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UPTAKE AND TRANSFORMATION OF TRICHLOROETHYLENE BY EDIBLE GARDEN PLANTS

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Abstract—Edible garden plants (carrots, spinach, and tomatoes) were grown to maturity inside continuous air-flow bioreactors, and were regularly irrigated with synthetic groundwater containing a mixture of ^{14}C-labeled and unlabeled TCE. Two dose levels were tested (about 560 μg/L and 140 μg/L). Following TCE exposure for 31 to 106 days, different plant tissues and bioreactor compartments were analyzed for the ^{14}C label. Radiolabel recoveries ranged from about 50% for low-dose reactors to about 70% for high-dose reactors. Most of the recovered ^{14}C label volatilized (74–95%) and was trapped in the Orbo® tubes that filtered the air exiting the reactors. A portion of the recovered label (5–25%) was sorbed to the soil. Although the percentage of the recovered ^{14}C label found in plant material was relatively small (1–2%), the concentration of ^{14}C label in edible plant tissue was higher than in the surrounding soil. On a harvest weight basis, accumulation factors ranged from 2.6 in high-dose tomato reactors to 32 in low-dose spinach reactors. If the radiolabel found by combustion of plants was TCE, the concentrations in edible tissue would range from 152 ppb for high-dosed tomatoes to 580 ppb for high-dosed spinach. However, neither TCE nor its commonly reported transformation products were detected by Purge & Trap GC-MS. Furthermore, the ^{14}C label found in plant tissue could not be extracted into the organic solvent CS_2 or into the inorganic solvent 10 N H_2SO_4. This suggests that TCE was taken up, transformed, and bound to plant tissue. Bound residues are generally believed to have lower toxicological effects than the parent compound.

Key words—trichloroethylene, phytoremediation, vegetative uptake, bound residue

INTRODUCTION

Trichloroethylene (TCE) is one of the most prevalent groundwater contaminants in the United States (Westrick *et al.*, 1984). TCE and other chlorinated aliphatics fall into a class of chemically stable compounds commonly known as "safety solvents". Because they are resistant to combustion and explosion, TCE and similar compounds were widely used as industrial solvents and degreasers for most of the twentieth century. The combination of extensive use and chemical stability has led to widespread contamination of groundwater and soil by this ubiquitous and recalcitrant pollutant.

TCE is cytotoxic to the human liver; necrosis and fatty liver are primary signs of chronic TCE exposure (Plaa, 1986). Although other chronic effects are not well understood, the National Cancer Center has found that TCE induces hepatocellular carcinomas in mice (Williams and Weisburg, 1986). In addition, vinyl chloride, an intermediate product in the reductive dechlorination of TCE, is a proven human carcinogen (Creech and Johnson, 1974). Consequently, regulatory agencies have set stringent standards or guidelines for TCE in drinking water, such as 5 ppb by USEPA, 30 ppb by WHO, and 50 ppb by Environment Canada. While TCE ingestion through drinking water is a common route of exposure to humans, there are other exposure pathways that deserve consideration. One exposure pathway that has received limited scientific attention is the human ingestion of TCE contaminated plants. This pathway may be important when TCE plumes move off-property and individuals use the contaminated groundwater to irrigate their gardens.

The uptake, transformation, and fate of TCE in vegetation are only partially understood, which precludes a comprehensive health risk assessment of contaminated sites. A recent study by Anderson and Walton (1995) indicated that 21% of the ^{14}C activity added as TCE to an enclosed bioreactor was assimilated by the soybean species *Glycine max*. The uptake of ^{14}C was closely related to the amount of water taken up by *Glycine max* and by other plant species tested. Potential transformation of the TCE within the plants, however, was not investigated.

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TCE transformations in eukaryotic systems have been investigated for several decades (for review of early work, see Bonse and Henschler, 1976). The enzyme cytochrome P-450 has monooxygenase activity, and was implicated in the oxidation of TCE to cytotoxic compounds in mammals and yeasts (Ensley, 1991). Another pathway, the reductive coupling of TCE to glutathione, results in mutagenic compounds in mammalian tissue (Henschler, 1990). Both cytochrome P-450 (Nelson *et al.*, 1988) and glutathione (Hopkins, 1995) are found in plants. Therefore, the transformation of TCE within plants is plausible, and such transformations might bear similarities to the better understood mammalian metabolic pathways.

Microbial transformations of TCE have also been extensively investigated (Vogel *et al.*, 1987). These transformations are relevant to the exposure pathway under consideration because plants may assimilate microbial TCE metabolites that are produced in or that diffuse to the rhizosphere. Some metabolites could be as harmful or more harmful to humans than TCE itself. Under anaerobic conditions, TCE can be reductively dechlorinated to dichloroethylene (DCE) and vinyl chloride, a potent carcinogen (Vogel and McCarty, 1985). Under aerobic conditions, TCE can be cooxidized via monooxygenase activity employing ammonia (Arciero *et al.*, 1989), isoprene (Ewers *et al.*, 1990), methane (Fox *et al.*, 1990), propane (Wackett *et al.*, 1989), or toluene (Folsom *et al.*, 1990) as the primary substrate. Similar to oxidation by P-450, these aerobic transformations result in the formation of a highly reactive epoxide which could be further transformed to cytotoxic products such as dichloroacetic acid, triochloroethanol, and trichloroacetic acid (Henschler, 1990; Vogel *et al.*, 1987). Although the rhizosphere is predominantly aerobic, anaerobic niches are not uncommon in aerobic soil (Hutchinson and Mosier, 1979). Therefore, both aerobic and anaerobic microbial transformations are plausible in soils, and their products merit consideration when examining plant uptake of TCE and its metabolites.

