Phytoremediation of Trichloroethylene with Hybrid Poplars

Milton Gordon,[1] Nami Choe,[1] Jim Duffy,[3] Gordon Ekuan,[2] Paul Heilman,[2] Indulis Muiznieks,[2] Marty Ruszaj,[3] B. Brook Shurtleff,[1] Stuart Strand,[1] Jodi Wilmoth,[1] and Lee A. Newman[1]

[1]University of Washington, Department of Biochemistry, Seattle, Washington; [2]Washington State University–Puyallup, Puyallup, Washington; [3]Occidental Chemical Corp., Grand Island, New York

Axenic tumor cultures of poplar cells, clone H11-11, were grown in the presence of [^{14}C]-trichloroethylene (TCE) (uniformly labeled). The cells were capable of metabolizing TCE to produce trichloroethanol, di- and trichloroacetic acid. Some of the carbon from TCE was found in insoluble, nonextractable cell residue, and small amounts were mineralized to [^{14}C]CO_2. Poplar cuttings grown in soil and exposed to TCE produced the same metabolites. In field trials, trees were planted in soil in test cells and exposed to TCE via underground water injection during the growing season. During the growing season, at least 95% of the TCE was removed from the influent water stream in cells containing trees. Mass balance studies conducted in the laboratory indicated that 70 to 90% of the TCE was transpired; however, greenhouse and field study results showed that less than 5% of the total TCE taken up by the plants is transpired. These results show that significant TCE uptake and degradation occur in poplars. Poplars appear to be useful for *in situ* remediation of TCE-contaminated sites under proper conditions. — *Environ Health Perspect* 106(Suppl 4):1001–1004 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-4/1001-1004gordon/abstract.html*

Key words: phytoremediation, trichloroethylene, poplars, hybrid poplars, mineralization of TCE

Trichloroethylene (TCE) is one of the most widespread contaminants in the environment of the United States (*1*). 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 (*2*) has lent urgency to cleanup efforts. The principal methods for remediating 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 (*3*). Other techniques use bacteria to degrade TCE, although this often requires inducers such as toluene or phenol for degradation to occur (*4*).

Recent research investigating the use of plants to remediate environmental pollution is yielding promising results. With some species of plants the major site of TCE degradation appears to be in the rhizosphere, and the actual role of the plant in remediation may be to supply nutrients to the rhizospheric organisms (*5,6*).

We have investigated two clones of poplar (*Populus trichocarpa* × *P. deltoides*, clones H11-11 and 50-189) as a means to remediate TCE pollution. Poplar was chosen for a variety of reasons. Poplar has a very wide geographical distribution and can be grown from southern Alaska into Central America. 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 in culture. Exogenous genes can be incorporated into the poplar genome, thus there is the potential of incorporating genes that will more completely mineralize TCE or other pollutants. The College of Forest resources at the University of Washington and Washington State University at Puyallup have worked with poplars for over 20 years, and has accumulated an enormous amount of background concerning the physiology and genetics of this genus. The absorption surface of roots in a stand of poplars is enormous and can approach 300,000 km/ha (*7*). The water usage under the warm, arid conditions of eastern Washington State is about 140 cm per year in a stand of 5-year-old trees at a density of 1750 trees/ha (*7*).

The investigations discussed in this chapter first utilized axenic cultures of *Populus trichocarpa* × *P. deltoides* H11-11 tumor cells, then small plants treated with water containing TCE, and, finally, a number of experimental cells that mimicked field trials. We also developed a bioreactor that enabled us to account for the distribution of most of the TCE metabolized by small H11-11 rooted cuttings.

Axenic Tumor Cell Experiments

Transforming shoots of H11-11 with *Agrobacterium tumefaciens* A281 produced the axenic H11-11 tumor cells. Tumor cells are used because they can be easily grown on simple minimal medium (Murashige and Skoog basal salt medium) (*8*). The cells were grown on the above medium at various degrees of saturation of TCE with shaking and illumination for 3 or 5 days at 22°C. The vital stain, Trypan blue (Figure 1),

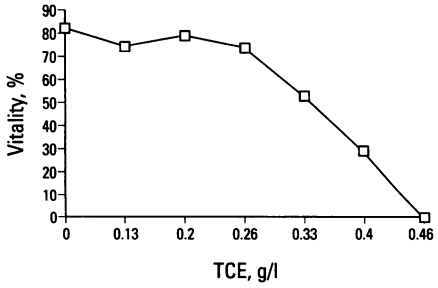

Figure 1. Toxicity of TCE towards H11-11 tumor cells. Viability was determined by Trypan Blue exclusion, as only dead cells are stained by this dye. One hundred cells per point were counted. The error per point is ±2%

This paper is based on a presentation at the Symposium on the Superfund Basic Research Program: A Decade of Improving Health through Multi-Disciplinary Research held 23–26 February 1997 in Chapel Hill, North Carolina. Manuscript received at *EHP* 11 December 1997; accepted 19 May 1998.

