APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1980, p. 165-171
0099-2240/80/01-0165/07$02.00/0
Vol. 39, No. 1

Tenax-GC Extraction Technique for Residual Polychlorinated Biphenyl and Polyaromatic Hydrocarbon Analysis in Biodegradation Assays

M. P. SHIARIS, T. W. SHERRILL, AND G. S. SAYLER*

Department of Microbiology and the Graduate Program in Ecology, The University of Tennessee, Knoxville, Tennessee 37916

A rapid Tenax-GC extraction technique has been evaluated for use in conjunction with aqueous biodegradation assays for polyaromatic hydrocarbons and polychlorinated biphenyls. The method was quantitatively efficient and reproducible for phenanthrene, but variable and not quantitative for Aroclor 1254 (polychlorinated biphenyls). Aqueous sample volumes and varying concentrations of organic matter influenced polychlorinated biphenyl and polyaromatic hydrocarbon extraction efficiency. Phenanthrene recovery was decreased by soil extract but unaffected by spent bacteriological culture medium. Both types of organic matter caused significant reduction of Aroclor 1254 recovery. Polyaromatic hydrocarbon and polychlorinated biphenyl biodegradation assays, performed with reservoir samples, supported the laboratory evaluation. The study demonstrated the utility of the Tenax-GC extraction technique for phenanthrene analysis in biodegradation assessment; however, Tenax-GC extraction was not appropriate for Aroclor 1254 biodegradation studies.

The nearly ubiquitous contamination of surface waters by polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbons (PAH) has stimulated numerous investigations into the sources, occurrence, and biodegradation of these environmental contaminants (2, 6, 11, 14). Analytical techniques employed in the extraction and concentration of these aquatic toxicants are numerous and may include solvent batch extraction (9), synthetic resin adsorption (10), urethane foam plug extraction (3, 12), or membrane filter extraction (8).

In studies on the environmental biodegradation of both PCB and PAH in aquatic habitats, it became obvious that a rapid, relatively inexpensive, yet efficient extraction procedure would greatly facilitate the processing of large numbers of biodegradation samples. Recently, Tenax-GC, a polymer of diphenylphenylene oxide, was demonstrated to be effective in the extraction of PAH from aqueous samples. A number of variables could adversely affect the routine efficiency and use of Tenax-GC extraction of test contaminants in biodegraded samples (1, 15). Of particular importance in these studies are the interaction of the elution solvent with extractant and the resulting contamination of the extracted test contaminant. In addition, the presence of particulate matter and dissolved organic compounds, indigenous to natural water samples or from bacterial metabolism, could significantly reduce the extraction efficiency and recovery of the test contaminant. Such effects have previously been encountered by other investigators using a variety of other extraction techniques (1, 3, 12, 15). Consequently, studies were undertaken to assess the use of Tenax-GC as an extraction polymer for biodegradation samples and to determine factors affecting the efficient recovery of both PAH and PCB.

MATERIALS AND METHODS

Chemicals. Aroclor 1254 (Monsanto Co., St. Louis, Mo.) (54% chlorine by weight, predominantly tri-, tetra-, and pentachlorobiphenyls) was employed as a model PCB mixture in the PCB Tenax extraction and biodegradation studies. Glass-distilled, pesticide-grade hexane (Burdick and Jackson Laboratories, Inc., Muskegon, Mich.) was the solvent used for all PCB extractions. Reagent-grade phenanthrene (PHE; Eastman Organic Chemicals, Rochester, N.Y.) was used as a model compound for examining low-molecular-weight PAH Tenax extraction efficiency. Pesticide-grade hexand and acetone (Fisher Scientific Co., Pittsburgh, Pa.) were employed in all PAH extraction studies and glassware preparation. [9-^{14}C]PHE, 13 mCi/mmol (New England Nuclear Corp., Boston, Mass.), and [*U*-^{14}C]PCB (54% chlorine by weight), 313 mCi/mmol, were used in extraction efficiency studies.

Preparation of Aroclor 1254 and PHE-saturated aqueous solutions. Saturated aqueous solutions of Aroclor 1254 and PHE were prepared in the following manner: acetone-washed, 4.5-liter virgin glass carboys were filled with 3.0 liters of distilled water. To each carboy, containing a Teflon stir bar, 1.0 g of Aroclor 1254 or PHE was added. The carboys

were sealed with an aluminum foil-covered rubber stopper and autoclaved for 1 h at 120°C. Each carboy was then placed on a magnetic stir plate and stirred for a period of 2 months at room temperature. The carboys were then allowed to equilibrate unstirred for an additional 2 months, at which time saturation was assumed complete. Moderate care was taken to avoid exposure of the carboys to sunlight or intense fluorescent light.