This study addresses the fate of TCE in common garden vegetables irrigated with TCE contaminated water. The primary objective was to determine if garden vegetables could take up TCE, accumulate it, or transform it. Emphasis was placed on determining the concentration of TCE and its potential metabolites within various plant components at harvest time. In so doing, information was obtained concerning the existence or absence of potentially harmful compounds in edible plant material. While this information is of primary value for health risk assessment, it has implications for the potential use of plants as bioindicators of site contamination and for the use of plants in the *in situ* treatment of hazardous wastes (i.e. phytoremediation).

MATERIALS AND METHODS

Plant selection and experimental design

Three types of garden plants were selected for this study. The tomato plant (*Lycopersicum* sp., Epoch) represented an edible fruit. The carrot plant (*Daucus carota sativa* sp, Nante) was an edible root. The spinach plant (*Spinacia oleracea* sp, Bloomsdale) represented an edible leaf. Due to differences in growth rates, each species was planted, grown, and harvested independently of the others. All seeds were planted and allowed to germinate prior to TCE dosing. During this period, the seedlings were watered with TCE-free tap water. Weeds (*Portulaca oleracea*, *Euphorbia supina*, and *Amarantus* sp.) were periodically removed from the soil during this initial phase. To enhance mass balance closures of volatile TCE during the dosing period, all plants were transferred and grown to maturity inside continuous air-flow bioreactors. Tomatoes were placed into the bioreactors 46 days after planting and harvested 169 days after planting. Carrots were placed into the bioreactors after 26 days and harvested after 87 days. Spinach was placed into the bioreactors 19 days and harvested 49 days after planting. Two different concentrations of [^{14}C]TCE were used in this study, 560 μg/L and 140 μg/L. For each plant type, one set was fed the higher concentration and another set was fed the lower concentration. Four replicates were prepared for each treatment set to address variability and reproducibility. Six additional control reactors without plants (three nonsterile and three sterilized soil reactors) were also operated to evaluate the effect that plants had on soil sorption and microbial degradation of TCE. Soil was sterilized by autoclaving twice for 1 h at 121°C and poisoned with mercuric chloride (1 g/kg) prior to insertion into the bioreactors. All controls were treated with the higher TCE concentration.

Bioreactor construction

Each plant was grown in glazed ceramic pots (Bear Pottery) containing 3–4 kg of garden soil. The pots had drainage dishes for excess feedwater, and were enclosed in modified aquarium vessels (Fig. 1). The size of the vessels varied according to plant type. Each tomato bioreactor was 416 L in volume, while a 114-L bioreactor was employed to grow each carrot and spinach plant, and for the soil (control) reactor. Each vessel was attached to an aluminum base plate containing ports to accommodate tubing for watering/dosing, cold water flow into hollow condenser coils, condensate removal, and air flow into and out of the bioreactors. All tubing and connecting ports were constructed of metal and/or Teflon® to minimize sorptive and leaching effects. Hollow copper condensation coils were mounted in the bioreactors to conduct cold water and condense excess evapotranspiration and control humidity levels. A Teflon® feed port was used for the introduction of water with a glass gas-tight 100 mL syringe (SGE, GT-100 mL).

A continuous flow of air, via a negative pressure vacuum pump system, was supplied to all bioreactors to supply CO_2 for photosynthesis. Air entered the bioreactors through inlet ports containing glass wool filters. Opposite the inlet ports, outlet ports carried the air to the trapping system. Evacuated air first passed through a series of two Orbo® tubes in series (Supelco, Orbo Charcoal: 32 Large) containing activated carbon to capture any volatilized [^{14}C]TCE or metabolites. Preliminary tests indicated that employing three Orbo® tubes in series significantly reduced the air flow without improving the trapping efficiency. Orbo® tubes were shown to be greater than 96% efficient in capture tests (data not shown). Air was then bubbled through an NaOH (1 N) trap to capture any $^{14}CO_2$ produced from [^{14}C]TCE. An air stone was used to produce fine bubbling and enhance mass transfer, and the pH was