This work was generously supported by grants from Occidental Chemical Corporation, the U.S. Environmental Protection Agency 10#R822329-01-3, the National Institute of Environmental Health Sciences Superfund grant 2P42 ES04696-11, and the Department of Energy grant DE-FG07-96-ER20256.

Address correspondence to M. Gordon, University of Washington, Dept. of Biochemistry, Box 357350, Seattle, WA 98195-7350. Telephone: (206) 543-1769. Fax: (206) 685-8279. E-mail: miltong@u.washington.edu

Abbreviations used: DNAPL, dense nonaqueous phase liquids; MTBE, methyl tertiary butyl ether; PVC, polyvinyl chloride; TCE, trichloroethylene.

Table 1. Concentrations of TCE and metabolites found in supernatant and axenic cells exposed to TCE.

	TCE	Chloral hydrate[a]	Trichloroethanol	Dichloroacetic acid	Trichloroacetic acid
Control 1					
Pellet	ND40	ND40	ND40	ND10	ND10
Supernatant	ND40	ND40	ND40	ND10	ND10
Control 2					
Pellet	ND40	ND40	ND40	ND10	ND10
Supernatant	ND40	ND40	ND40	ND10	ND10
Exposed, batch 1					
Pellet	ND40	ND40	60	12000	ND10
Supernatant	2000	ND40	760	1600	ND10
Exposed, batch 2					
Pellet	ND40	ND40	80	39000	130
Supernatant	ND40	ND40	110	3800	30

ND, not detected at stated limit. Concentrations given are nanograms of TCE or metabolites per gram wet weight of sample. Errors are ± 5%. [a]Chloral hydrate is the monohydrate of trichloroacetaldehyde.

Table 2. Comparison of TCE treated and control plants.[a]

Plant parameter	% of control
Height	80–92
Stem weight	70–72
Leaf area	75–78
Number of leaves	50–80
Root weight	52–70
Length of fine roots	22–32

[a]Measurements of plants exposed to TCE (50 mg/l) were compared to control plants grown under the same greenhouse conditions for 8 months.

determined the toxicity of TCE, towards the H11-11 cells. To study the metabolites of TCE, the poplar cells were incubated with 80 mg TCE/l. The suspension was then centrifuged and a sample was extracted at 20°C with 1 N H_2SO_4/10% NaCl, then 3 times with methanol, and then 3 times with methyl tertiary butyl ether (MTBE). In order to test for the presence of di- and trichloroacetic acid, a second batch of cells was extracted with 1 N sodium hydroxide (NaOH), the extracts acidified, back-extracted with MTBE, and the organic acids in the ether extract esterified with diazomethane. The metabolites were then analyzed by gas chromatography with an electron capture detector.

The cell cultures were also tested for the formation of ^{14}C-CO_2 from TCE. The gases evolved during 4- or 10-day incubations with ^{14}C-TCE were trapped in 1 N NaOH and confirmed to be CO_2. The cells and media were separated by centrifugation and the amount of ^{14}C remaining in the media was determined. Insoluble extracted cell residue was combusted and the resulting CO_2 analyzed for radioactivity. The results of these analyses are given in the accompanying table (Table 1). The products from the TCE metabolism, trichloroethanol, di- and trichloroacetic acid, and CO_2, are also among the products produced by the activity of rat and mouse hepatic cytochrome P450 (*9*). These findings suggest that the oxidative metabolism of TCE in poplars is similar to the processes in mammals ("green liver" hypothesis).

