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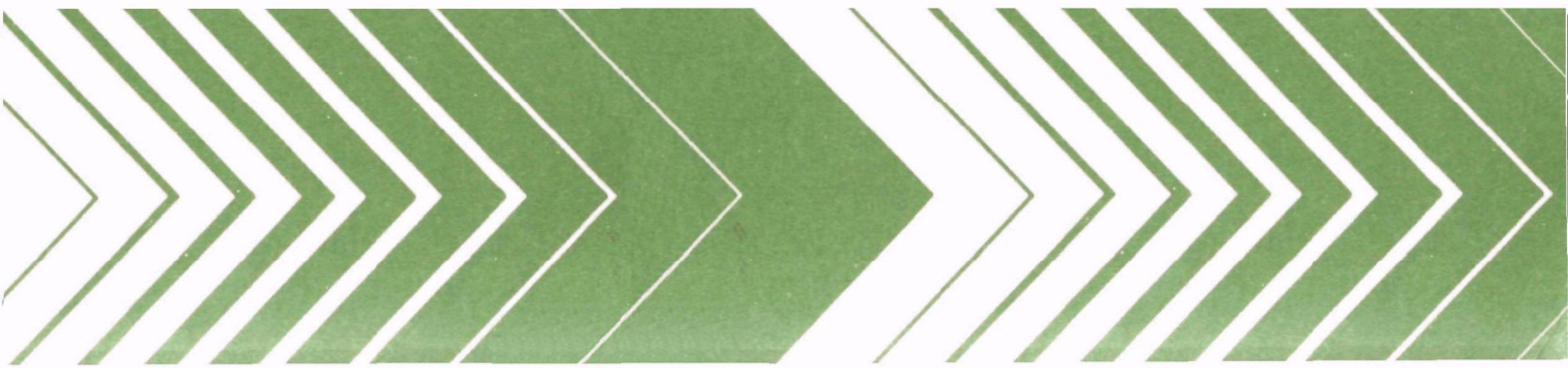
Environmental Monitoring
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P.O. Box 15027
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September 1979

Research and Development



Benzene Vapor Depletion in the Presence of Plants



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BENZENE VAPOR DEPLETION IN THE PRESENCE OF PLANTS

by

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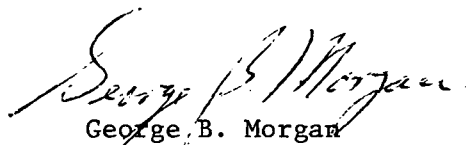
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FOREWORD

Protection of the environment requires effective regulatory actions that are based on sound technical and scientific information. This information must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of specific pollutants in the environment requires a total systems approach that transcends the media of air, water, and land. The Environmental Monitoring and Support Laboratory-Las Vegas contributes to the formation and enhancement of a sound monitoring data base for exposure assessment through programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment
- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This study is designed to determine whether plant systems deplete atmospheric benzene. Depletion of benzene by Eichhornia crassipes, Beta vulgaris saccharifera, and Beta vulgaris cicla in soil and water cultures was observed. This study contributes to the knowledge of biological sinks as deactivators of carcinogenic materials. Such research aids in the identification of permissible ambient levels of these compounds.



George B. Morgan
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SUMMARY

Three plant species, Eichhornia crassipes in a nutrient hydroponic culture, Beta vulgaris saccharifera, and Beta vulgaris cicla in soil and in water cultures, were found to deplete benzene from the air. Following benzene depletion, plant tissues were extracted and no benzene was detected. This suggests that benzene was completely utilized within the test system and that it was degraded to other chemicals.