Organic matter preparation. To study the effects of naturally occurring dissolved organic matter on the efficiency of Tenax extraction, two representative sources of dissolved organic matter were prepared. An extract of soil organic matter was prepared by adding 10% (wt/vol) soil to 1.0 liter of distilled water. The mixture was heated to 80 to 90°C for 1 h with moderate stirring, and residual soil was removed by clarification through cheesecloth. The clarified soil extract was prefiltered through Whatman no. 1 filter paper and passed through a 0.45-μm Gelman cellulose membrane filter (Gelman Instrument Co., Ann Arbor, Mich.). The resulting extract was sterile and dark golden in color.

A second source of dissolved organic matter was prepared from a spent bacteriological culture medium. A 500-ml amount of yeast extract peptone glucose broth (13) was inoculated with 10 ml of raw lake water. The mixed bacterial culture was incubated for 48 h at 20°C with constant agitation. The bacterial suspension was removed by centrifugation at 3,000 × *g* for 10 min, and the spent culture supernatant was filtered through a 0.2-μm pore size membrane filter (Nuclepore Corp., Pleasanton, Calif.). The dissolved organic carbon concentration of each of the extracts was determined by wet persulfate combustion (4). The dissolved organic carbon concentrations for the soil extract and the culture media extract were 390 mg of C liter^{-1} and 970 mg of C liter^{-1}, respectively.

Tenax-GC extraction column. A 60/80-mesh Tenax-GC (Applied Sciences Laboratories, Inc., State College, Pa.) extraction column was prepared by packing a long-stem (15-cm) glass filtration funnel with 10 cm of Tenax-GC (Fig. 1). The extraction polymer was held in place with pyrex glass wool plugs at either end. The final dimensions of the column were 10 by 0.4 cm. The column was inserted into a nylon mini-adapter (Ace Glass Inc., Vineland, N.J.) and fitted with a Teflon O-ring seal and vacuum adapter (threaded female port with a 20/40 ground-glass male port). A 15-ml concentration tube (Kontes, Vineland, N.J.) with a 24/40 ground-glass neck was used as a receiving vessel for the column eluate. A negative pressure of 5 lb/in^2 was applied to the column to facilitate the extraction procedure.

Gas chromatography. Aroclor 1254 concentrations were determined by injecting 1 μl of hexane eluate into a Perkin-Elmer 3920 gas chromatograph equipped with a ^{63}Ni electron capture detector. The GC column was packed with 5% SE-30 on Chromosorb WHP 100/200 mesh in a glass column (108 inches [ca. 274.32 cm] by 0.25 inches [ca. 0.635 cm] outer diameter; 2 mm inner diameter). Nitrogen was employed as the carrier gas at a flow rate of 60 ml/min, and the temperature parameters were as follows: injector, 290°C; column, 240°C isothermal; and detector, 300°C.

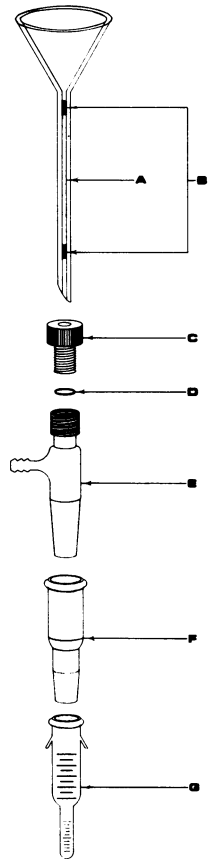

FIG. 1. *Tenax extraction apparatus. Components: (a) Tenax-GC column, 10 by 0.4 mm; (b) pyrex glass wool plugs; (c) nylon fitting; (d) Teflon O-ring, (e) vacuum adapter (mini-adapter, Ace Glass Co.); (f) 24/40-19/22, ground glass adapter; (g) 15-ml concentrator tube (Kontes).*

Aroclor 1254 was quantitated from the total area of all the major PCB peaks with the aid of a Perkin-Elmer M-2 integrating calculator.