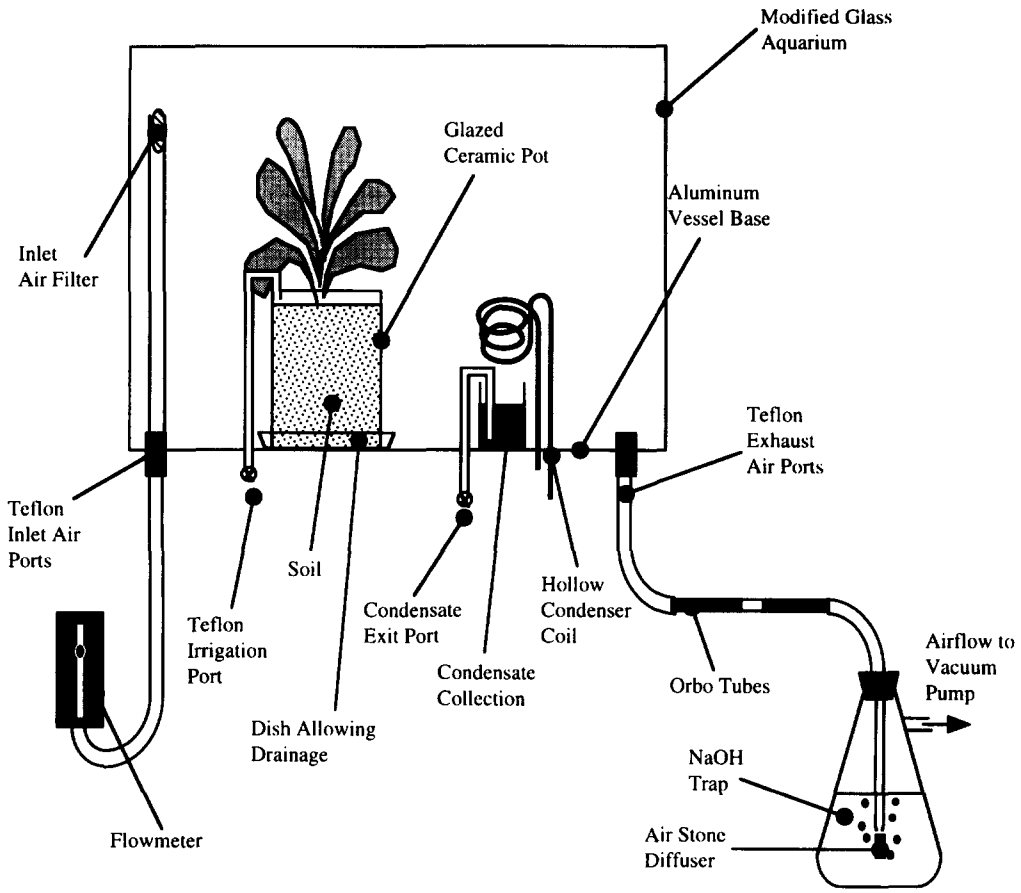

Fig. 1. Schematic representation of experimental bioreactor.

maintained above 1 l to enhance the capture efficiency. In a similar study, NaOH traps proved to be over 95% efficient at trapping $^{14}CO_2$ (Nair *et al.*, 1993). Vacuum pumps (Gast, 91 L/min Vacuum Pump) located downstream of the traps were used to control air flow through the bioreactors. Approximately three to six chamber air exchanges occurred per day.

Soil used for all bioreactors was obtained from a garden overlying a TCE-plume in Sunset, Utah. The soil had a medium/fine texture, and a low organic carbon content (1.5%). Upon arrival, this soil had no detectable levels of TCE ($<5\ \mu g/kg$). All experiments were conducted in a temperature-controlled greenhouse (average temperature of 32°C) equipped with a ventilation system. To mimic natural conditions, plants were grown during the summer using natural sunlight.

Dosage and feeding methodologies

[^{14}C]TCE (Sigma Chemical Company) was stored at 4°C in a stock solution consisting of 90 mL deionized H_2O, 231 mg [^{14}C]TCE, and 25 mL ethanol. The specific activity of the [^{14}C]TCE in the stock solution was 0.433 μCi/mg TCE, while the TCE purity was >99.5%. Immediately prior to distribution to the bioreactors, the stock solution was diluted in a 500 mL gas-tight syringe (Dynatech, Magnum Pressure-Lok 500 mL) with synthetic groundwater designed to resemble the pH, bicarbonate alkalinity, and ionic composition of groundwater at the Utah site. This synthetic groundwater was formulated with KNO_3 (0.2 mM), $CaCl_2$ (2.0 mM), $MgSO_4$ (1.0 mM), and $NaHCO_3$ (6.0 mM), and the pH was adjusted to 6.8 with 5% HCl. The irrigation water was analyzed prior to each feeding event for radiolabel concentration. Results of these liquid scintillation analyses were recorded for mass balance calculations.

TCE-spiked feedwater was fed using the 500-mL gas-tight syringe via a gas-tight, luer-lock system (Hamilton, HV 3-2 Plug Valve). Water was applied until the soil was saturated, as indicated by visible moisture in the drainage dish. Plants were watered on an "as needed" basis, which ranged from 3 days to 1 week depending upon sunlight, humidity, and temperature within the greenhouse.

Condensed humidity, collected in 800 mL beakers placed under the condenser coils, was removed from individual bioreactors as needed using a 100 mL gas-tight syringe (SGE, GT-100 mL). Collected condensate was analyzed by liquid scintillation for dissolved radiolabel.