INTRODUCTION

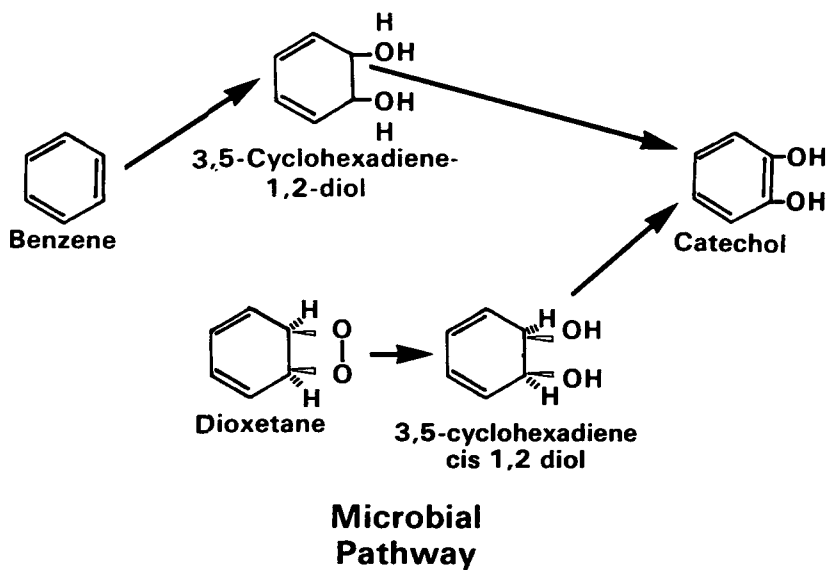
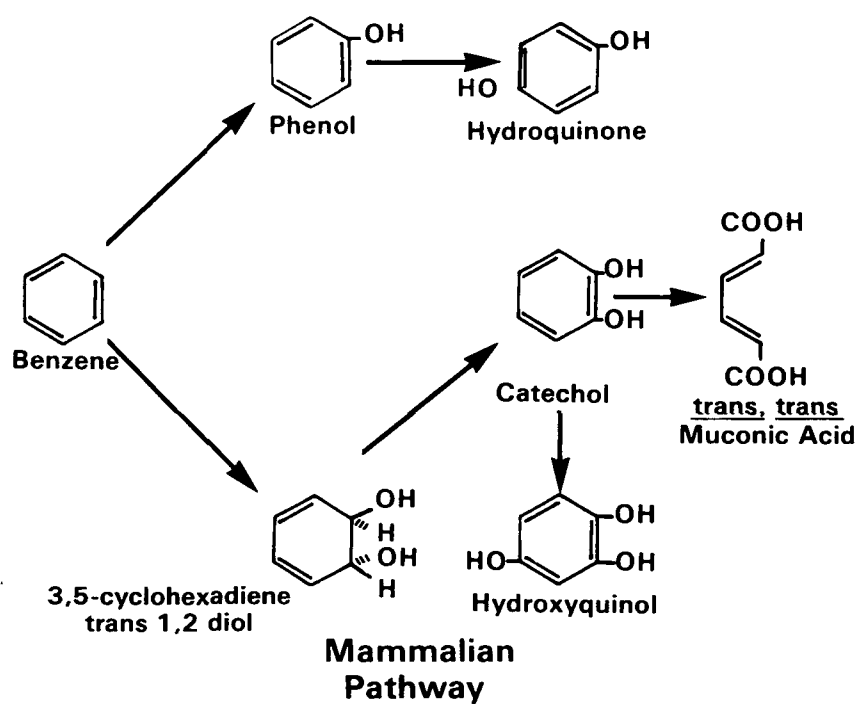
Of all the chemicals declared as suspected carcinogens (NIOSH, Letkiewicz, 1976), benzene is produced in the largest quantities [5×10^9 kilograms (kg) annually (Bertke et al., 1977)]. Among its varied applications, benzene is used in the manufacture of fuels, industrial solvents, dyes, polymers, explosives, pesticides, and disinfectants (Gibson, 1968; Technical Services Division of U.S. EPA, 1976; Fishbein, 1976). It is also found to occur as a natural product in some raw foods such as avocado (Jansen and Olson, 1969) other fruits, vegetables, fish, dairy products, and eggs (Mara and Lee, 1977). It also occurs as a component of cigarette smoke (Schmeltz and Hoffman, 1976) and is found in canned and irradiated beef and Jamaican rum (National Cancer Institute, 1977).

Much attention has been given to the sources of environmental contamination by benzene because it is listed in Section 112 of the Clean Air Act. Several literature reviews and task reports have been undertaken for the U.S. Environmental Protection Agency in which large volumes of data and literature were assembled and reviewed (Manning and Johnson, 1977; Mara and Lee, 1977; Fentiman et al., 1978; and Neher et al., 1977). Benzene has a high vapor pressure, 100 millimeters (mm) of mercury at 26°C , and large quantities are lost by volatilization into certain industrial environments. However, most benzene emissions (81%) are attributed to volatile losses from gasoline (Bertke et al., 1977).

Despite the large amount of literature describing the production and release of benzene, little is known about its environmental fate. Available data indicate that benzene is minimally reactive photochemically. Thus, it must be assumed that benzene is essentially unchanged chemically in the atmosphere before reaching receptor sites (Manning and Johnson, 1977).

Inhalation is the major means of exposure to benzene in animals (Saita, 1973). It has been shown to damage the hematopoietic system resulting in maladies ranging from anemias to benzene-induced leukemia (Uyeki et al., 1977; Saita, 1973; Moloney, 1977). The mechanisms involved in these blood disturbances are unclear, but are likely the result of lymphocyte chromosome abnormalities (Sawicki, 1977). An unstable metabolite, possibly benzene oxide, is thought to interact with DNA to initiate this damage (Lutz and Schlatter, 1977).

Man metabolizes benzene in much the same way as experimental mammals, primarily involving mixed function oxidases (Marquardt, 1977). In the mammalian system stable metabolites include phenol, catechol, and muconic acid (Figure 1) (Gibson, 1971). Certain microorganisms readily oxidize the benzene ring (Evans, 1963; Gibson, 1971; Marr and Stone, 1961); however,



**Benzene Metabolism in Mammals and Soil Microbes,
Adapted from Gibson, 1971; Marr and Stone, 1961.**

Figure 1. Benzene metabolism in mammals and soil microbes, adapted from Gibson, 1971; Marr and Stone, 1961.

these pathways are different from those in mammals (Figure 1) (Gibson, 1971; Marr and Stone, 1961). For instance, phenol is not an important intermediate and dioxetane is proposed as an intermediate metabolite (Gibson, 1971). Pseudomonas aeruginosa and Mycobacterium rhodochrous degrade benzene via 3,5 cyclohexadiene-1,2 diol and catechol to carbon dioxide and water (Marr and Stone, 1961). Another soil microbe, Pseudomonas putida, degrades benzene by way of the cis isomer of 3,5 cyclohexadiene-1,2 diol and catechol (Gibson, 1971). Marr and Stone (1961) found that microbes capable of oxidizing benzene were rendered incapable of this oxidation when the benzene was removed. The inducibility of substrate-specific oxidizing microbes led Rao and Bhat (1971) to suggest the incorporation of such microbes for the purpose of removing benzene, and thus detoxifying, waste waters. An alternate use would be as a biological indicator of aromatic hydrocarbons, or specifically to evaluate the benzene burden of a polluted area (Caparello and LaRock, 1975).