Quantitation of PAH in the hexane elutions was performed with a Perkin-Elmer 3920 gas chromatograph equipped with a flame ionization detector. The GC column was packed with 3% SE-52 on Chromosorb WAW DAMCS 80/100 mesh in a column (108 by 0.25 inches outer diameter; 2 mm inner diameter). Nitrogen was employed as the carrier gas at a flow rate of 50 ml/min with the following temperature parameters: injector, 300°C; column, 180 to 240°C at 8°C/min; and detector, 310°C. PHE was quantitated by comparison to a standard peak with the aid of the integrating calculator.

Tenax extraction efficiency and substrate recovery. Radiolabeled PCB and PHE were employed to determine the net recovery and extraction efficiency of the Tenax extraction technique. In PHE extraction efficiency studies, 50 to 100 μg of [9-^{14}C]PHE, in 10 μl of acetone, was added to 100-ml volumes of filter-

sterilized lake water in 250-ml, screw-cap Erlenmeyer flasks. After mixing and equilibration, a 1.0-ml portion was removed and placed in a 15.0 ml, 0.8% Omnifluor in dioxane, liquid scintillation cocktail and counted on a Tracor 6892 liquid scintillation counter, using a preset ^{14}C window. The remaining sample was extracted via the Tenax column and the extracted aqueous sample was again subsampled and counted. The Tenax column was eluted with two 5.0-ml volumes of hexane, and a portion was removed from each eluate and counted before and after evaporative concentration to 1.0 ml (Kuderna Danish concentrator and Kontes tube heater block). PCB extraction efficiency was determined in an identical manner, except that a concentration of 5 μg of [U-^{14}C]PCB 100 ml^{-1} of filter sterilized lake water was employed.

Additional studies were performed for PHE extraction efficiency and recovery in which gas-liquid chromatographic quantitation was employed to determine residual PHE in various sample fractions. These studies, which simulated degradation assessment studies, used 100 ml of filter-sterilized lake water to which 10 mg of Celite and 100 μg of PHE were added. The samples were vacuum filtered through Whatman no. 1 filter circles and the Tenax column and eluted with two 5.0-ml volumes of hexane. The filters were returned to their respective flasks and extracted with 20 ml of hexane in the presence of 10 g of Na_2SO_4. The filter extracts and the column eluates were subjected to gas-liquid chromatographic analysis for residual PHE before and after evaporative concentration to 1.0 ml final extract volume.

Effects of aqueous sample loading and dissolved organic matter on Tenax extractions. A series of experiments were performed to determine the effects of aqueous sample size and dissolved organic matter on the recovery of PHE and Aroclor 1254 (PCB) by Tenax extraction. Three volumes, 50, 100, and 200 ml, of PCB- or PHE-saturated water were extracted by the prescribed technique. Each eluate of triplicate samples for each volume extracted was concentrated to 1.0 ml and was analyzed via gas-liquid chromatography for quantitating substrate recovery. For assessing the influence of dissolved organic matter on Tenax extraction efficiency, 0.01, 0.1, 1.0, and 5.0% solutions of either soil extract or culture medium extract were prepared in 100 ml of PCB- or PHE-saturated water. Extraction, concentration, and quantitation techniques employed were identical to those described vide supra.

Biodegradation assay. Water samples (100 ml each), taken from Center Hill Reservoir in central Tennessee, were placed in Teflon-lined, screw-cap, 250-ml Erlenmeyer flasks and supplemented with 100 μg of PHE or 10 μg of Aroclor 1254. A 10-mg amount of Celite (Fisher) was added to each flask as an inert carrier to reduce losses caused by volatilization. Autoclaved water samples were treated in an identical manner and used as sterile controls for assessment of PAH degradation. The assays were performed in triplicate and incubated at 25°C in the dark for 4 to 8 weeks. Upon termination of the assays, a Whatman no. 1 filter paper circle was placed in the bowl of the Tenax extraction column. Filtration and elution of the Tenax column extraction of the filterable particulates was performed as previously described. Biodegradation was calculated by comparing the total substrate recovery of the degradation flasks to the recovered substrate in the sterile control flasks.

Data analysis. Significance between the controls and organic matter treatments was tested by analysis of variance. The Student-Neuman-Keules multiple-range test was used to test for significant differences among the different organic matter treatments.

