Bull. Environ. Contam. Toxicol. (2004) 72:1006–1011

DOI: 10.1007/s00128-004-0343-y

Bulletin of Environmental Contamination and Toxicology

Toxicity of Benzene, Toluene, Ethylbenzene, and Xylene (BTEX) Mixtures to *Sorghum bicolor* and *Cucumis sativus*

Y.-J. An

School of Earth and Environmental Sciences (BK21), Seoul National University, Sillim-dong, Gwanak-gu, Seoul 151-742, Korea

Received: 1 July 2003/Accepted: 3 September 2003

Monocyclic aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX) compounds are major volatile constituents of gasoline and commonly found in gasoline-contaminated soils. High levels of BTEX compounds in soils have often been detected near gasoline stations due to the leakage of gasoline from underground storage tanks. Soil pollution by gasoline has become a significant environmental concern due to its adverse ecological effects. BTEX compounds are more toxic than liquid alkanes, and well-known toxicants to a wide range of terrestrial biota as well as aquatic organisms. They are released into environment as a mixture, and not as a single compound. Although there are previous reports on the single phytotoxicity of benzene and toluene (Hulzebos et al. 1993; Ugrekhelidze et al. 1997), there is little information available on the mixture toxicity of BTEX compounds to plants.

In this study, the mixture toxicity of BTEX compounds to the terrestrial plants, *Sorghum bicolor* (sorghum) and *Cucumis sativus* (cucumber) was evaluated. The aim was to determine the lowest-observed-adverse-effect-concentration (LOAEC) in soil microcosm studies. Plants have been used as bioindicators for toxicity assessment in terrestrial ecosystem (Gorsuch et al. 1991). *Sorghum bicolor* was recommended by the Organization for Economic Cooperation and Development (OECD) and *Cucumis sativus* are recommend by the U. S. Environmental Protection Agency (EPA) and FDA as test species in plant toxicity assessment (Fletcher 1991).

MATERIAL AND METHODS

Benzene (99.5% purity), toluene (>99.5% purity), ethylbenzene (99.0% purity) were purchased from Showa Chemical Co., Ltd in Japan. Xylene (94.8-95.8% purity) was obtained from Duksan Pure Chemical Co., Ltd in Korea. All chemicals are reagent grade and used without further purification. Selected physical and chemical properties of BTEX are listed in Table 1.

Seeds of *Sorghun bicolor* and *Cucumis sativus* were supplied by Hanarum Seed Company (Seoul, Korea). Production year (place) of *Cucumis sativus* and *Sorghun bicolor* were 2000 (Kyungsang-Do), and 2001 (Jeonla-Do), respectively. Seed germination tests using control soils had a greater than 85% germination.

A sandy loam soil (pH 4.3, 0.3 percent organic matter) was collected from Ahn Mountain (Seoul, Korea). Soil was sieved and dried for 24 hours at an oven temperature of 105 °C. Soil texture was measured using a soil texture test kit (Model 1067; LaMotte, Chestertown, MD, USA), by determining sand, silt and clay fraction. Soil pH was measured by shaking air-dried soil in distilled water for one hour and waiting for one hour prior to the pH assay with a digital pH meter (Model 420A; ORION Research Inc., Boston, USA) (Sparks et al. 1996). Percent organic matter content of the test soil was measured using an organic matter soil test kit (Model ST-OR 5020, LaMotte Company, Chestertown, MD, USA) according to the manufacturer's manual.

Table 1. Selected physical and chemical properties of BTEX compounds[a].