Comparatively little research on benzene degradation in plants is available. Some reports indicate that plants can metabolize aromatic rings such as benzene, phenol, toluene, and catechol (Prasad and Ellis, 1978; Jansen and Olson, 1969). The products of benzene metabolism in plants are reported to be phenol, muconic acid, and carbon dioxide (Durmishidze et al., 1974; Jansen and Olson, 1969), suggesting a pathway similar to that in mammals. Unlike microorganisms, there have been no reports of plants being inducible to benzene metabolism.

Complete knowledge of the fate of benzene in the environment is important due to its abundance and carcinogenicity. This study was undertaken to expand this knowledge by observing benzene depletion rates in plant systems. In addition, an attempt was made to identify the interactions of benzene with plants.

MATERIALS AND METHODS

Three plant species, Eichhornia crassipes (water hyacinth), Beta vulgaris cicla (Swiss chard), and Beta vulgaris saccharifera (sugar beet) were studied in this series of experiments. They were chosen for the convenience afforded by leaf size and ease of propagation. In addition, water hyacinth has been found to metabolize phenol (Wolverton and McKown, 1976). Water hyacinth plants were asexually propagated in Hoaglund nutrient solution in a glass house. The hyacinths were transplanted to 400 ml of distilled water in the test vessels (Figure 2) and allowed to grow 10 days prior to study. Three to 5-week-old Beta plants grown in peat/vermiculite (Jiffy Mix) were rinsed of all rooting material possible, without damaging the roots, and were placed in 5 or 10 milliliters (ml) of distilled water in the necks of the smaller test vessels (Figure 2), and allowed to grow for at least 3 days before experimentation.

Test vessels for exposing plants to benzene consisted of 0.65-liter (Beta spp.) or 2.4-liter (Eichhornia) borosilicate glass bottles with short, 2.5-centimeter (cm) diameter, necks. Openings had a Teflon (TFE) lip seal with a Teflon lined screw-type lid. Each container had a 1-mm diameter hole in the side and/or top for injecting treatment material and air sampling.

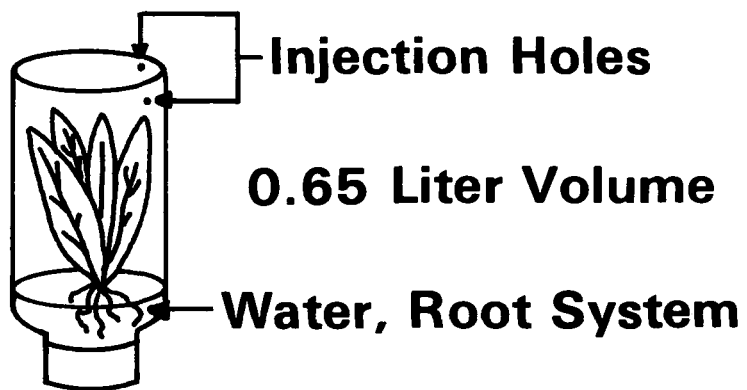
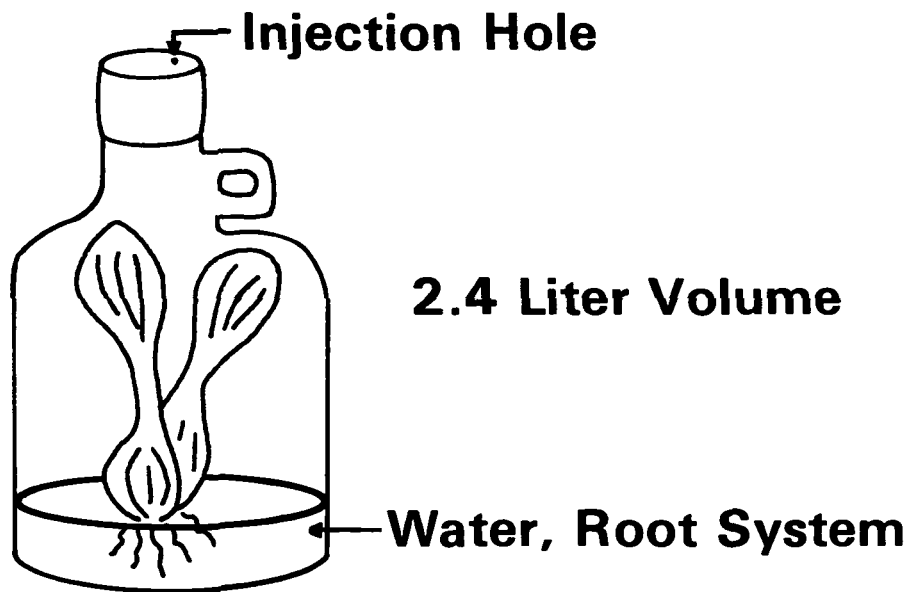


Figure 2. Microcosms for exposure of Swiss chard, sugar beets, and water hyacinths.

These holes were sealed with cellulose acetate tape when not in use. Carbon dioxide (CO₂) was added daily to the test vessels to allow photosynthesis. The desired benzene concentration was achieved by an injection of liquid benzene with subsequent volatilization within the vessel. The starting concentration was verified by gas chromatographic (GC) analysis. Experimental controls were prepared by injecting benzene into bottles containing the same volume of water as the test vessels, but with no plants. Benzene loss was negligible (Appendix 1). A standard benzene concentration was established by injecting a similar amount of benzene into an empty bottle. Benzene concentration was determined by comparing GC signal peak heights of samples to those of the standards. Depletion rates were determined by performing a linear regression analysis on the resulting concentrations. Upon dropping to a zero concentration, the vessels were re-injected with the original amount of benzene.

