
EVALUATION OF TOXICITY OF TRICHLOROETHYLENE FOR PLANTS

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ABSTRACT Trichloroethylene (TCE) exposure of several species of plants was studied. Although earlier studies indicated that the root systems of plants could tolerate an aqueous phase concentration of 1 mM for a day, toxicity to whole plants was observed at somewhat lower levels in the gas phase in this study. The tested species included pumpkin (*Cucurbita maxima*), tomato (*Lycopersicon esculentum*), sweet potato (*Dioscoria batata*), tobacco (*Nicotiana tabacum*), soybean (*Glycine max* L. Merr), and alfalfa (*Medicago sativa*). Damage was observable as wilting or failure of the gravitropic response of shoots at levels above about 0.2 mM in the gas phase, which corresponds to 0.5 mM in the aqueous phase. Plants were usually killed quickly at gas phase concentrations above 0.4 mM.

KEYWORDS: trichloroethylene, toxicity, gas phase

INTRODUCTION

Plants have the potential to remediate a number of toxic compounds but there is very little literature on their tolerance to high levels of common solvents. If plants are to be used for clean-up of compounds such as trichloroethylene (TCE), they must survive in its presence. Relatively large plants (50 g or more fresh weight) of alfalfa (*Medicago sativa*), hybrid poplars (*Populus deltoides x nigra*), and saltcedars (*Tamarix parviflora*) can tolerate exposure of their root systems to a 1 mM aqueous concentration of TCE, other halogenated solvents, and some gasoline constituents [1] for at least one day. Other common gasoline constituents including ethylbenzene, xylene, and trimethylbenzene were toxic to some of the above species of plants at 1 mM in the aqueous phase. In those experiments, survival was observed at a later time while transpiration rate was monitored continuously over a one-day exposure time.

The present studies were done to measure the damage induced by exposure of entire plants to a range of concentrations of a representative contaminant, TCE. Several species were examined under a range of conditions. The general method described here allows for rapid screening of plant tolerance.

METHODS

General culture conditions

All experiments were done under continuous fluorescent light at a room temperature of 23 +/- 2°C unless noted otherwise. Treatment containers were 1.2 liter bottles having metal lids with a sealing gasket around the outside edge and a vaccine stopper septum in the middle.

Toxicity evaluation with soybean

Seeds of cultivar KS 4390 soybean (*Glycine max* L. Merr) were surface sterilized by soaking in a 1:10 dilution of household bleach for 10 min. After five rinses with

sterile water the seeds were sown in pots with a 1:1 perlite:vermiculite mixture watered with Hoagland's solution. After two weeks under cool white fluorescent lights (14 h photoperiod) at room temperature of 21-25°C, the seedlings were transferred to treatment bottles. Each plant received 10 ml sterile water and then TCE to give levels of 0.1, 0.5, 1, and 2 mM in the jar. After 48 hours the relative electrolyte leakage was determined by relative conductivity of water extracts of leaf discs before and after freezing [2].

Toxicity evaluation with tobacco

Seeds of tobacco (*Nicotiana tabacum*) were surface sterilized and then germinated in 125 ml Erlenmeyer flasks on 0.8% agar-solidified MS medium [3]. After four weeks under cool white fluorescent lights (14 h photoperiod) at a room temperature of 21-25°C, plants were transferred to sterilized treatment bottles and grown for an additional two weeks. Then TCE was injected to give final concentrations of 15, 30, or 100 µM. Equivalent amounts of TCE were added to jars containing the agar medium but without plants. Samples were taken for gas chromatography at two and seven days of incubation.

Toxicity evaluation with sweet potato

Cuttings of sweet potato (*Dioscoria batata*) having three to four leaves (1-2 g wt) were rooted in distilled water. After a week under continuous fluorescent lights at 21-25°C they were transferred to treatment bottles. Each plant got 1 ml water and an amount of TCE to give a final concentration of up to 0.9 mM. Bottles were kept upright and observed visually at daily intervals. After a week the surviving plants were retrieved and placed in distilled water. No further deaths occurred.

A second test was done with sweet potato cuttings but with the roots placed into small tubes containing 5 ml water. Levels of TCE ranged up to 0.67 mM. The bottles were placed nearly horizontal for this experiment so that the gravitropic response could be observed. The plants were exposed for two days, then the bottles were opened and ventilated for 10 minutes after which TCE was added again.

