

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

DETERMINATION OF SOIL ADSORPTION PARTITION
COEFFICIENTS OF ^{14}C -LABELED CARCINOGENIC
ORGANIC CHEMICALS BY LIQUID SCINTILLATION

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INTRODUCTION

We are becoming more aware of the consequences resulting from the manufacture, transport, and use of organic chemicals. A number of these chemicals are carcinogenic, mutagenic and/or teratogenic, and it is therefore advisable to determine their environmental movement and fate.

Chemicals that are released, either by design or accident, into the terrestrial environment come in contact with the soil, and the soil can possibly become a repository for the fugitive chemical. If this happens, the chemical could become an integral part of soil and thus move with the soil during erosion episodes, or the chemical could pass through soil profiles and come in contact with ground water. Of course, the possibility exists that the chemical can be degraded into less harmful byproducts.

The degree of retention of a chemical in soils can be predicted by determining the soil adsorption partition constants for the chemicals of interest. Partition constants describe the distribution of a chemical between the soil and the soil-water solution.

When we determine the transport and fate of chemicals in soils, it is desirable to use environmental concentrations of the chemical. Unless there has been a spill, environmental concentrations are generally in the range of nanograms of chemical per gram of soil (ppb). When we deal with such a low

concentration of chemicals, available analytical procedures are often not sensitive enough to give quantitative results. However, by using chemicals with a ^{14}C label in conjunction with liquid scintillation it is possible to improve detectability by an order magnitude or more. Another advantage of using ^{14}C -labeled chemicals is that the amount of chemical sorbed to the soil can be directly determined by a simple process of oxidizing the soil and trapping the resultant $^{14}\text{CO}_2$ in a specially prepared liquid scintillation cocktail. This method avoids the inconvenience and uncertainties associated with trying to extract some portion of an unlabeled chemical from the soil. Labeled chemicals are particularly useful when dealing with volatile organic chemicals. Because there are fewer preparatory steps, less of the chemical is likely to be lost.

The authors have had experience in determining the partitioning constants between soil, benzene, carbon tetrachloride and ethylene dibromide, and we anticipate investigating several other suspected organic carcinogens. In this report we use benzene to illustrate steps involved in the investigation of organic carcinogen-sorption to soils.

METHODS

^{14}C -radiolabeled benzene was obtained dissolved in distilled H_2O from New England Nuclear. Each sealed ampule contained 0.10 mCi of uniformly labeled benzene (specific activity of 54.0 mCi/m mol) mixed with 20 ml H_2O . New England Nuclear analyzed this mixture after we had stored some of the solutions for over one year and found no deterioration of the benzene.

Before use, the radiolabeled benzene from an ampule was diluted to 210 ml with distilled water, and this stock solution was stored in a 210 ml amber bottle with a screw cap Supelco mininert valve. The valve allowed the use of a syringe to remove solution in any desired amount.

A 10^5 -ppb stock solution of stable benzene was prepared by mixing 0.1 g of benzene with 1 liter of distilled water. Labeled benzene solutions used in the studies were prepared by mixing stock solutions of labeled and stable benzene with a required amount of water in sealed serum vials.

The batch equilibration sorption study was conducted with 1 g of soil (Hastings soil series from Nebraska and Overton soil series from southeastern Nevada) at a ratio of 1:25 adsorbent:water-benzene solution. This ratio was required because of the need for a minimal headspace in the 25-ml

centrifuge tubes that were used, since a restricted headspace reduced the volatile loss of benzene from solution during shaking.

Three solution concentrations of benzene were used for the sorption study: 10, 100 and 1,000 ppb benzene in 25 ml of liquid. The soil solutions were shaken for the desired period of time (1, 16, 42, 64, or 135 h) and the soil was removed from solution by refrigerated centrifugation (5°C at 2,000 G). Benzene remaining in the supernatant was determined by liquid scintillation counting of 5 ml of the liquid. After decanting the supernatant from the centrifuge tube, the tube and its contents were reweighed to determine the amount of the water-benzene solution remaining in the soil pellet. The amount of benzene entrapped in the pellet was then calculated, and this value was used as a correction to obtain the amount of benzene that was actually sorbed to soil.