After experiments were complete, the plants were extracted by grinding them in 50% acetonitrile and 50% distilled water with a ceramic mortar and pestle. This mixture was vacuum-filtered before being extracted with an equal volume (5 ml/g tissue) of petroleum ether. Some extracts were concentrated by evaporation before analysis.

Gas chromatographic analyses were performed on a model 222 Tracor gas chromatograph with a flame-ionization detector. A 1.8-meter x 2-mm (inside diameter) glass column was used. For separation of benzene from ether, 10% Pennwalt-223 plus 4% potassium hydroxide on 80/100 mesh Gas-Chrom packing was used in the column. Benzene in air samples was separated using a column packing of 25% diethylene glycol succinate on 60/80 mesh WAW-DMCS Chromosorb. The carrier gas was helium at a flow rate of 60 ml/minute at the detector. Detector and injection port temperatures were 265° C and the column temperature was 150° C.

During these experiments, it became evident that a time lag occurred between the initial benzene exposure and the maximum benzene depletion rate. To test the importance of benzene concentration on the depletion rate, plants were exposed to various benzene concentrations for a predetermined time using a flow-through exposure system. Four stainless steel cylinders were evacuated and enough liquid benzene was drawn into them to yield 0, 0.18, 0.27, and 0.61 milligrams per liter (mg/liter) of benzene. Carbon dioxide was added in the same way to make final concentration of 300 parts per million (ppm). Finally the cylinders were pressurized to 400 pounds per square inch with CO₂-free compressed air. These exposure gases were attached through pressure regulators and a manifold of needle valves and the flow rates were adjusted to 10 ml/minute through each test vessel.

Three test vessels were fumigated with each of the different benzene concentrations for 3 days. The sources were then disconnected and the benzene depletion rates of each were determined as previously outlined.

A third study was undertaken to determine the depletion rate of benzene on a larger scale with mature sugar beets. Six-month-old sugar beet plants grown in 30-cm pots filled with Jiffy Mix were placed in a 2.26-m³ sealed growth chamber. The CO₂ concentration was maintained at 300 ppm and benzene

was added daily to reestablish a concentration of 0.6 mg/liter. The benzene concentration in this chamber was monitored by GC and the depletion rate calculated from the slope as in the other studies.

Leaf weight and areas were used to confirm uniformity of test plants and to determine relationships between plant size and depletion rates. Plants were weighed after blotting the roots on absorbent paper towels. Leaf area was obtained with a Li-Cor (R) portable leaf area meter. Dry weights were determined after oven-drying the plants for 24 hours at 80° C.

RESULTS AND DISCUSSION

Benzene vapor concentration decreased in the presence of water hyacinth, sugar beet, and Swiss chard (Figures 3, 4, and 5). This depletion appears to be a linear phenomenon even when approaching zero concentration. Linearity suggests that benzene depletion was not limited by gaseous diffusion but by a rate-limiting reaction site or chemically reactive substrate. Linear regression was used to calculate the slopes and these values are used to describe the benzene uptake rates.

Data from the initial experiments show typical changes in benzene depletion rates for a series of plants observed at intervals during repeated benzene fumigations (Figure 6). The rate of benzene depletion was determined repeatedly for up to 25 exposures with some plants. Succeeding rate determinations on the same plant did not always yield the same result. Differences also occurred between replicates and large differences were evident between species. For the two Beta species there was a time lag before the rate of benzene depletion increased. Maximum depletion rates were reached at 16 days of exposure. The lag period was shorter in the Eichhornia tests. Benzene depletion was slow the first day but increased rapidly the second and third days followed by a decline. Experimentation to date has not allowed a conclusive explanation for the time lag or for the subsequent decreased absorption. However, factors suspected in causing this characteristic depletion pattern include: 1) the deterioration of the epicuticular wax and cuticle by benzene exposure; 2) the induction of an enzyme system responsible for the degradation or metabolism of benzene; 3) establishment of a substantial microbial population which utilizes benzene; 4) and changes in the vigor of the plants. Current research is directed toward the identification of the responsible mechanism.

The decreasing rate of benzene depletion coincided with the appearance of leaf damage. The conditions of the microcosm were not ideal for plant growth. Maintenance of CO₂ concentration on only a daily basis, lack of nutrients, no air turbulence, and high humidity all precluded normal plant growth. Therefore, the decrease in benzene depletion rates was assumed to be related to plant deterioration, each species having a different tolerance to these conditions.

Analysis of petroleum ether extracts of benzene-exposed sugar beet and water hyacinth plants yielded no benzene in the plants. This indicates that