Property	Benzene C_6H_6	Toluene $C_6H_5CH_3$	Ethylbenzene $C_2H_5C_6H_5$	Xylenes $C_6H_4(CH_3)_2$
Molecular weight	78.11	92.13	106.2	106.2
Specific gravity (g/cm^3)[b]	0.8765	0.8669	0.8670	0.8802-0.8611
Water solubility (mg/L)	1780	515	152	160-220
Vapor pressure (Pa)	12700	3800	1270	1100-1170
Log K_{ow}[c]	2.13	2.69	3.13	3.15-3.20
Log K_{oc}[d, e]	1.9	2.0	2.5	2.6
% Volume in gasoline[e]	1	5	<1-1.5	8-10

[a] Data from Mackay et al.(1999) unless otherwise noted
[b] measured at 20 °C
[c] Octanol-water partition coefficient
[d] Sorption partition coefficient
[e] Day (2000)

General procedures for acute toxicity test were adapted from An et al (2002). The acute toxicity tests were conducted in a closed wide mouth glass jar test unit (volume = 2.7×10^{-4} L, ID = 7.0×10^{-2} m) at ambient temperature of 30 ± 1 °C. Each BTEX concentration in soils (e.g., treatment) was prepared in four replicates. The soil used in the tests was a sandy-loam soil with 40% adjusted moisture content. Ten plant seeds were planted just below the surface of the soil in test units. Each test unit contained 0.1 kg soil as dry weight and 0.04 L of test solution with a specific BTEX concentration. Test solutions were mixed with the soil as drop-wise additions and silicon-lined lids were tightened to prevent volatilization of BTEX. After an incubation period of five days, the plants were separated from the soil by adding some water to a test unit to make a slurry. Test units were checked for seed germination and seedling growth. A seed was considered to have germinated when shoot sprouts were observed. Growth was measured by shoot and root length. Exposure concentrations for the test plants were 0, 11, 22, 45, 90, and 180 mg BTEX/kg soil as dry weight as shown in Table 2. The ratios of benzene, toluene, ethylbenzene and xylene were adjusted based on the percent volume of each hydrocarbon in gasoline as shown in Table 1. Distilled water without BTEX was used as the control.

LOAEC values for seedling growth were determined using Dunnett's procedure for multiple comparisons (Dunnett 1955). The Dunnett program (version 1.5)

(USEPA 1999) was used to calculate the minimum difference between the control and the treatment means detected as statistically significant. The software was obtained from US EPA's Ecological Monitoring Research Division (EMRD), Environmental Monitoring Systems Laboratory (EMSL). A 95% significant level ($p < 0.05$) was used for all comparisons.

Table 2. Exposure concentrations of BTEX used in this study.
(Unit: mg /kg soil as dry weight)

No[a]	Benzene	Toluene	Ethylbenzene	Xylenes	BTEX
1 (control)	0	0	0	0	0
2	0.7	3.5	0.7	6.3	11
3	1.4	6.9	1.4	12.7	22
4	2.8	13.9	2.8	25.3	45
5	5.6	27.7	5.6	50.7	90
6	11.2	55.5	11.3	101.4	180
Ratio[b]	1	5	1	9	

[a] Each treatment was prepared in four replicates
[b] based on the % volume in gasoline as shown in Table 1

RESULTS AND DISCUSSION

Fig. 1A shows the shoot and root growth of *Sorghum bicolor* exposed to BTEX in soil expressed as a percent of the growth of plants in the control treatment. There are not an observed difference of plant growth between 0 and 45 mg BTEX/kg soil as dry weight. This indicated that BTEX used would not be toxic to *Sorghum bicolor* unless the concentrations were greater than 45 mg BTEX/kg soil as dry weight. The 5-day LOAECs for shoot and root growths of *Sorghum bicolor* determined by Dunnet's multiple comparison were 90 mg BTEX/kg soil as dry weight in this study.

Fig. 1B shows the percent growth of *Cucumis sativus* compared to the control treatment. After five days incubation the lower levels of BTEX have enhanced plant growth over the control. This may be explained by the fact that BTEX might serve as a carbon source for plant growth. The 5-day LOAECs for shoot and root growths of *Sorghum bicolor* determined were 90 and 180 mg BTEX/kg soil as dry weight, respectively.