Benzene associated with the adsorbent pellet was determined by oxidizing the organic materials at 900°C in a stream of oxygen. For this work a Harvey Biological Oxidizer, model OX-100, was used. Gasses from the oxidizer were bubbled into a CO₂-gathering liquid scintillation cocktail. The efficiency of CO₂-trapping was determined before each day's samples were oxidized. The amount of ¹⁴C was then determined by liquid scintillation analysis. The liquid scintillation cocktail was made by mixing 900 ml of the stock cocktail (1.5 g bis-MSB, 7.0 g PPO, 1 liter p-xylene, and 400 ml triton N-101) with 100 ml of Packard Carbo-sorb. Usually this cocktail became cloudy due to the addition of H₂O during the CO₂-gathering process. This problem was eliminated by the addition of 1 ml of methanol to the scintillation vial containing the cloudy liquid.

Adsorption-partition constants were determined for benzene by use of the following log form of the Freundlich isotherm: $\log x/m = 1/n \log C + \log K$, where X is the weight of benzene sorbed (ng), m is the weight of adsorbent (g), C is the equilibrium concentration of benzene in solution, and K and 1/n are constants. K is a measure of the degree or strength of adsorption while 1/n is used as an indication of whether adsorption remains constant (as indicated by a 1/n value of unity) or decreases with increasing adsorbate concentrations.

To check the methods used in the sorption studies, activated charcoal ground to pass a 5-mm sieve was used as an adsorbent. In a batch equilibration study 50-mg quantities of charcoal were incubated 16 h with 2,500 ng benzene. It was found that 98% of the benzene was sorbed, thus indicating that the methods used for the batch equilibration provided sufficient opportunity for benzene to be sorbed. It was also found that benzene did not adsorb to the glass centrifuge tubes.

RESULTS

Initial benzene sorption on the soils was determined after 16 h of incubation. Table I shows the Freundlich constants which were determined for the adsorbents. The $1/n$ values are at unity or very close to it, indicating that sorption should be linear within the range of increasing benzene concentrations used for this study. This assumption was shown to be correct when plots of the Freundlich isotherms were made (Figures 1 and 2).

A budget for ^{14}C benzene showed that all benzene was accounted for either in the solution or on the adsorbents. Other incubation periods were tested to determine if sorption was time-dependent. Figure 3 is a plot of the Freundlich K values with time. Both soil adsorbents had increasing K values with time (Figure 3).

Since microbial degradation of benzene by oxidation is known to occur the possibility of microbial metabolism of benzene was studied by incubating sterilized soils with 1,000-ppb sterilized benzene solutions for 136 h. As a control, sterilized soils re-inoculated with microbes were also incubated. Data from this study (Table II) show that 2 percent and 1 percent of the benzene was sorbed to the sterilized Hastings and Overton soils, respectively, while 24 percent of the carbon from the original benzene was sorbed to the nonsterile soils. In the initial study, after 136 h the apparent increased sorption of benzene by the nonsterile soils (Figure 3) resulted in 25 percent and 33 percent of the ^{14}C being sorbed to the Hastings and Overton Soils respectively. These data implicate microbes as the source of benzene degradation, and suggest that decay products, rather than benzene, are the primary residue sorbed on soils that have been incubated for extended periods of time.

TABLE I. *Freundlich Constants for Benzene Sorption after 16 Hours of Incubation*

<i>Adsorbent</i>	<i>K</i>	<i>1/n</i>
<i>Hastings Silty clay loam</i>	2.4	0.89
<i>Overton Silty clay loam</i>	1.8	0.94