Toxicity evaluation with tomato

Tomato (*Lycopersicon esculentum*) cultivar VF-1 was grown from seed. Plants at the four to six leaf stage (~1 g wt) were transferred to distilled water for a few days adaptation prior to exposure to TCE. Single plants were placed in treatment bottles. Each bottle received 2 ml water and TCE to give concentrations up to 0.68 mM. Bottles were kept upright.

A second experiment was done using levels of TCE in 0.09 mM increments up to 0.45 mM while each jar received 5 ml water. Two plants were tested at the lowest and highest levels. The bottles were placed nearly horizontal so that the gravitropic response could be observed.

A third experiment was done in which the root systems of small rooted cuttings were placed in small test tubes containing 5 ml water. TCE was added to the bottles to give levels up to 0.68 mM. The bottles were placed near horizontal.

Toxicity test with alfalfa

Small well-nodulated plants of alfalfa (*Medicago sativa L.*) were obtained from a field stand initiated in the fall of 1995. Their tap roots were trimmed to about 12.5 cm and the tops were trimmed to 5 cm. Pairs of plants were placed in treatment bottles with 5 ml water. TCE was added to levels of 0.09

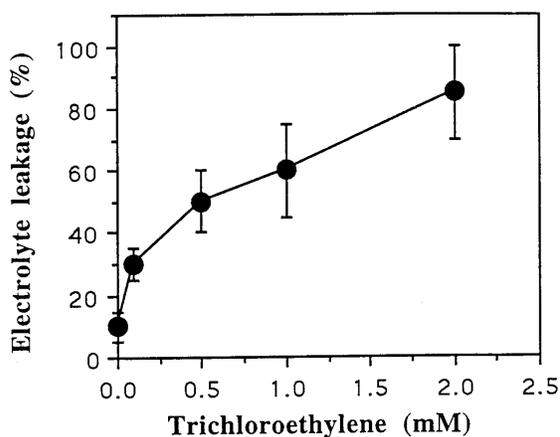


FIGURE 1. RELATIVE ELECTROLYTE LEAKAGE FROM SOYBEAN SEEDLINGS EXPOSED TO DIFFERENT CONCENTRATIONS OF TCE.

to 0.45 mM, in 0.09 mM increments. Bottles were kept upright.

Toxicity evaluation with pumpkin

Seedlings of pumpkin (*Cucurbita maxima*) were obtained at the one true leaf stage. They had been grown in a mix of perlite:vermiculite 1:1 with Hoagland's solution, under continuous fluorescent light in the laboratory at 25°C. Individual seedlings were placed in treatment bottles with 5 ml water. TCE was added to give concentrations from 0.09 to 0.45 mM. The bottles were placed nearly horizontal so that the gravitropic response could be observed.

Two additional experiments were done using the same range of TCE concentrations, but placing the root system of the plant into a test tube with 5 ml water. The bottles were placed near horizontal.

RESULTS AND DISCUSSION

Soybean

Aseptically grown soybean seedlings were exposed to levels of TCE up to 2 mM in the gas phase surrounding the entire plant.

Severe damage was evident at 0.5 mM as shown by electrolyte leakage (Figure 1). Plants were killed at higher concentrations.

Tobacco

Plants, regenerated from callus, and growing aseptically on agar medium, were tested with levels of TCE ranging up to 100 µM. No visual evidence of damage was observed and the plants remained healthy for a week. There was no significant decrease of TCE levels in the gas phase between two and seven days, except at the lowest added TCE level, suggesting that the plants did not rapidly metabolize TCE. No dichloroethylene or other more reduced products were observed in the jars. However, observable metabolism is highly dependent on tissue mass/volume ratios unless isotopic tracers are used. With intact plants in sealed containers, depletion of CO₂ becomes a problem unless there is a regulated source of CO₂ or the containers that are used have a large gas volume per amount of photosynthetic tissue.

Tomato

When seedlings were exposed to TCE, in the first experiment there was little observable effect at 0.045 or 0.09 mM. At 0.18 mM the plant survived but did not show a normal gravitropic response. At 0.27 or 0.45 mM the plants were wilted after one day and died by the second day. At 0.68 mM the plant was dead within one day. The second experiment, testing levels up to 0.45 mM, showed killing of all plants exposed to 0.18 mM or above after 2 days. Exposure of the whole plant to TCE but with root systems in tubes of water (experiment #3) gave somewhat higher tolerance in a one day exposure. At or below 0.18 mM the gravitropic response was normal; with 0.27-0.45 mM leaves were significantly damaged; at 0.68 mM the plant died. Slow dissolution

of TCE in the water may have decreased the damage to root systems.