RESULTS

Tenax extraction efficiency and substrate recovery. Approximately 99% of the [9-^{14}C]PHE and 96% (combined aqueous phase and glassware residual) of the [*U*-^{14}C]PCB added to the reaction vessels were recovered upon initial subsampling before Tenax extraction (Tables 1 and 2). After Tenax extraction, greater than 98% of the PHE was recovered in the hexane eluate of the Tenax column (Table 1). However, only 67% of the added PCB (78% of the aqueous phase subsample) was recovered in the hexane eluate, whereas an additional 16.6% was apparently not extracted and recovered in the aqueous phase residual (Table 2). It was also found that concentrating the hexane eluate of the Tenax column (from 5.0 ml to a final analytical volume of 1.0 ml) resulted in analytically reproducible losses of 45 and 50% of the PHE and PCB,

TABLE 1. *Comparative analytical recovery of PHE after Tenax extraction*

Method	PHE	
	Concn (μg/liter)	Recovered (%)
[^{14}C]PHE analysis[a]		
Aqueous phase	500	99.1 ± 1.4
Before extraction	1,000	98.4 ± 1.1
Column eluate	500	98.7 ± 21.0
Before concn	1,000	117.2 ± 11.2
Column eluate	500	51.4 ± 8.0
After concn	1,000	56.3 ± 0.8
GLC analysis[b]		
Column eluate before concn	1,000	64.9 ± 1.8
Filter extract before concn	1,000	31.3 ± 4.2
Total PHE before concn	1,000	96.2 ± 1.1
Column eluate after concn	1,000	31.0 ± 3.3
Filter extract after concn	1,000	10.5 ± 1.4
Total PHE after concn	1,000	41.5 ± 3.5

[a] PHE recovery quantitated by using liquid scintillation counting.

[b] PHE recovery quantitated by gas-liquid chromatography (GLC). Experimental design identical to biodegradation assessment, except sterile water rather than raw lake water was employed.

respectively (Tables 1 and 2). Separate experiments later demonstrated that this loss could be avoided by evaporating the eluate to a minimum volume of 2.0 ml.

Comparatively, similar results for Tenax-PHE extraction were obtained from simulated biodegradation assays using sterile water in which PHE was quantitated by gas-liquid chromatographic analysis (Table 1).

Approximately 96% of the total PHE added to the biodegradation vessels was recovered by Tenax extraction and extraction of the filter residue (ca. 65 and 31%, respectively). Concentration of these extracts resulted in similar evaporative losses as previously described.

Effects of aqueous sample size and organic matter on Tenax extraction. PHE- or Aroclor 1254-saturated solutions, 50-, 100-, or 200-ml sample sizes, were extracted via Tenax extraction and eluted with 5.0 ml of hexane. Each eluate was concentrated to 2.0 ml for PCB analysis or 1.0 ml for PHE analysis and quantitated by gas-liquid chromatography. The recovery of PCB or PHE was found to be variable for both substrates (Table 3). The net recovery of PCB from 50- and 100-ml through 200-ml solutions extracted was linear. Net recovery from 50- and 100-ml volumes of PHE-saturated water was linear; upon increasing the sample volume twofold (100 to 200 ml), only a 20% greater level of PHE was recovered.

The effect of organic matter, either soil extract or spent culture medium, significantly affected both Tenax PCB and PHE extraction (Table 4). Soil extract and culture medium significantly decreased PCB extraction efficiency by an average of 41.6 and 27.8%, respectively ($\alpha = 0.01$). Soil extract caused a 12% reduction in Tenax PHE extraction efficiency; however, culture medium had no effect on PHE extraction efficiency. For both Tenax PCB and PHE extraction, neither soil extract nor culture medium resulted in

TABLE 2. *Analytical recovery of [U-^{14}C]PCB after Tenax extraction*

Sample	Mean recovery (%)[a]
Aqueous phase (before extraction)	89.4 ± 6.7
Aqueous phase residual (after extraction)	16.6 ± 7.4
First eluate (before concn)	67.2 ± 6.8
First eluate (after concn)	34.4 ± 6.6
Second eluate	2.5 ± 2.7
Reaction vessel residual	6.8 ± 2.4

[a] Relative to the known total disintegrations per minute added to the reaction mixtures.

TABLE 3. *Effect of aqueous sample size on Tenax extraction efficiency of Aroclor 1254- and PHE-saturated solutions*

Substrate (ml)[a]	Recovery[b]		Concn (liter^{-1})
	Mean (μg)	C_V (%)	
Aroclor 1254[c]			
50	4.0	7.1	80.0
100	7.3	6.1	73.0
200	17.9	20.5	89.5
PHE[d]			
50	49.1	4.0	983.2
100	98.7	2.1	987.4
200	117.8	18.6	589.0

[a] Aqueous samples taken from 3.0-liter stock solution of either Aroclor 1254- or PHE-saturated distilled water.
[b] Mean recovery and coefficient of variation (C_V).
[c] Column eluates concentrated to 2.0 ml.
[d] Column eluates concentrated to 1.0 ml.