Orbo® tubes that filtered the evacuated air were changed periodically. For low dose bioreactors, Orbo® tubes were replaced before each new watering event. For high dose bioreactors, Orbo® tubes were replaced before being watered and 1 day after being watered, as a large portion of the TCE had been found to volatilize within 24 h of application. After removal from the bioreactors, Orbo® tubes were capped and stored at 4°C until analysis.

Analytical methods

Liquid scintillation (LS), biological oxidation with liquid scintillation (BO–LS), and gas chromatography with mass spectrometry (GC–MS) were the methods used to analyze the fate of the [^{14}C]TCE in the various system compartments.

LS analysis was performed on a Beckman LS6000IC liquid scintillation counter. For feedwater, condensate, and

NaOH trap solutions, a 1 mL aliquot was added directly to 15 mL Scintiverse I Cocktail (Fisher) in a 20 mL scintillation vial. For Orbo® tube analysis, the activated carbon was extracted with 10 mL carbon disulfide for 30 min. A 100 μL aliquot of the CS_2 extract was then added to 15 mL Scintiverse I in a 20 mL scintillation vial. Wipes of plant and bioreactor surfaces were tested by inserting the wiped filter paper directly into 15 mL Scintiverse I cocktail. All samples were then counted twice in the scintillation counter, until a coefficient of variation ≤0.5% was obtained or a 15 min maximum time period elapsed. For each type of sample, blank standards and nonlabeled plant tissue were counted to determine background levels for data calculations. The limit of quantification by BO–LS was dependent on sample size, and it was about 2 nCi/kg (i.e. 5 μg TCE equivalents/kg) for 2 g samples.

BO–LS of plant and soil samples was accomplished with an R. J. Harvey OX-600 Biological Oxidizer. Upon harvest, plant samples were immediately flash frozen in liquid nitrogen for at least 1 min to minimize volatilization of analytes before oxidation. No detectable radiolabel was extracted from plant material during N_2 immersion. Plant samples were weighed, and triplicate 2-g samples were inserted into the oxidizer and combusted in pure O_2 at 900°C for 4 min. Six soil core samples were taken from each pot at harvest. Soil samples were immediately weighed and combusted. Effluent CO_2 from the oxidized soil and plant samples was trapped in 15 mL of R. J. Harvey Carbon 14 Cocktail. Ten mL aliquots were then analyzed by LS. The biological oxidizer provided a recovery of ≥98% and a reproducibility of ±0.5%.

GC–MS was performed at the University of Iowa Hygienic Laboratory on a HP 5890 GC and HP 5972 MS using EPA method 8260A. Selected soil, plant tissue, and Orbo® tube samples were sent directly from the greenhouse to the Hygienic Laboratory for subsequent analysis. Plant tissue samples were flash frozen in liquid nitrogen, and Orbo® tube samples were extracted into carbon disulfide as previously described before transport. Volatile organics were extracted from plant tissue and soil prior to GC–MS analysis using a Tekmar 3000 Purge & Trap, as described in EPA Method 8260A. The limits of quantification by GC–MS analysis for TCE, DCE, and vinyl chloride were all 5 μg/kg. Additional extractions of polar and nonpolar metabolites were obtained from selected plant tissue (5 g) using 20 mL of 10 N sulfuric acid or 20 mL of carbon disulfide, respectively, prior to radiolabel analysis by BO–LS.

RESULTS AND DISCUSSION

Radiolabel results

At harvest, all bioreactor compartments were analyzed for ^{14}C label to investigate the fate of the label and establish mass balances. These compartments included plant material, Orbo® tubes, soil, condensate water, NaOH traps, and bioreactor material surfaces (condenser coils, bioreactor walls). No radiolabel was detected in the condensate water (<11 nCi/L, or 26 μg/L as TCE), NaOH traps (<11 nCi/L), or on the bioreactor material surfaces (<1 pCi/cm²). Radioactivity was detected in Orbo® tubes, soil, and plant material (Table 1).

Radiolabel recoveries ranged from 45 to 50% for low dose reactors, and from 62 to 73% for high dose reactors. In all cases, most of the recovered ^{14}C label volatilized (74–95%) and was found in the Orbo® tubes that filtered the air exiting the reactors. A portion of the recovered label (5–25%) was sorbed to the soil. Plant tissue represented a relatively small potential TCE sink. That is, the mass of the soil (3–4 kg) was two to three orders of magnitude larger than the mass of the plants (about 7 g for carrots, 4 g for spinach, and 95 g for tomatoes), and only a small percentage of the applied radiolabel was found in plant tissue (1–2%). Considering the volatility of TCE, the continuous air flow design of the bioreactors, and the extended time over which this experiment was run, it is likely that the unrecovered radiolabel leaked from the system. It is highlyunlikely that the missing radiolabel remained in the plant tissue or in the soil after analysis because these samples were totally oxidized. Overall, the mass balances for this experiment compare favorably with published recoveries of less than 50% for similar uptake studies with volatile organic compounds (Anderson and Walton, 1995; McFarlane *et al.*, 1990; Strand *et al.*, 1995).