Germinations of *Sorghum bicolor* and *Cucumis sativus* were not adversely affected by BTEX up to 190 mg/kg soil as dry weight. This indicated that the germination is not good protocol to assess plant toxicity, as previously reported in several studies (An 2004; Dorn and Salanitro 2000; Dorn et al. 1998).

In both plants, shoots were generally more susceptible to BTEX than roots. These observations suggest that BTEX might be transported within the plants from the roots to the shoots. Same phenomenon was previously observed for methyl *tert*-butyl ether, which was more toxic to shoots than roots of plants (An et al. 2002).

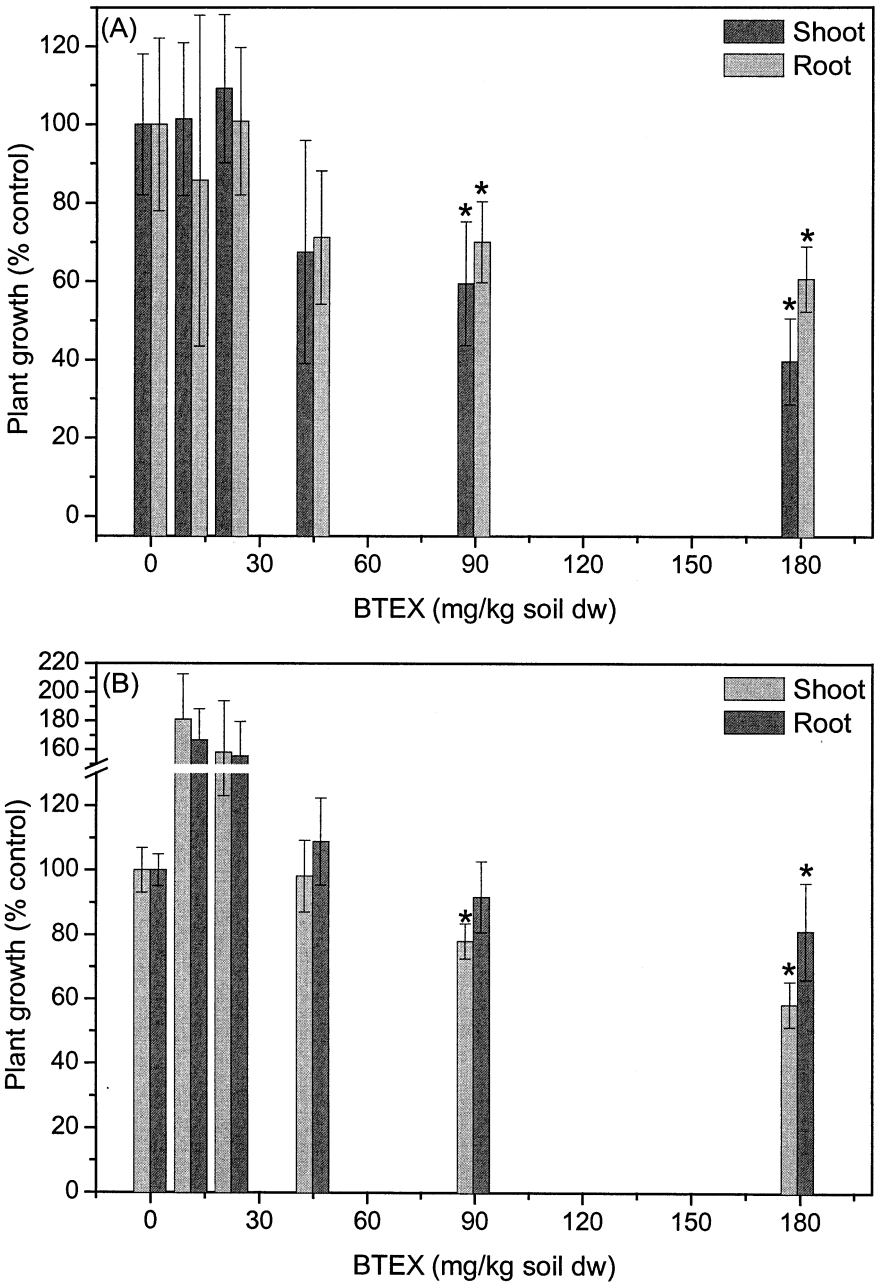

Figure 1. Seedling growth of (A) *Sorghum bicolor* and (B) *Cucumis sativus* as a percent control after 5 days in soil containing different concentrations of BTEX. Bars represent one standard deviation of the mean of four replicates. Significant differences from controls ($p<0.05$) are marked with an asterisk.

