TOXICITY ASSESSMENT OF VOLATILE ORGANIC COMPOUNDS FOUND IN SOIL AND GROUND WATER AT A HAZARDOUS WASTE LANDFILL SITE

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INTRODUCTION

Throughout the U.S., including Georgia, a variety of organic solvents have been detected in ground water. Many of these organic solvents are acutely toxic and carcinogenic. As a result, there is great concern regarding the environmental fate of these compounds and their associated toxicities. However, it is currently recognized that chemical analysis alone is inadequate in assessing the environmental fate and toxicity of such hazardous chemicals. Short-term microbial toxicity assays of environmental components represent a more direct estimate of toxicity potential and overall health risks to humans and animals.

Our laboratory is currently evaluating microbial toxicity assays for use under field conditions to assess mutagenic and toxic hazards associated with organic solvents at a chemical waste landfill. The site is the University of Georgia's hazardous waste landfill which is located adjacent to the Botanical Gardens in Athens. The site is approximately 225 by 100 feet and consists of trenches 10 to 12 feet deep. This landfill received laboratory chemical, low-level radioactive, and biological wastes from before 1969 to 1979. Ground water samples from 30-foot downgradient wells (in the direction of ground water flow) have contained numerous compounds; most notably, the volatile organic compounds, chloroform, methylene chloride (i.e., dichloromethane), toluene, xylene, and trichloroethylene (Law Environmental, 1988).

Herein, we present results of laboratory investigations with these five organic solvents which demonstrate that microbial assays can be used to detect the acute toxicity and genotoxicity of these compounds. The acute toxicity of the organic solvents was monitored with assays based on the inhibition of de novo β-galactosidase biosynthesis in the Escherichia coli strains C3000 and K12 OR85. Genotoxicity was evaluated with the Ames Salmonella-mammalian microsome test.

METHODOLOGY

The five organic solvents used in this study (i.e., chloroform, methylene chloride, toluene, xylene, and trichloroethylene) were of HPLC, spectrophotometric or ultrapure grade. Dilutions of the test compounds were made with 100% or 5% dimethyl sulfoxide (DMSO; HPLC grade --glass distilled; filter sterilized).

Dilutions of test compounds were screened for the ability to inhibit de novo β-galactosidase biosynthesis in E. coli C3000 and K12 OR85 as per the methods of Dutton et al. (1988) and Reinhartz et al. (1987), respectively. The Ames Salmonella-mammalian microsome assay was used to determine the mutagenicity of the test compounds. The protocol followed was that of Maron and Ames (1983), with modifications intended to contain the volatile compounds, such as methylene chloride, during exposure of the test bacteria. Containment was accomplished by taping the plates (Distlerath et al., 1984), or placing the plates open-faced in Tedlar bags in which a test organic solvent was subsequently allowed to evaporate (Hughes et al., 1987). The latter method proved to have several disadvantages, the most important being microbial contamination of the open-faced plates despite the sterilization of the Tedlar bags (presumably from the manual insertion of plates into the bags). As a result, we developed a modification of the Tedlar bag method whereby a plastic plate containing the test bacterium and agar was inverted over a glass plate containing the test compound, and they were incubated in a Tedlar bag. In this manner, the test bacterium was optimally exposed to the volatile compounds, while minimizing the chances for microbial contamination of the assay system.

The mutagenicity results presented herein were obtained with Salmonella tester strain TA100, in the absence (-S9) and presence (+S9) of microsomal activation mix containing the Aroclor 1254-induced rat liver homogenate fraction S9. The tester strain was checked for appropriate responses to known mutagens in DMSO. A positive mutagenic response was defined as a dose-related response with one or more doses producing at least a 2-fold increase in revertant colonies per plate as compared to the concurrent spontaneous count per plate.