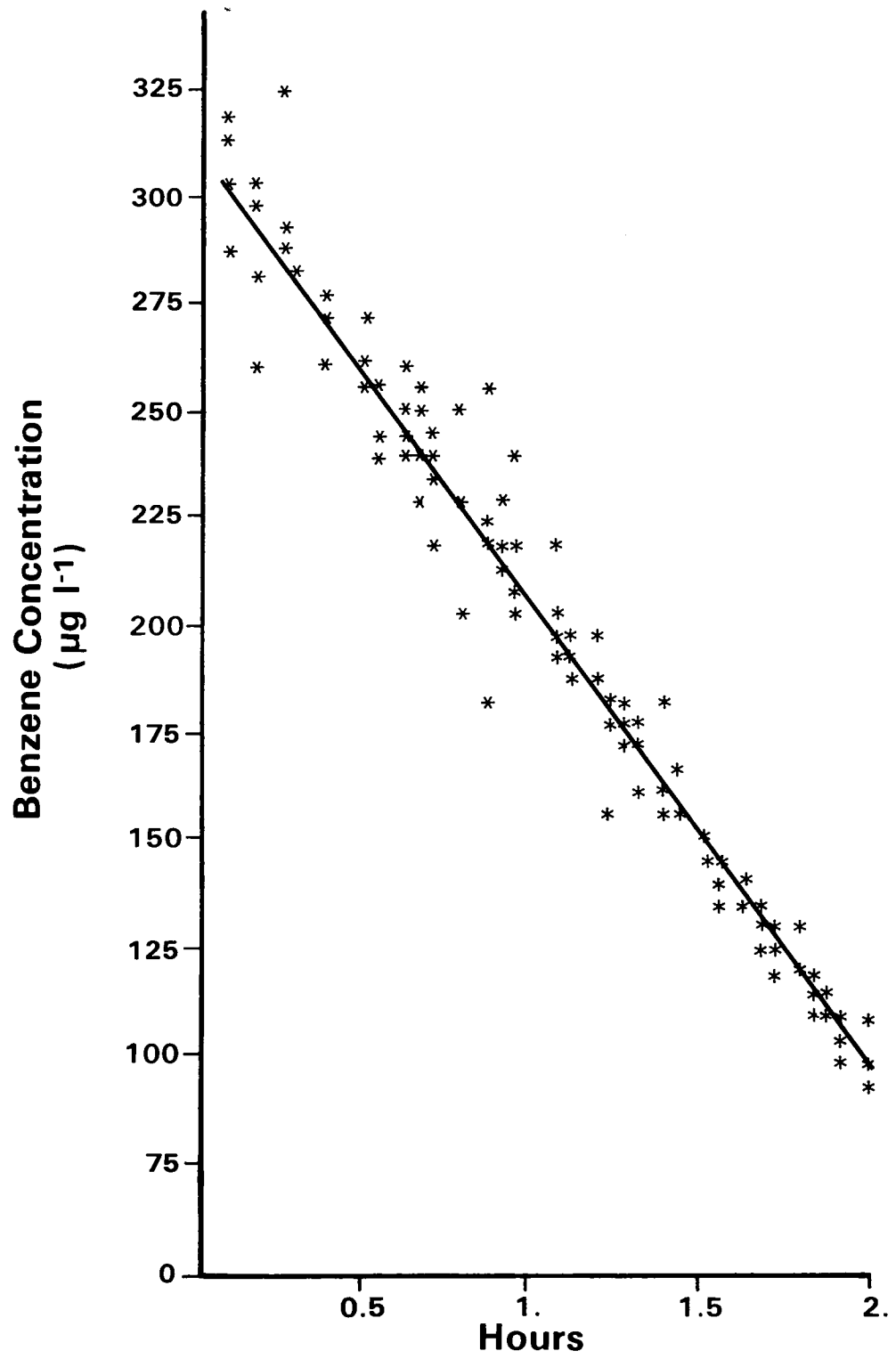


Figure 3. Linear depletion of atmospheric benzene by sugar beets.