Microbial TCE mineralization did not play a significant role in the fate of TCE. This notion is supported by the absence of $^{14}CO_2$ in the NaOH traps, and the similar radiolabel recoveries in both soil and Orbo® tubes in control reactors (without plants) containing either nonsterilized or sterile soil (Table 1). This is consistent with the recalcitrance of TCE to microbial degradation in aerobic systems lacking adequate primary substrates such as methane, toluene, or phenol (Vogel *et al.*, 1987).

A higher proportion of radiolabel was found sorbed to soil in reactors containing spinach or carrot plants compared to control reactors without plants (Table 1). For example, high-dose spinach bioreactors exposed to TCE for the same length of time at the same concentration had 10.6 ± 3.1% of the

System	Total radiolabel (μCi)	Recovery in air (%)	Recovery in soil (%)	Recovery in plants (%)	Total mass balance closure (%)
Carrots, high	0.58	63.1 ± 7.4	9.1 ± 2.1	0.6 ± 0.4	73%
Carrots, low	0.15	44.7 ± 10.0	4.4 ± 1.6	0.7 ± 0.4	50%
Spinach, high	0.29	50.4 ± 6.6	10.6 ± 3.1	1.1 ± 0.6	62%
Spinach, low	0.08	34.3 ± 8.8	11.6 ± 3.4	0.6 ± 0.6	46%
Tomatoes, high	2.14	62.1 ± 1.3	4.6 ± 1.0	0.5 ± 0.1	67%
Tomatoes, low	0.52	42.2 ± 2.3	2.8 ± 0.4	0.5 ± 0.2	45%
Non-sterile soil	0.24	57.2 ± 4.9	5.4 ± 1.3	NA	62%
Sterilized soil	0.32	60.0 ± 5.3	3.3 ± 1.3	NA	63%

NA, not applicable. Recoveries are reported as the mean ± one standard deviation of at least three samples. Specific activity of the added radiolabel was 0.433 μCi/mg TCE.

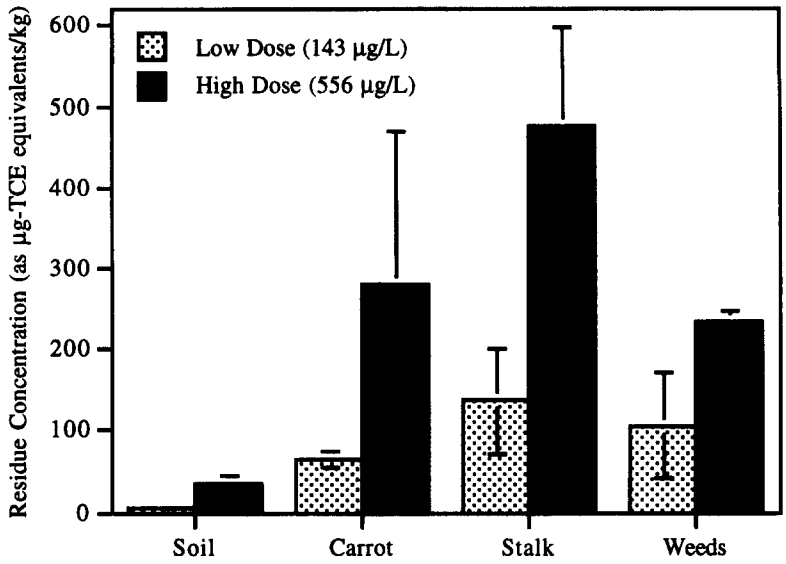

Fig. 2. ^{14}C residue concentration in carrot plants. To put into perspective the mass of TCE taken up by the plant, radiolabel concentration is expressed on a trichloroethylene weight-equivalent basis. Error bars depict ± one standard deviation from the mean of at least three plant bioreactors.

added radiolabel sorbed to the soil, whereas control bioreactors had 4.4 ± 1.3% sorbed to the soil. This result can be explained, in part, by the exudation of organics through plant roots and turnover of organic root biomass, which would increase the organic content and sorption capacity of the soil.

Radiolabel concentrations measured in various components of carrot, spinach, and tomato plants are depicted in Figs 2, 3, and 4, respectively. To put into perspective the amount of TCE assimilated, radiolabel concentrations are expressed on a TCE weight-equivalent basis. Nevertheless, as shown by GC–MS analyses discussed below, this radiolabel is not associated with original TCE; rather, it is likely associated with bound transformation product residue. Radiolabel concentrations in carrot stalks and edible roots were not statistically different at the 95% significance level (Fig. 2). In contrast, radiolabel concentration in high-dosed spinach roots were significantly higher than the concentration in the leaves (Fig. 3). This trend was also observed in tomato plants (Fig. 4), though the concentration in the roots was not significantly higher than that in the leaves.