TABLE 4. *Comparative effect of dissolved organic matter on the Tenax-GC extraction of Aroclor 1254 and PHE*

Organic matter	Aroclor 1254 recovered (μg)	Relative recovery efficiency (%)[a]	Phenanthrene recovered (μg)	Relative recovery efficiency[a]
Soil extract				
0.01%	2.8 ± 3.6	38.2	38.8 ± 2.6	79.0%
0.1%	2.5 ± 1.2	33.9	43.6 ± 0.5	88.9%
1.0%	2.6 ± 2.9	35.7	43.7 ± 0.2	89.0%
5.0%	3.1 ± 6.4	42.5	45.3 ± 4.0	92.3%
Mean efficiency		37.6 ± 3.7		87.3 ± 5.6
Culture medium				
0.01%	3.5 ± 5.3	47.9	48.0 ± 3.9	97.8%
0.1%	3.7 ± 2.5	50.6	50.4 ± 0.2	102.6%
1.0%	3.4 ± 4.0	46.6	52.9 ± 5.2	107.7%
5.0%	3.0 ± 1.2	41.2	50.3 ± 0.7	102.4%
Mean efficiency		41.5 ± 4.0		102.6 ± 4.0

[a] Extraction efficiency relative to the recovery of Aroclor 1254 or PHE from 100 ml of Aroclor 1254- or PHE-saturated water (Table 3).

a significant dose effect upon increasing the organic matter concentration.

Biodegradation assessment. The results of 2-month PCB biodegradation assessment indicated no apparent Aroclor 1254 biodegradation in those samples supplemented with 100 μg of Aroclor 1254 liter^{-1} as compared to sterile control samples (Table 5). Net recovery of Aroclor 1254 was 69.2%, which was higher yet insignificantly different from that of control flask (net recovery, 48.7%). It was observed that there was a significant difference in localization of the Aroclor 1254 in the control samples versus the biodegradation samples. In control samples, the majority of the Aroclor 1254 was associated with filterable particulates (56.2%), whereas the next greater fraction was associated with glass surfaces (31.5%). In the biodegradation samples, the majority of the Aroclor was recovered from glass surfaces (67.8%), whereas only 17.2% was associated with filterable particulates. In both the control and biodegradation samples, less than 20% of the total recovered PCB was actually in the aqueous phase.

The applicability of Tenax extraction to PHE biodegradation assessment is demonstrated in Table 6. The net recovery of PHE in sterile control samples was found to be 77.5 ± 10.5%. Approximately 15% of the PHE was recovered in the Whatman no. 1 particulate fraction. The Tenax extraction accounted for 62.5% of the total PHE recovered, which compares well with the 61.6% PHE recovery for 100 ml of PHE-saturated water (Table 1).

The relative biodegradation of PHE in six diverse samples was variable, ranging from 66.3 to 90.4%, relative to the sterile controls (Table 4). For the six biodegradation samples, there was an average of an 11-fold greater recovery of Tenax-extractable PHE as compared by PHE associated with extracted particulates.

DISCUSSION

Tenax-GC extraction efficiencies ranging from 98.5 to 99.6% have been demonstrated for high-molecular-weight PAH at concentrations as low as 0.08 μg liter^{-1} (9). Comparable results for Tenax PHE analytical extraction efficiency (i.e., mean recovery using either GC or scintillation quantitation, 106%) were obtained in the present study for higher substrate concentrations employed in biodegradation assessment.

Two volatilization analytical losses were observed in this study which can be accommodated by the investigator. An apparent loss of substrate by volatilization (ca. 2 and 4%, respectively, for PHE and PCB [Tables 1 and 2]) was encountered in efficiency studies, using membrane-filtered lake water. Herbes has previously shown that up to 15% of a radiolabeled anthracene can be lost in short-term incubations (7). However, in the presence of particulate matter (yeast cells), it was demonstrated that the surface adsorption-desorption phenomena occur ki-

TABLE 5. *Recovery of Aroclor 1254 after 2-month biodegradation assessment*

Sample type	Net recovery (%)[a]			Total recovery (μg)[a]
	Aqueous phase	Filterable particulates	Glass residual[b]	
Sterile controls	12.3 ± 6.0	56.2 ± 0.1	31.5 ± 8.6	4.9 ± 2.0
Biodegraded	19.6 ± 3.0	17.2 ± 10.0	67.8 ± 9.8	6.9 ± 1.8

[a] Mean ± standard deviation; initial substrate concentration, 10 μg in 100 ml of lake water.
[b] Residual recovered from surfaces of the reaction vessel.