The specific toxic and mutagenic activities of the test chemicals were expressed as percent

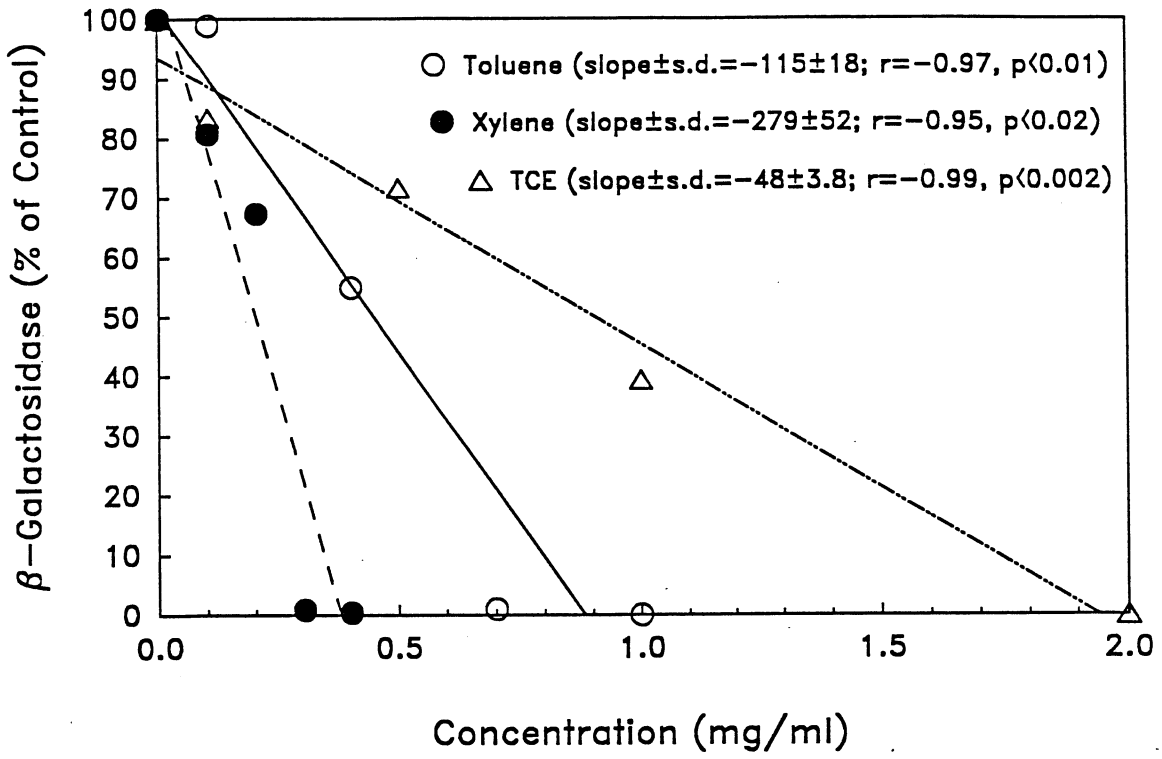

Figure 1. Dose-Related Inhibition of β-Galactosidase Biosynthesis in E. coli (C3000) by Toluene, p-Xylene, and Trichloroethylene (TCE). The concentrations of the test compounds are the final concentrations to which the bacteria were exposed. The chemicals were dissolved in 100% DMSO, resulting in exposure of the bacteria to a final DMSO concentration of 10% in all trials. Regression analysis data are shown which include slope (± standard deviation of the slope), correlation coefficient (r), and probability of significance (p).

reduction in β-galactosidase biosynthesis per mg/ml of the compound, and net revertants per mg of the compound, respectively. These activities were based on the slopes (± standard deviation of the slopes) of the dose-response curves as determined by least-squares regression analysis. The statistical significances (probabilities) of the resulting slopes and correlation coefficients (r) for the dose-response curves were determined.