Examination of Figs 2–4 reveals a relationship between feedwater radiolabel concentration and plant radiolabel concentration. High-dose reactors received approximately four times more TCE than low-dose reactors, and the corresponding ^{14}C-label concentrations in plant material were also about four times higher (within statistical error). Another similarity was the assimilation of radiolabel in all plant tissues at concentrations higher than the surrounding soil. The ratios of radiolabel recovery from plant material (roots, leaves, stems, and fruit) to radiolabel recovery from soil for various plant types and dosages were calculated based on the harvest (wet) weights of soil and plants (Table 2). For reference, Table 2 also depicts the concentration of ^{14}C label (expressed as µg-TCE equivalents/kg-soil) found in the corresponding soils. The accumulation factor was much higher for spinach than for tomatoes or carrots, despite the shorter exposure time (31 days for spinach versus 106 days for tomatoes and 61 days for carrots). This could be due to differences in metabolic rate or translocation biochemistry, but further research would be required to determine the specific cause. The accumulation factors summarized in Table 2 are comparable to reported accumulation factors of chlorinated compounds taken up by barley plants (Kloskowski *et al.*, 1981).

GC–MS results

GC–MS analyses were run on selected spinach, tomato, soil, and Orbo® tube samples. Using a purge and trap extraction technique, samples were analyzed for TCE and its commonly reported metabolites, *cis*-1,2-dichloroethylene, vinyl chloride, trichloroacetic acid, dichloroacetic acid, and 2,2,2-trichloroethanol. The presence of TCE in the soil and Orbo® tube samples was confirmed by GC–MS analysis, but no metabolites were detected. This further supports the notion that microbial transformation did not play a significant role in the fate of TCE in this experiment. No TCE or potential metabolites (≤ 5 µg/L) were detected in the spinach or tomato samples. As described above, however, a relatively high radiolabel concentration was found in these same samples by combustion of plant material. The radiolabel could not be extracted from tomato stems or leaves with either carbon disulfide or 10 N sulfuric acid, which suggests that TCE and/or transformation

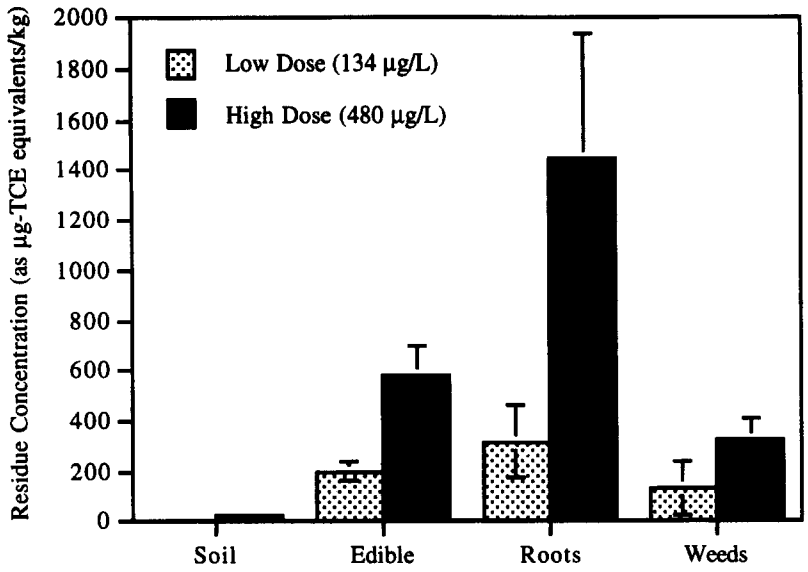

Fig. 3. ^{14}C residue concentration in spinach plants. Error bars depict ± one standard deviation from the mean of at least three plant bioreactors.

products were covalently bound to the plant tissue. This irreversible covalent bonding could be analogous to the formation of bound pesticide residues reported by Klein and Scheunert (1982).

Fate of TCE

The hypothesis that TCE was taken up, transformed, and bound to plant material is supported by both experimental and theoretical considerations. Experimental evidence includes the detection of radiolabel by combustion of plant tissue, and the lack of detection of free TCE or its commonly-reported metabolites in the same tissues by an equivalently sensitive purge and trap GC–MS analysis. In other words, the radiolabel detected was not associated with volatile organics such as TCE or vinyl chloride. In addition, most of the radiolabel (>90%) remained in tomato stems after extraction with stringent solvents. Strand *et al.* (1995) recently found that while TCE is predominantly transformed to aerobic metabolites in poplar trees, a substantial portion binds irreversibly to plant tissues. In addition, ongoing TCE phytoremediation research with freshwater plants at the USEPA laboratory in Athens, GA has found that although significant radiolabel was