Sweet potato

When cuttings were exposed to TCE in the first experiment, the plant at 0.9 mM was obviously dead and browned within one day. At 0.27 mM the plant appeared to wilt and roots not in water desiccated within two days. There was progressive yellowing over time, even at 0.09 mM. When removed to water after five days all plants exposed to 0.27 mM or less survived. In a second experiment, plants survived a two-day exposure at levels up to 0.45 mM but not at 0.68 mM. The gravitropic response was normal at levels up to 0.36 mM. Re-exposure of the same plants after replacing the air in the bottle resulted in extensive damage at concentrations of 0.27 mM and above.

Alfalfa

When field-grown seedlings were exposed to TCE, there was rapid (within 1-2 days) photobleaching of leaves at all levels at or above 0.18 mM. Plants at 0.09 mM appeared relatively normal for 5 days.

Pumpkin

When seedlings were exposed to TCE in the first experiment, there appeared to be relatively little damage up to 0.36 mM. However, when the plants were removed from the containers and placed in water, those that had been exposed to higher levels of TCE were severely damaged, presumably due to desiccation upon removal from the water-saturated atmosphere. The gravitropic response of the plants when laid horizontal resulted in the root systems being at least partially lifted out of the small amount of water present in the bottle. In a second experiment with the plant roots placed in a

tube of water, so that only the stem could be lifted by the gravitropic response, there was not obvious damage at TCE levels up to 0.45 mM. However, in both experiments there appeared to be loss of the TCE from the bottles, at about 25% per day. A third experiment showed leaf damage at 0.22 mM and plant death at 0.45 mM.

DISCUSSION

Several species of plants were more susceptible to TCE in the gas phase surrounding their leaves than expected on the basis of their apparent root tolerance in short term experiments. Those earlier experiments usually began with a 1 mM aqueous phase concentration which corresponds to a 0.38 mM gas phase concentration at room temperature. Toxicity was observed with some species in some experiments at or below this concentration. The highest TCE concentration used here (0.9 mM in the gas phase) is about 1/5 of the water-saturating amount and is within the range of concentrations that may be found near a source of nonaqueous TCE in ground water. We have observed similar concentrations when water is flowed over TCE-saturated sand in a plant growth chamber.

Under common atmospheric conditions leaves are seldom exposed to such high concentrations because even if high concentrations were transported from the roots, they would rapidly be diluted by mixing with the air surrounding the leaves. In unsaturated soils two factors promote dilution [4, 5]. Air diffuses down to mix with TCE moving upward through the soil and, more importantly, TCE rapidly diffuses down a concentration gradient to the unconfined atmosphere where the TCE concentration is effectively zero. Thus, roots in an unsaturated zone are in contact with a

lower TCE concentration than those in a saturated zone. However, long term exposure within roots in the saturated zone, or in stems, which may not be so accessible to air, could show toxicity similar to that observed in closed containers. The plant leaf is triphasic with lipid, water, and gas phases. The partitioning of TCE between those three phases is a complex one unless it is at equilibrium. In roots and stems presumably there is little gas phase so that the $\log K_{ow}$ is the relevant variable for partitioning of TCE into the membranes.

We do not know the mechanism by which TCE proves toxic but the increase in electrolyte leakage that was observed in plants exposed to TCE suggests membrane leakiness. The failure of the gravitropic response, which may indicate a loss of turgor pressure or cell enlargement under the influence of auxin, suggests that plasma membrane properties are altered by dissolution of TCE within the membrane. The rapid bleaching of alfalfa leaves might indicate interaction with photosynthetic systems as well. Rapid desiccation of plants after removal from the treatment atmosphere may indicate an inability to regulate water loss, or deficiency in water transport. We have not proved that the TCE effect is not mediated by other factors in the closed containers but the survival of control plants and those treated with lower levels of TCE indicates that we are most likely looking at a direct TCE effect.

The susceptibility of different species was rather similar, varying only a few-fold. Thus it should be simple to screen a wide range of solvents with one or a few species and determine the likely extent of toxicity that would be observed under field conditions.

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