Whole Plant Experiments

Cuttings of the two hybrid clones noted above were rooted in PVC (polyvinyl chloride) pipes, 20.5 cm diameter, containing a 30-cm bottom layer of sand with 60 cm soil (Sultan silty clay loam) overlay. The plants were dosed with water alone, or with 50 mg TCE/l added directly to the sand layer through an inner watering tube. A total of 8 g TCE was added over a period of 8 months. During the period of growth, transpiration was assayed by enclosing the leaves in plastic bags and pulling the air through a charcoal filter for 0.5 hr. The results were highly variable and indicated transpiration values from undetectable to about 1.6 µg TCE/leaf/hr, with an average of approximately 1.0 µg TCE/leaf/hr. After 8 months, the plants in PVC pipes were harvested and various morphologic measurements were taken (Table 2). The major difference noted was that the density of the fine roots in the sand layer of TCE-exposed plants was less than that of the controls. The plant tissues were analyzed for metabolites of TCE (Table 3) using the same methods that were used for cell analyses. The nature and levels of the chlorinated metabolites suggest that the TCE is oxidized as it moves from bottom roots to the aerial sections of the plant, especially the leaves. These results, together with the products derived from the axenically cultured poplar cells, strongly argue for a role of the plant in the metabolism of TCE, in addition to the well-known degradation of TCE by soil microorganisms.

Mass Balance Studies

The mass balance of TCE in hybrid poplar trees was determined in a bioreactor. The leaves, stem and roots of the plant were

Table 3. Concentration of TCE and metabolites found in tissues of plants exposed to TCE under greenhouse conditions.

	Tissue	Clone[a]	TCE	Chloral hydrate[b]	Trichloro-ethanol	Dichloro-acetic acid	Trichloro-acetic acid
Control 1	Leaves	A	ND40	ND40	ND40	ND10	ND10
	Stems		ND40	ND40	ND40	ND10	ND10
Control 2	Leaves	B	ND40	ND40	ND40	ND10	25
	Stems		ND40	ND40	ND40	ND10	ND10
Control 3	Leaves	B	ND40	ND40	ND40	ND10	ND10
	Stems		15	ND40	ND40	ND10	ND10
TCE 1	Leaves	A	13	ND40	180	ND10	1100
	Stems		770	ND40	140	ND10	31
TCE 2	Leaves	B	49	ND40	19	180	7200
	Stems		1900	ND40	170	ND10	22
TCE 3	Leaves	B	27	ND40	24	ND10	2100
	Stems		1300	ND40	125	ND10	100
TCE 4		A					
	Roots: Upper		13	ND	200	320	44
	Middle		150	ND	110	25	21
	Lower		640	ND	31	270	44

Concentrations are nanogram of TCE or metabolite per gram wet weight of sample. ND, not detected at stated limit. Errors are ± 5% of stated value. [a]Clone designations: A, *Populus trichocarpa* x *P. deltoides*, hybrid number H11-11; B, *Populus trichocarpa* x *P. deltoides*, hybrid number 50-189. [b]Chloral hydrate is the monohydrate of trichloroacetaldehyde. [c]Control, control plants receiving only water. [d]TCE, plants dosed with 50 ppm TCE.

enclosed in separate sections of the chamber to allow the study of plant uptake, incorporation, transpiration, and rhizosphere degradation without interference from soil volatilization. The major problems we faced were due to the volatility of TCE and its suspected metabolites, and the absorption of these materials by commonly used laboratory materials such as rubber, tygon, and various sealants. The chamber is shown in Figure 2. It is constructed of glass, aluminum foil, Teflon, and inert inorganic materials. Note that the separate sections for roots, stems, and leaves are independently aspirated to prevent volatile transpirates from leaking into other chambers. Controls without plants show only 0.03% leakage of the TCE into the foliage chamber.

Small rooted poplar cuttings (approximately 20 cm tall) were planted in a peat moss/vermiculite mixture in the root section and TCE was added to the root section to a final concentration of 5 mg/l and spiked with 2×10^6 dpm ^{14}C-TCE. After 7 days approximately 0.8% of the added ^{14}C was detected in the transpirate.

Several conclusions can be drawn from these three laboratory experiments. First, TCE can be taken up by the poplars. Second, the plants transpire some of the material, and some is metabolized to known extractable metabolites, as found in Tables 1 and 2. Finally, some nonextractable material is fixed in tissue; the nature of incorporation in tissues is under investigation. We believe that the contribution of each of these pathways towards the destruction of TCE depends upon variables such as concentration of TCE, size of tree, nature of soil microflora, type of soil, temperature, light intensity, relative humidity, wind velocity, etc.