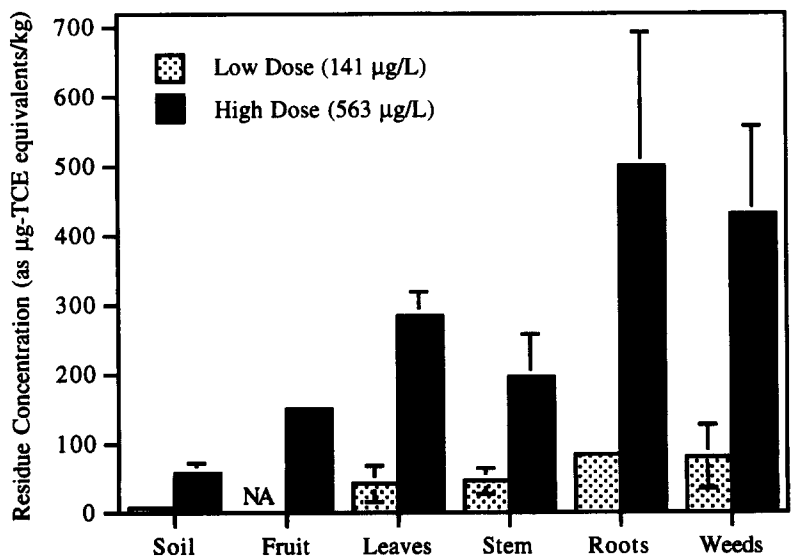

Fig. 4. ^{14}C residue concentration in tomato plants. Low-dose plants did not fruit, and only one out of four high-dose plants fruited within the reactors. Error bars depict ± one standard deviation from the mean of at least three plant bioreactors.

Table 2. Radiolabel accumulation factors (μg/kg in plant/μg/kg in soil)

	Low-dose tomatoes	High-dose tomatoes	Low-dose carrots	High-dose carrots	Low-dose spinach	High-dose spinach
Roots	10	8	14	8	53	67
Leaf	5	5	—	—	38	30
Stem	6	3	30	14	—	—
Fruit	—	2	—	—	—	—
Soil residue concentration (μg/kg)[a]	8 ± 1	58 ± 16	4 ± 2	36 ± 10	6 ± 3	21 ± 6

[a]Soil residue concentration is based on radiolabel data and expressed as μg trichloroethylene/kg soil. Error is expressed as ± one standard deviation from the mean of at least three samples. Accumulation factors are based upon harvest (wet) weights of soil and plants.

detected by combustion of [^{14}C]TCE-exposed plants, no readily extractable TCE or metabolites were detected (Nzengung *et al.*, 1995).

Plants are known to possess cytochrome P-450, which has monooxygenase activity analogous to that responsible for TCE epoxidation in mammalian liver (Nelson *et al.*, 1993). The resulting TCE epoxide is highly reactive (Vogel *et al.*, 1987). When an epoxide bond is broken, a carbocation intermediate can be formed. This electrophilic carbon atom would be highly susceptible to nucleophilic attack by nitrogen atoms in plant proteins, oxygen atoms in polysaccharides, or carbon double bonds in unsaturated phospholipids, for example (Fig. 5a). TCE could also be nucleophilically attacked by sulfur atoms in glutathione (Fig. 5b). Such nucleophilic attacks would initiate a cascade of electrophile-yielding reactions that ultimately end with the products being covalently bound to plant tissue. While these theoretical mechanisms have not been investigated for TCE in plants, such transformations are known to covalently bond TCE to mammalian (Van Duren and Banerjee, 1976) and prokaryotic (Wackett and Householder, 1989) tissue, and are plausible in plants. Indeed, conjugation of other xenobiotics (e.g. atrazine) with glutathione has been observed in plants (Lamoreaux *et al.*, 1970).

The health effects of TCE bound residue in plants are not known; however, several pesticide bound residues are considered to be of little toxicological concern to monogastric animals and may not present any problems (Khan and Dupont, 1986). Although the comparison of TCE residue and innocuous pesticide residue leads to optimistic expectations of negligible health risks, further studies of TCE translocation biochemistry would be necessary to confirm this notion.

The fate and potential transformations of TCE in the experimental system described in this paper are in keeping with the current thought concerning the fate