RESULTS AND CONCLUSIONS

The dose-related inhibition of β-galactosidase biosynthesis in E. coli C3000 by the five organic solvents is shown in Figures 1 and 2. As can be seen, a statistically significant linear dose-response relationship was obtained for each solvent. There were, however, marked differences in the toxic activities of the five test compounds. The three least volatile compounds (toluene, xylene, and trichloroethylene) proved to be the most toxic, while the most volatile compounds (chloroform and methylene chloride) displayed lower toxicity. These results may reflect the loss of the more volatile compounds during incubation with the test bacterium (thus, reducing the concentration of the compound to which the organism is exposed), rather than the inherent lower toxicity of these compounds. It should also be noted that the results shown in Figures 1 and 2 are for the test compounds dissolved in 100% DMSO. Markedly lower toxicities were observed when the test compounds were dissolved in deionized water or in a lower % DMSO. Since DMSO is not very toxic to E. coli (Reinhartz et al., 1987) and is controlled in the assay, it is believed that DMSO enhanced the uptake of the test compounds by the bacteria, thereby increasing the toxicities of these compounds. A comparison of the IC_{50} (mean concentration of chemical resulting in a 50% inhibition of β-galactosidase biosynthesis) for the test compounds using E. coli strains C3000 and K12 OR85 is shown in Table 1. The E. coli K12 OR85 assay system is a commercially-available kit known as the Toxi-Chromotest. This assay is conducted in microtiter plate wells without

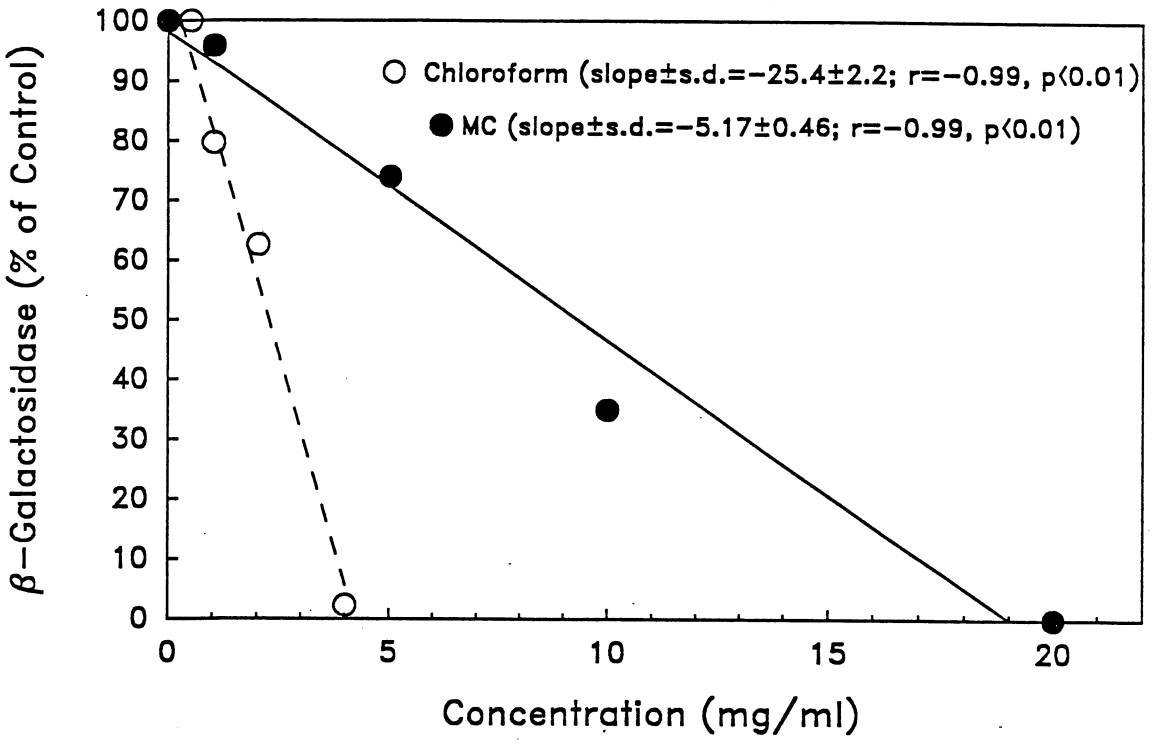

Figure 2. Dose-Related Inhibition of β-Galactosidase Biosynthesis in E. coli (C3000) by Chloroform, and Methylene Chloride (MC). See Legend to Figure 1.

Table 1. Comparison of the Inhibition of β-galactosidase Biosynthesis in E. coli C3000 and E. coli K12 OR85 (Toxi-Chromotest) by the Test Organic Solvents.