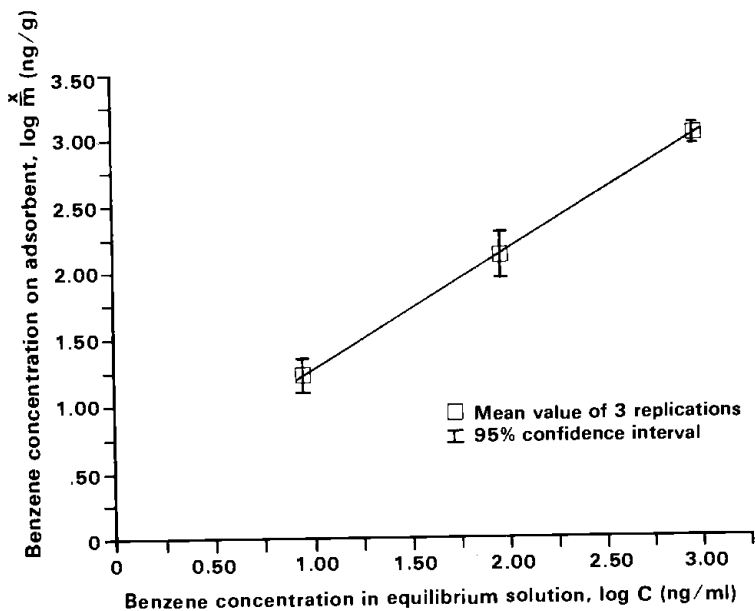


FIGURE 1. Freundlich isotherm for sorption of benzene on Overton clay loam.

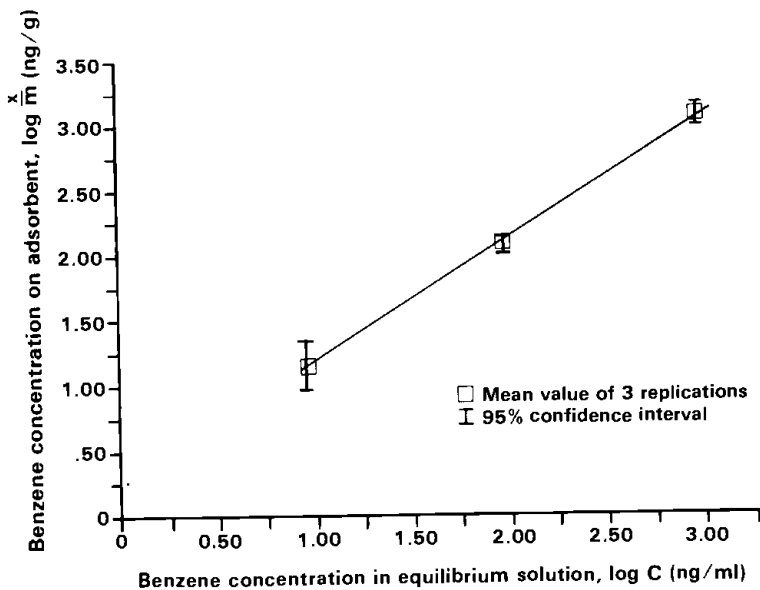


FIGURE 2. Freundlich isotherm for sorption of benzene on Hastings clay loam.

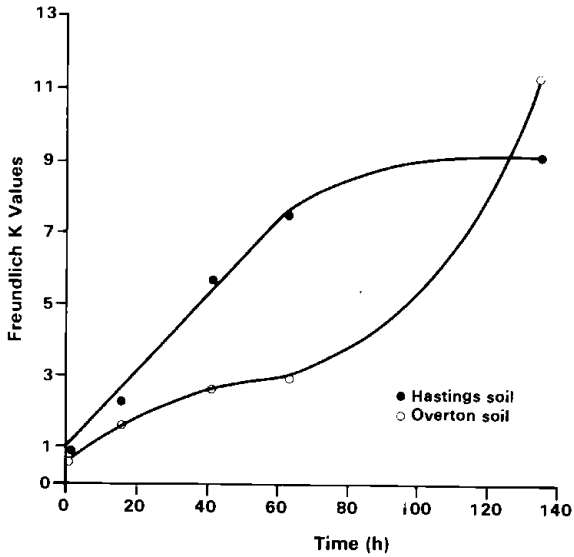


FIGURE 3. Increase of Freundlich K values with time.

TABLE II. Sorption of Benzene-Derived ^{14}C * in Sterile and Sterilized Reinoculated Soils During 136 h

Adsorbent	Sterilized	Sterilized Reinoculated
Hastings Silty clay loam	580 \pm 67 pCi	7495 \pm 193 pCi
Overton Silty clay loam	318 \pm 117 pCi	7480 \pm 430 pCi

* 30,694 picocurie (pCi) ^{14}C initially added to solution

SUMMARY

Sorption and degradation data, such as those generated for benzene, add much to our knowledge of the fate and transport of organic carcinogens. This study confirms the usefulness of using liquid scintillation counting to obtain data on organic carcinogens.