Westergreen, L.A. Newman, S. E. Strand and M. Q. Gordon. 2000. Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2E1. *Proc Natl. Acad. Sci. U S A* 2000 Jun 6; 97(12):6287-91.
- Friedman, S.T. and W.T. Adams. 1985. Estimation of gene flow into two seed orchards of loblolly pine (*Pinus taeda* L.). *Theor. and Appl. Genet.* 69:609-615
- Gordon, M., N. Choe, J. Duffy, G. Ekuan, P. Heilman, I. Muiznieks, L. Newman, M. Ruszaj, B. B. Shurtleff, S. Strand, and J. Wilmoth. 1997. Phytoremediation of Trichloroethylene with hybrid poplars. In: *American Chemical Society Symposium Series 664*, E. L. Kruger, T. A. Anderson, and J. R. Coats, eds. pp 177-185.

Meilan, R., and S.H. Strauss. 1997. Poplar Genetically Engineered for Reproductive Sterility and Accelerated Flowering. In: Micropropagation, genetics engineering, and molecular biology of *Populus*. (Klopfenstein, N. B.; Chun, Y.W.; Kim, M.S.; Ahuja, M.R., eds.) U.S. Department of Agriculture, Forest Service Gen.Tech. Rep. RM-GTR-297. Fort Collins, CO. pp. 212-219.

Newman, L, S. Strand, J. Duffy, G. Ekuan, M. Raszaj, B. Shurtleff, J. Wilmoth, P. Heilman, M. Gordon. 1997. Uptake and biotransformation of trichloroethylene by hybrid poplars. *Environmental Science and Technology*. 31: 1062-1067.

Newman, Lee A., Xiaoping Wang, I. A. Muiznieks, G. Ekuan, M.Ruszaj, R. Cortellucci, D. Domroes, G. Karscig, T. Newman, R. S. Crampton, R.A. Hashmonay, M. G. Yost, P. E. Heilman, J.Duffy, M. P. Gordon, and S. E. Strand. 1999. Remediation of trichloroethylene in an artificial aquifer with trees: a controlled field study. *Environmental Science & Technology*, Vol 33(13): 2257-2265.

Rock, S. and P. Sayre. 1999. Phytoremediation of Hazardous Wastes: Potential Regulatory Acceptability. *Environmental Regulation and Permitting*, John Wiley & Sons, Inc., Vol 8(3): 33-42

Sorbet, Martha. 1998. Impact of contaminated litter from a TCE phytoremediation project and major metabolites of TCE on terrestrial isopods and aquatic amphipods. Thesis, Clemson University, SC

Strand, S.E., L. Newman, M. Raszaj, J. Wilmoth, B. Shurtleff, M. Brandt, N. Choe, G. Ekuan, J. Duffy, J.W. Massman, P.E. Heilman and M.P. Gordon. 1995. Phytoremediation of trichloroethylene from polluted aquifers using poplars. In *Proceedings of the International Poplar Symposium: Populus Biology and its Implications for Management and Conservation*, August 20 25, 1995, Seattle, WA. p. 88.

Zsuffa, L. 1976. Vegetative propagation of cottonwood by rooted cuttings. In *Proceedings of the Symposium on Eastern Cottonwood and Related Species* (B.A. Thielges and S.B. Land, Jr., Eds.), September 28 - October 2, 1976, Greenville, MS. pp. 99-108.

## Appendix 1. Sample Release Notification Letter

USDA, APHIS, PPQ (submit on letterhead)  
Biotechnology Evaluations  
Unit 133  
Riverdale Park, MD 20737  
E-mail: biotech@usda.gov  
FAX 301-73410

1. Reference Number: (leave blank for APHIS' use)

2. Applicant Reference Number:

3. Applicant/Responsible party:

Ma's Potatoes, Inc.	Dr. Ida Solanum
1992 Tuberosum Dr.	(315) 789-1011
Tatertown, NY 12345	fax (315) 789-1213

4. Duration of Introduction:

Release: February 21–September 1, 1994

5. Recipient: Potato, *Solanum tuberosum* cultivar Russet Burbank

6. Regulated Article:

a) designation of transformed line: VR67

category: VR

phenotype: PVY resistant

construct: pCP123

genotype:

promoter: enhanced 35S 5' from cauliflower mosaic virus (CaMV)

gene: anti-sense coat protein from PVY, strain O

enhancer: alcohol dehydrogenase (adh) intron 1 from *Zea mays*

terminator: nopaline synthase (nos) 3' from *Agrobacterium tumefaciens* T-DNA

selectable marker:

promoter: 35S 5' from CaMV

gene: phosphinothricin acetyltransferase (bar) from *Streptomyces hygroscopicus*

terminator: nos 3' from *A. tumefaciens* T-DNA

b) designation of transformed line: VR19

category: VR

phenotype: PVY resistant construct: pCP456

genotype:

promoter: 35S 5' from CaMV  
gene: coat protein from PVY, strain O  
terminator: nos 3' from A. tumefaciens T-DNA

selectable marker:

promoter: 35S 5' from CaMV  
gene:  $\beta$ -glucuronidase (uidA) from E. coli  
terminator: 35S 3' from CaMV  
promoter: 35S 5' from CaMV  
gene: neomycin phosphotransferase (nptII) from E. coli Tn5  
terminator: 35S 3' from CaMV

c) designation of transformed line: VR327

category: VR

phenotype: PVY resistant

construct: pCP123 and pCP456

genotype: (see descriptions above)

7. Mode of Transformation: disarmed A. tumefaciens for line VR67; electroporation for line VR19; microprojectile bombardment for line VR327

8. Introduction:

Release:

NUMBER OF STATES/TERRITORIES AND SITES: ID(1), ME(1), WI(1)

Russ Burbank's Farm, Bingham County, ID, 1.5 acres;

Pa's Potato Farm, Hancock County, ME, 1 acre; and

Potato Research Farm, Oneida County, WI, 1 acre

9. Certification: I certify that the regulated article will be introduced in accordance with the eligibility criteria and the performance standards set forth in 7 CFR 340.3. The above information is true to the best of our knowledge. If there are any changes, we will contact APHIS.

Signature \_\_\_\_\_

Date \_\_\_\_\_

Name Typed \_\_\_\_\_