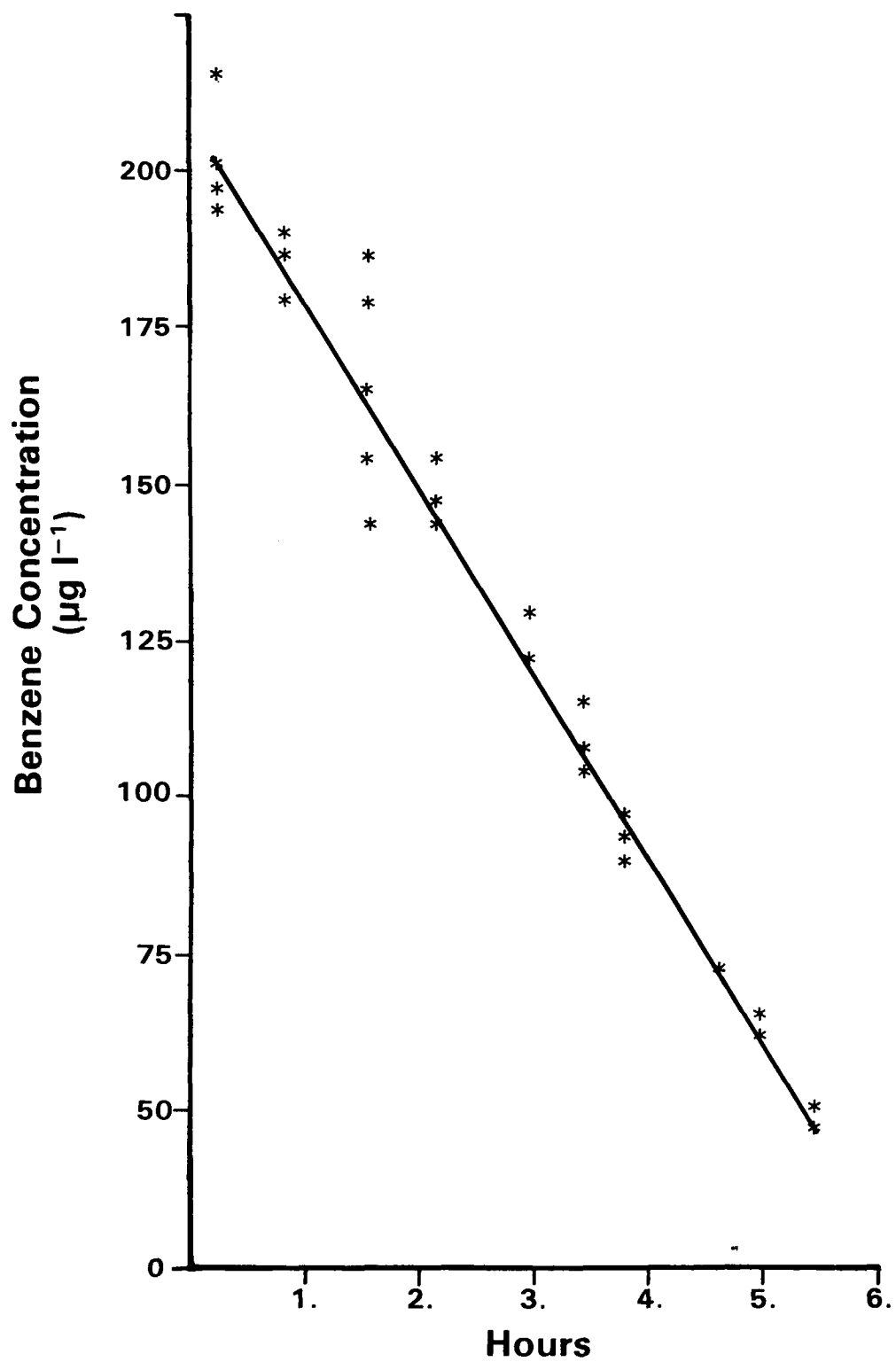


Figure 4. Depletion of atmospheric benzene by Swiss chard.

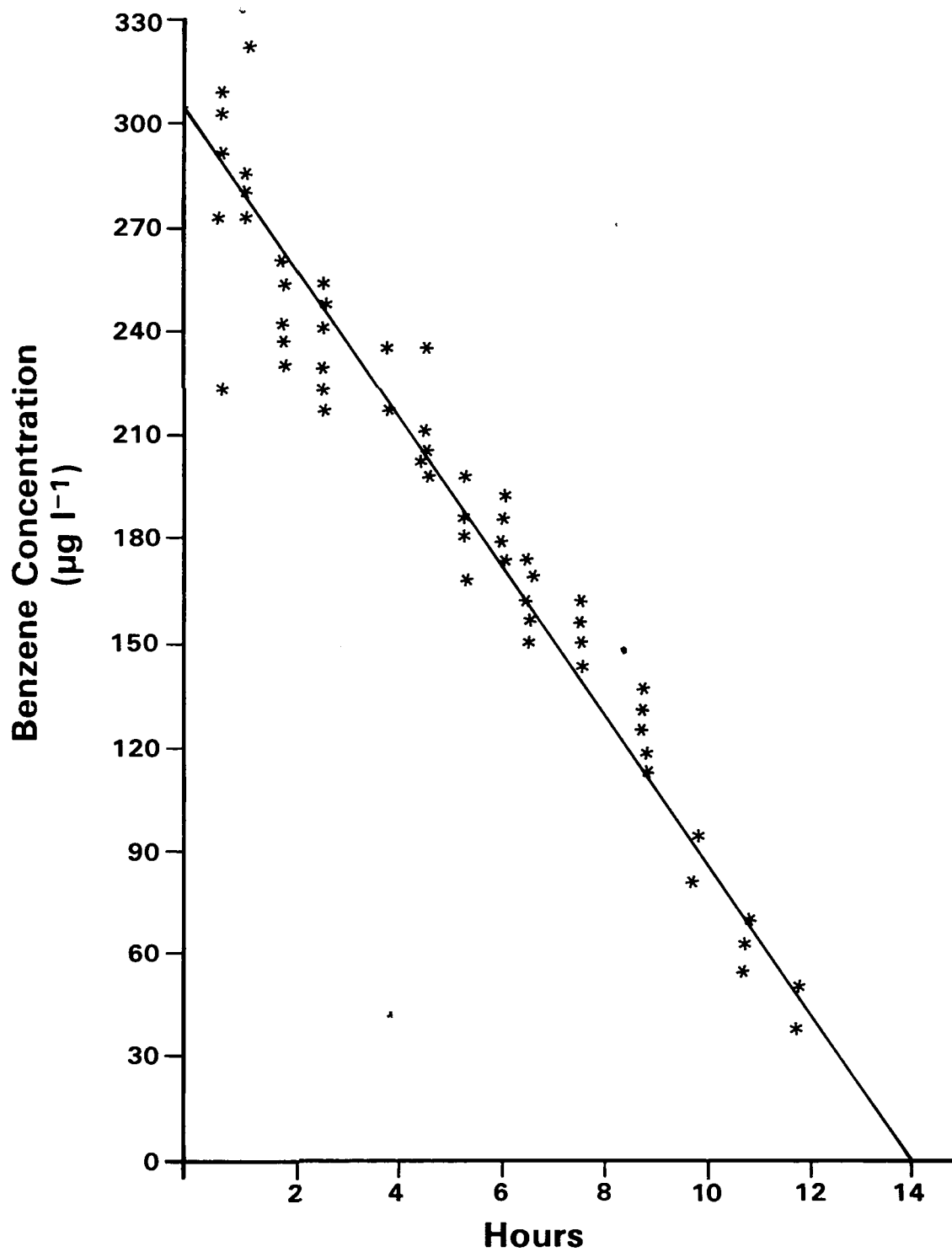


Figure 5. Depletion of atmospheric benzene by water hyacinth.