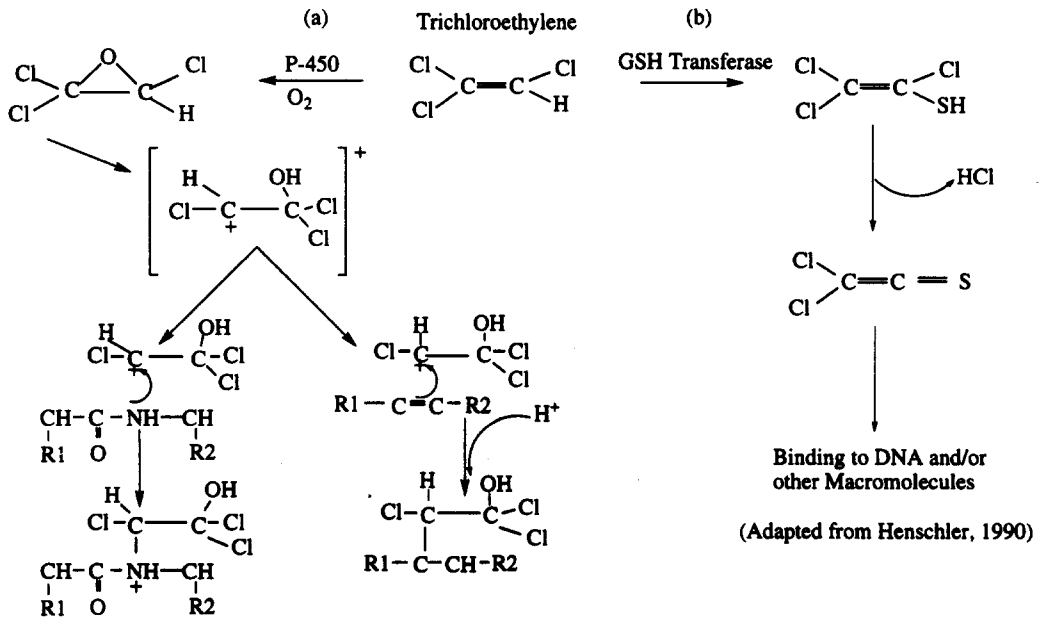

Fig. 5. Theoretical binding mechanisms for TCE. Pathway (a) shows TCE oxidation by cytochrome P-450 to an epoxide, which then goes on to bind with plant matrix proteins (left) and unsaturated carbon chains (right). Pathway (b) shows TCE reductively coupled with glutathione, and the resulting thioacyl chloride goes on to bind with other macromolecules such as DNA.

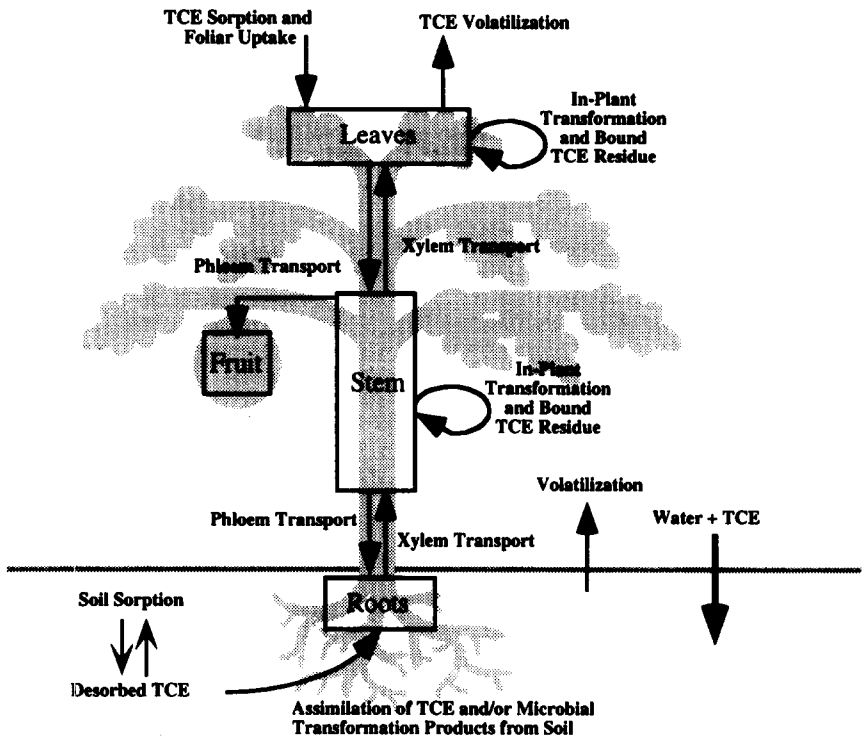

Fig. 6. Potential fate of TCE added to soil surrounding a tomato plant.

of contaminants in other vegetative systems (Schnoor *et al.*, 1995). Potential mechanisms for the uptake and transformations of TCE in a tomato plant are summarized in **Fig. 6.** In this figure the potential transformation of TCE in the rhizosphere is shown, as well as the uptake of TCE or its metabolites into the roots, xylem transfer of the compounds to the leaves, volatilization from the leaves, foliar uptake, phloem transfer to the fruit, and bound metabolic residue formation throughout the plant. While quantification of these potential mechanisms would be necessary for the rational development of phytoremediation technologies, the observed sequestration of TCE into bound residue suggests the potential for plants to enhance the cleanup of TCE contaminated sites.

CONCLUSIONS

The results of the experiments described above led to the following conclusions:

1) TCE dissolved into water and fed to plants in enclosed bioreactors was translocated into the plants.
2) The assimilated radiolabel, applied as [^{14}C]TCE, was irreversibly bound to plant tissue.
3) Most of the added radiolabel volatilized from the soil during plant irrigation and was recovered in Orbo® tubes that sorbed radiolabeled organics from the air.
4) The concentration of radiolabel detected in plant tissues was dependent upon the concentration of [^{14}C]TCE in the feedwater, the plant species, and in some cases the type of tissue (i.e. roots, stem, leaf).
5) Although the ^{14}C label was easily traced in these experiments, the exact chemistry of the end products was not determined. Additional research into the properties and chemical structure of bound TCE residue would provide important information concerning the mechanism of TCE breakdown in plants.
6) Microbial mineralization was less than detectable in these experiments.
7) Because of the potential for in-plant contaminant transformation, plants may not serve as accurate bioindicators of organic chemical contaminants in the environment.
8) Due to the observed sequestration and potential transformation to innocuous compounds, investigating the use of deep-rooted trees (e.g. poplars) for phytoremediation of shallow, TCE-contaminated groundwater might be a fruitful avenue of research.

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