Controlled Field Trials

A controlled field trial was set up in collaboration with the Occidental Chemical Corporation. Double-walled cells, 3.7 m × 6.1 m × 1.5 m deep, were constructed of 60-gauge high-density polyethylene sheeting. The bottoms had about 0.3 m sand overlaid with 1.1 m Sultan silt loam. In order to ensure a uniform flow of input water, a T-shaped input pipe was used at the bottom of the influent pipe and a 1/40 slope was oriented toward the effluent well. Four cells were each planted with fifteen 30-cm plants of poplar clone H11-11. Two of these cells were dosed with water containing 50 mg/l TCE; the other two cells received only water. The fifth cell, which had no vegetation, also received water containing 50 mg/l TCE. Each of the cells received the same volume of liquid; however, variable amounts of liquid were pumped out of the cells on a daily basis to maintain a level of about 20 cm of liquid in the bottom of the cells.

About 9 weeks after injection of TCE into the cells, breakthrough of TCE and related metabolites, particularly *cis*-1,2-dichloroethylene, occurred in the cells that did not contain trees. The occurrence of this compound is indicative of anaerobic bacterial dehalogenation of TCE, perhaps because of ethanol, which was initially used as a solvent for the TCE in the stock solutions. The use of ethanol was discontinued, and the concentrations of these compounds decreased. In cells with trees, very little TCE or metabolites could be detected in the effluent until after leaf drop (Figure 3). Throughout the course of this first year of the experiment, a total of 957 mmol TCE was added to each dosed cell. Of this amount a total of 366 mmol was recovered from the unplanted cell (cell 8, 38% recovery), while 21 and 61 mmol of TCE were recovered from cells 3 and 5, which were planted with hybrid poplars (2 and 6%, respectively). The stems, roots, and leaves of the trees exposed to TCE showed the same products of oxidative metabolism which were seen in the greenhouse studies and tissue culture experiments (Tables 1, 2). The 1-year-old trees (approximately 3.5 m tall) were very effective in removing TCE from the input water. These experiments are being continued.

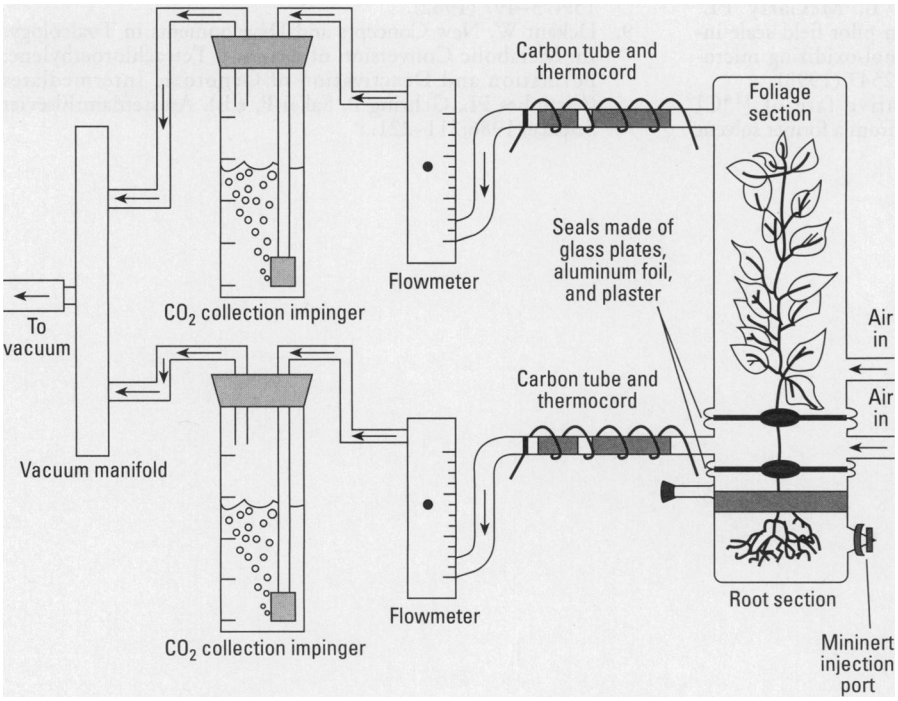

Figure 2. Diagram of bioreactor. The temperature of the carbon tubes was left at 40°C to prevent condensation of water.