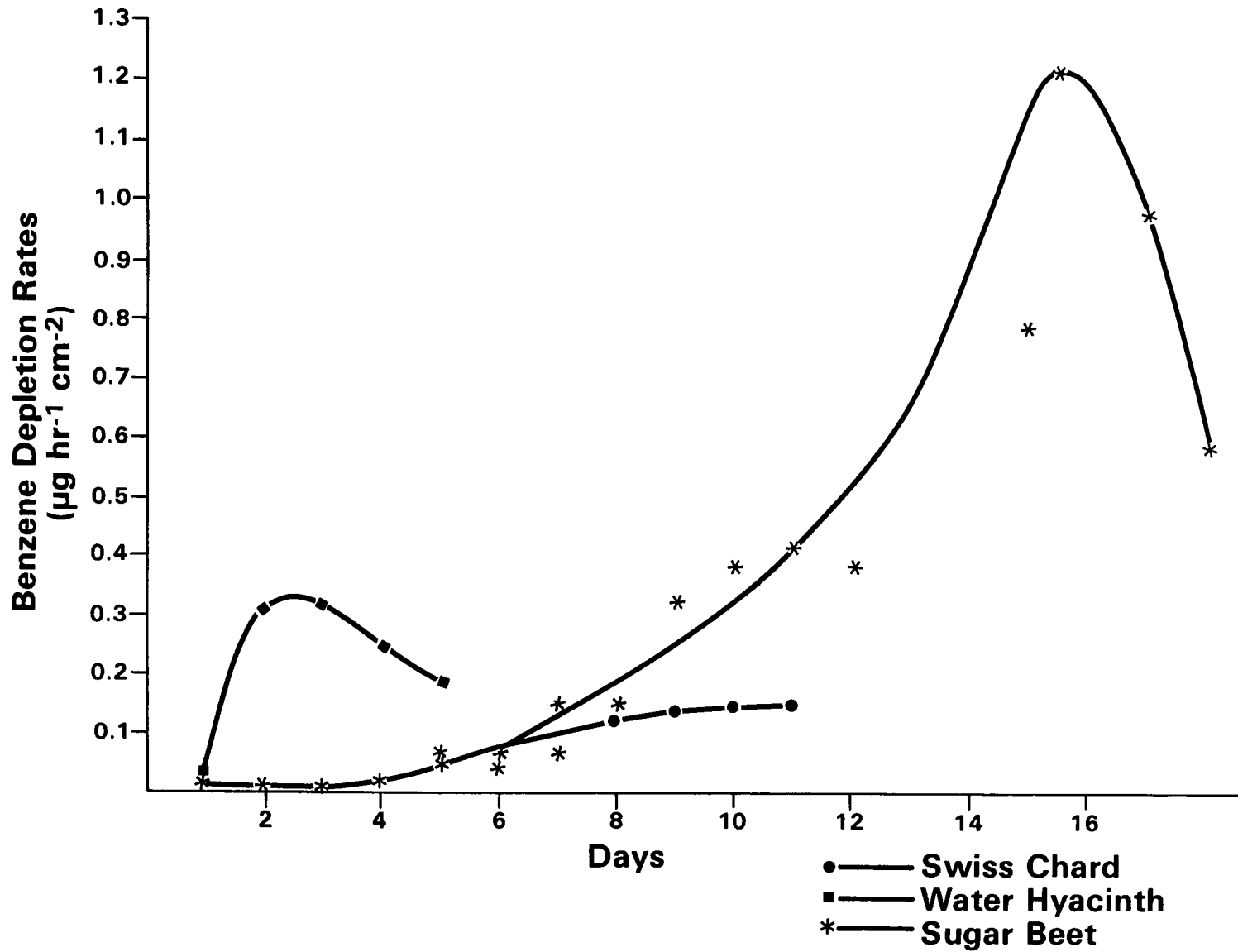


Figure 6. Benzene depletion rates of plant systems during repeated exposures.

benzene was converted to another compound, and not solubilized from the atmosphere by a plant component. This finding parallels the interpretation of the linear uptake which we suggest indicates chemical or a site-limiting reaction.

The study designed to evaluate the effect of different benzene concentrations on depletion rates indicated a relationship between exposure concentration during the lag phase and the benzene absorption rate. Although there was considerable variation in the depletion rates between replicates, the test vessels exposed to the higher concentrations had faster depletion rates (Figure 7). The cause of this is unknown, but could be explained by the induction of an enzyme system, or ingrowth of a microorganism population that is capable of metabolizing benzene.