It was demonstrated that BTEX would be toxic to *Sorghum bicolor* and *Cucumis sativus* when BTEX concentrations in soil were greater than 90 mg BTEX/kg soil as dry weight, which is the LOAEC determined in this study. BTEX contamination near gasoline filling stations is of concern in Korea. Soils contaminated by gasoline usually show BTEX concentration much greater than 90 mg /kg soil as dry weight, and sometimes several thousands of mg /kg soil as dry weight in Korea (Mail Business Newspaper, 2000).

This study was limited to the acute toxicity (5 days) of BTEX to plants. Lower levels of BTEX (<90 mg BTEX/kg soil as dry weight) may have chronic adverse effects after long-term exposure.

Acknowledgments. This work was supported by the Korea Research Foundation Grant (KRF-2003-003-D00206). I thank Y.-M. Kim and T.-I. Kwon for their assistance in laboratory experiments.

REFERENCES

An Y-J (2004) Soil ecotoxicity assessment using cadmium sensitive plants. Environ Pollut 127:21-26

An Y-J, Kampbell DH, McGill ME (2002) Toxicity of methyl tert-butyl ether (MTBE) on plants (*Avena sativa, Zea mays, Triticum aestivum, and Lactuca sativa*). Environ Toxicol Chem 21:1679-1682

Day MJ (2000) Fate and Transport of fuel components below slightly leaking underground tanks. Soil Sediment & Groundwater MTBE special issue. 21-24

Dorn PB, Salanitro JP (2000) Temporal ecological assessment of oil contaminated soils before and after bioremediation. Chemosphere 40: 419-426

Dorn PB, Vipond TE, Salanitro JP, Wisniewski HL (1998) Assessment of the acute toxicity of crude oils in soils using earthworms, microtox, and plants. Chemosphere 37:845-860

Dunnett CW (1955) Multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc 50:1096-1121

Fletcher J. (1991). Keynote speech: A brief overview of plant toxicity testing.? Plants for Toxicity Assessment, M. A. Lewis, ed., American Society for Testing and Materials STP 1115, Philadelphia, 5-11.

Gorsuch JW, Lower WR, Lewis MA, Wang W (1991) Plants for Toxicity Assessment: Second Volume, ASTM STP 1115. Philadelphia, ASTM.

Hulzebos EM, Adema DMM, Breemen EMD-V, Henzen L, Dis WAV, Herbold HA, Hoekstra JA, Baerselman R (1993) Phytotoxicity studies with Lactuca sativa in soil and nutrient solution. Environ Toxicol Chem 12:1079-1094

Mackay D, Shiu W-Y, Ma K-C (1999) Ph*ysical-Chemical Properties and Environmental Fate Handbook,* CRC netBase, Chapman & Hall.

Mail Business Newspaper, July 4th, 2000, http://news.mk.co.kr

Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (1996) Methods of Soil Anlysis. Part 3.

Chemical Methods. Soil Science Society of America, Inc., Madison. WI, 487-489.
Ugrekhelidze D, Korte F, Kvesitdze G (1997) Uptake and transformation of benzene and toluene by plant leaves. Ecotoxicol. Environ. Safety 37:24-29
USEPA (1999) Dunnett Program Version 1.5 Users' Manual. U. S. EPA, Environmental Monitorign Systems Laboratory, Ecological Monitoring Research Division, Cincinnati, Ohio.