Conclusion

The results of the above experiments show that TCE has multiple fates in poplars. Although the mass balance data would have us believe that most of the TCE taken up by the plant is transpired, the field and greenhouse studies show that very little is transpired under field conditions. The compound also can undergo oxidation to chloromethyl derivatives such as trichloroethanol, dichloroacetic acid, trichloroacetic acid, or complete mineralization to CO_2, which are oxidative metabolites found in mammalian livers (*9*). Variable amounts of ^{14}C from TCE are found fixed in insoluble, non-extractable residues. Plant uptake coupled with TCE metabolism serves to remove TCE from groundwater and from the soil environment. The details of these reactions and methods for increasing the mass flow through these pathways are subjects of current investigations. We are also attempting to introduce enzymes into

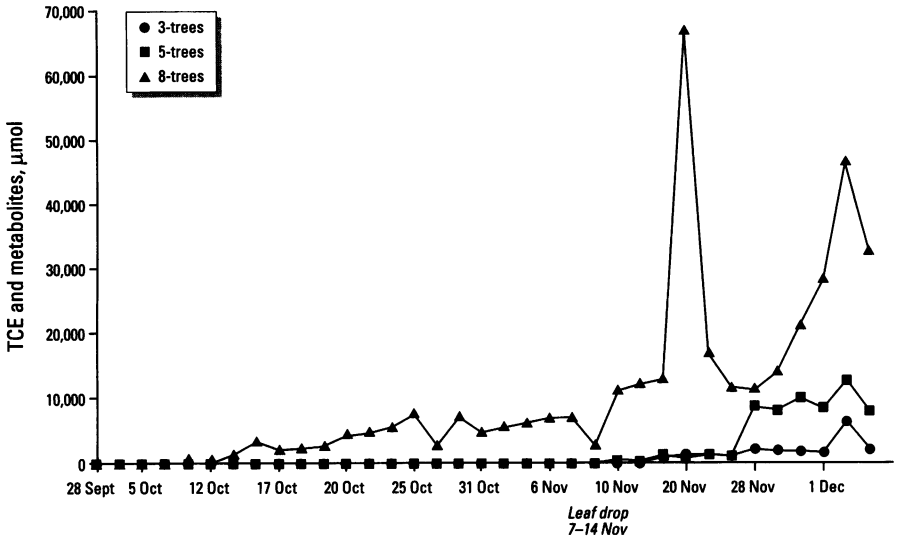

Figure 3. Micromoles of TCE and metabolites recovered in effluent water 28 September through 4 December 1995. Cells 3 and 5 contained trees. Cell 8 is the nonvegetation control. The spikes on 17 November and 1 December are due to flooding during periods of very heavy rainfall.

poplars to increase their tolerance to higher levels of TCE and to increase the fraction of TCE that is mineralized to CO_2.

These experiments show that roots of actively metabolizing poplars are able to intercept a plume of TCE-contaminated water and metabolize most of the TCE taken up. The system shows promise where there is sufficient space to plant trees and when the roots can reach the contaminated region.

NOTE ADDED IN PROOF. As a result of 2 more years of operation of the experimental field trial, we have found that approximately 99% of the TCE is removed from influent water. There is virtually no detectable transpiration of TCE on the part of the more mature trees, which are now 40 ft tall. An increase in chloride ion, derived from TCE dechlorination, appears in the soil surrounding the roots of the trees.

REFERENCES AND NOTES

1. Westrick JJ, Mello JW, Thomas RF. The groundwater supply survey. J Am Water Works Assoc 5:52–59 (1984).
2. National Cancer Institute. Carcinogenesis Bioassay of Trichloroethylene. NIH Publ no 76-01-6. Bethesda, MD:National Institutes of Health, 1976.
3. Travis CC, Doty CB. Can contaminated aquifers at superfund sites be remediated? Environ Sci and Technol 24:1464–1466 (1990).
4. Hopkins D, Munakan J, Semprini L, McCarty PL. Trichloroethylene concentration effects on pilot field-scale in-situ groundwater bioremediation by phenol-oxidizing microorganisms. Environ Sci Technol 27:2542–2547 (1993).
5. Anderson TA, Walton BT. Comparative fate of [^{14}C] trichloroethylene in the root zone of plants from a former solvent disposal site. Environ Toxicol and Chem 14(12):2041–2047 (1995).
6. Anderson TA, Guthrie EA, Walton BT. Bioremediation in the rhizosphere: plant roots and associated microbes clean contaminated soil. Environ Sci and Technol 27(13):2630–2636 (1993).
7. Paul Heilman. Personal communication.
8. Murashige T, Skoog F. A revised medium for rapid growth and bio-essays with tobacco tissue culture. Physiol Plant 15:473–497 (1962).
9. Dekant W. New Concepts and Developments in Toxicology. In: Metabolic Conversion of Tri- and Tetrachloroethylene: Formation and Deactivation of Genotoxic Intermediates (Chambes PL, Gehring P, Sakai F, eds). Amsterdam:Elsevier Science, 1986;211–221.