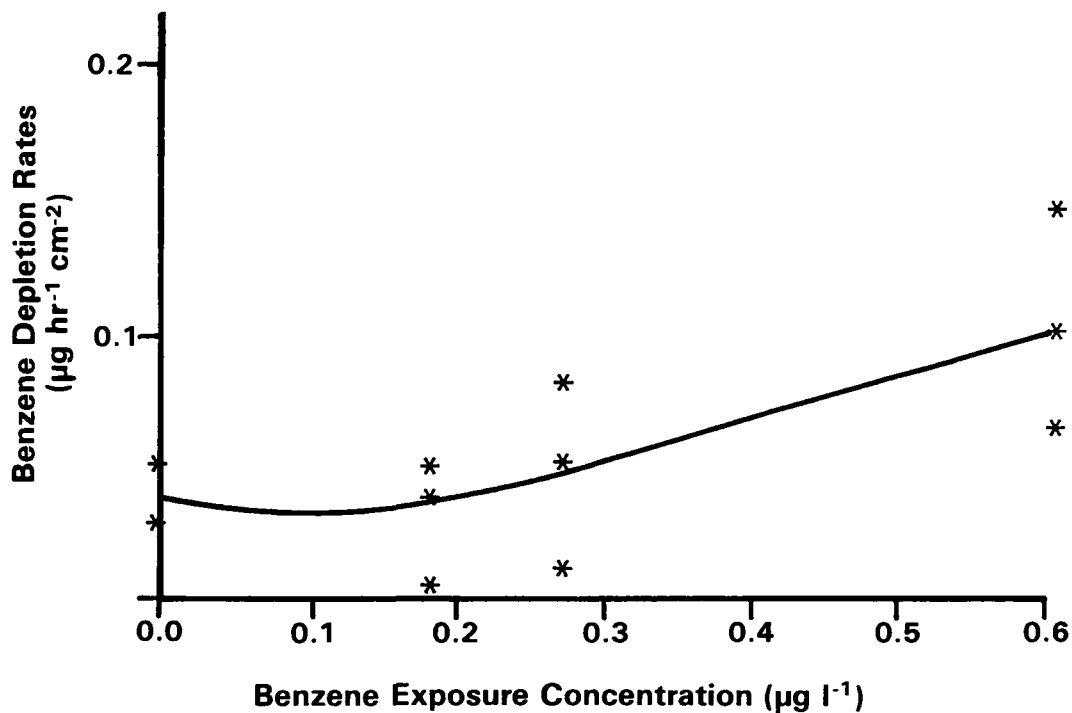


Figure 7. Effect of benzene exposure concentration on depletion rates by sugar beet plants.

These studies demonstrate that plant systems may play a major role in the removal of benzene from the environment. In all studies benzene depletion began after a time lag, the length of which varied with species. The absorption rate appears to be related to the concentration of the benzene exposure during the lag phase. Extracts of microcosm components yield no benzene, suggesting biotransformation of the compound. The component of the plant system which is responsible for the absorption and biotransformation of benzene from the atmosphere has yet to be determined, but clearly a sink exists. The possibility of the absorption of benzene by microorganisms on and in the plants and soil cannot be overlooked.

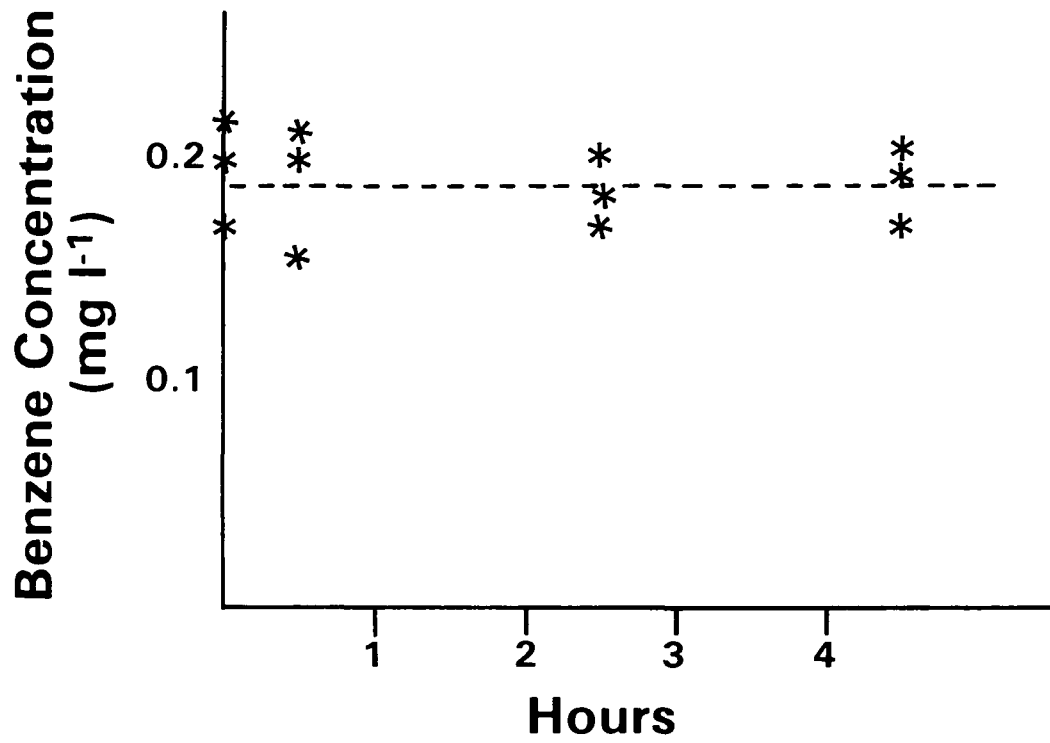
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APPENDIX A. Static benzene concentration reached in test vessel containing water only.

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

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16. ABSTRACT Three plant species, <u>Eichhornia crassipes</u> in a nutrient hydroponic culture <u>Beta vulgaris saccharifera</u> , and <u>Beta vulgaris cicla</u> in soil and in water cultures, were found to deplete benzene from the air. Following benzene depletion, plant tissues were extracted and no benzene was detected. This suggests that benzene was completely utilized within the test system and that it was degraded to other chemicals.				
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