Chemical	Mean IC_{50}[a] (mg/mL) in E. coli strains:	
	C3000	K12 OR85
p-Xylene	0.199	0.803
Toluene	0.448	0.891
Trichloroethylene	0.904	1.96
Chloroform	2.25	3.03
Methylene Chloride	9.32	14.2

[a]Mean concentration of chemicals resulting in a 50% inhibition of bacterial β-galactosidase biosynthesis relative to a control containing the same quantity of DMSO but no test chemical. These are the final concentrations of the test chemicals to which the bacteria were exposed. The chemicals were dissolved in 100% DMSO, resulting in exposure of the bacteria to a final DMSO concentration of 10% in all trials.

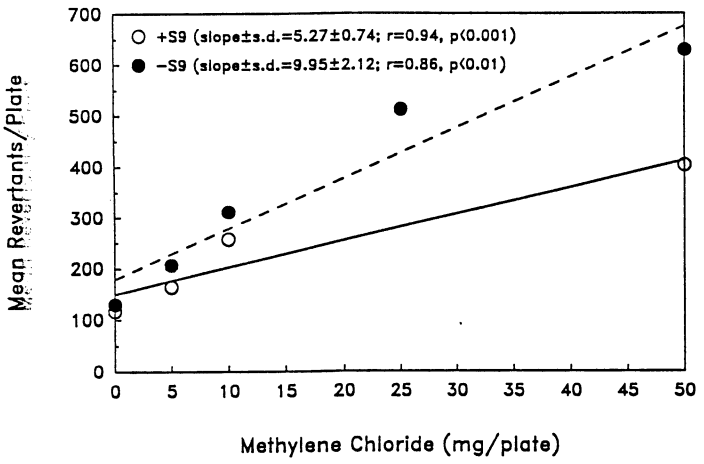

Figure 3. Dose-Related Mutagenic Responses Produced by Methylene Chloride in the Salmonella typhimurium Tester Strain TA100 (±S9). Methylene chloride was diluted in 100% DMSO. The Ames test was conducted by a modified Tedlar bag method as described in the text. Regression analysis data are shown which include slope (± standard deviation of the slope), correlation coefficient (r) and probability of significance (p).

continuous mixing, and, therefore, the bacterial cells may not have been fully exposed to the toxicants, particularly at the higher doses. As a result, we were able to obtain statistically significant linear dose-response data for only toluene and trichloroethylene, and the assay proved to be less sensitive than the E. coli C3000 system for all five test compounds (Table 1).

With respect to mutagenicity, initial testing has demonstrated that methylene chloride is mutagenic. Shown in Figure 3 are dose-related mutagenic responses produced by methylene chloride in strain TA100 in the presence and absence of metabolic activation (S9). It appears that the presence of S9 reduced the mutagenic response. This data was obtained with our modified Tedlar bag method. Lower mutagenic activity was demonstrated for methylene chloride in the taped assay method of Distlerath et al. (1984), and the Tedlar bag method of Hughes et al. (1987) provided poor results due to microbial contamination. Moreover, methylene chloride was not mutagenic in the conventional Ames test with preincubation (20 min.). Based on these results, it appears that our modified Tedlar bag assay is best for the detection of mutagenesis associated with volatile compounds. We are currently testing other volatile chemicals with the Ames Salmonella tester strains using this modified Tedlar bag method.

The results presented herein demonstrate that these short-term microbial assay systems can be used to detect the acute toxicity and mutagenicity of organic solvents. Previous chemical analysis of ground water at the University of Georgia's chemical landfill site has found the following compounds and concentrations: chloroform (40 mg/L), methylene chloride (28 mg/L), toluene (6.9 mg/L), xylene (2.3 mg/L), trichloroethylene (0.49 mg/L), and other assorted organic solvents in low mg/L concentrations (Law Environmental, 1988). Based on these results, the concentration of one to four liters of ground water from the landfill site into a few milliliters of DMSO should be sufficient to detect acute toxicity and mutagenicity in our microbial assay systems. The results of toxicity monitoring of ground water at this site will be presented.

ACKNOWLEDGMENTS

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