TABLE 6. *Tenax extraction recovery of residual PHE from biodegradation samples*[a]

Sample site	% PHE recovered[b]			Relative biodegradation (%)[d]
	Tenax extraction	Extracted particulates[c]	Total recovery	
Sterile control	62.5 ± 10.9	15.0 ± 4.5	77.5 ± 10.5	
1	24.9 ± 10.5	1.2 ± 0.2	26.1 ± 10.6	66.3 ± 13.7
2	5.6 ± 7.0	1.7 ± 0.9	7.5 ± 7.1	90.4 ± 9.2
3	15.6 ± 6.4	0.8 ± 0.4	16.4 ± 6.3	78.9 ± 8.1
4	14.9 ± 2.2	2.0 ± 0.9	17.0 ± 1.9	78.0 ± 2.4
5	13.3 ± 2.0	2.1 ± 1.3	15.5 ± 1.3	80.0 ± 1.8
6	11.6 ± 0.6	1.3 ± 0.3	12.9 ± 0.8	83.3 ± 1.0

[a] Six sites sampled, each biodegradation assay performed in triplicate.
[b] Average of three determinations ± standard deviation.
[c] Hexane extraction of prefiltered samples (see text for procedure).
[d] Biodegradation relative to the sterile control samples.

netically much faster than the rate of PAH volatilization in the presence of detrital matter; consequently, microbial cells in whole lake water would render the initial PAH substrate more susceptible to microbial attack as a result of longer residence time. A practical modification in the procedure described here would be the elimination of the negative pressure extraction step and the use of a moderate positive pressure to facilitate the column flow rate and reduce potential volatilization. Significant, reproducible analytical losses upon concentrating the column eluate could be accommodated by the investigator. However, by gently concentrating the elution volume to 2.0 ml rather than 1.0 ml, the volatilization of the extracted substrate is reduced to insignificant levels.

Calculation of the aqueous saturation concentration of PCB normalized to 78% relative efficiency yields a mean value of 134 μg liter^{-1}. This saturation concentration is in the range of values reported by other investigators and is approximately twofold greater than solubilities reported by Haque et al. (5). An estimate of PHE solubility (normalized to the 45% loss on concentration), from the linear values reported in aqueous saturation studies (Table 3), is 1.79 mg liter^{-1}, which compares with a solubility of 1.6 mg liter^{-1} reported by Wodzinski and Johnson (16). It is apparent that PHE recovery decreased significantly upon increasing the total PHE column load from 100 to 200 μg.

Those studies on the effects of soluble organic matter on substrate recovery by Tenax extraction potentially demonstrated the differential recovery of PHE and PCB. It is evident that Tenax PCB extraction is inefficient due to either a solubilization by surfactants or nonpolar components of the organic matter complex or to their adsorption onto the Tenax column effectively reducing adsorptive sites for PCB adsorption. These results suggest that the threshold effect of organic matter upon extraction efficiency lies below the 0.01% concentration. The equivalent dissolved organic carbon concentration at this concentration of soil extract in water is 39 μg of C liter^{-1}, which is far below the dissolved organic carbon levels encountered in natural surface waters.

In any case, it is apparent that although Tenax is effective for PAH extraction in biodegradation assessment, PCB extraction is inefficient and subject to serious analytical difficulties. These results are further supported by the individual PCB or PHE biodegradation studies. PCB biodegradation was demonstrated to be highly variable, due to both the extraction technique and perhaps wall growth and accumulation of PCB by microorganisms. In essence, if PCB biodegradation were occurring, it could not be detected. PHE biodegradation indicated substantial biodegradation (decomposition plus substrate transformation) relative to sterile control samples. In addition, replicate variability was low, which contributed to the sensitivity of the biodegradation assessment technique. It should be noted that biodegradation in this study represents total environmental loss, and not strict substrate metabolism. The results of these studies indicate the Tenax-GC extraction is a valuable tool for the analysis residual PHE substrate, after biodegradation, and suggest its potential use for other PAH substrates.

ACKNOWLEDGMENTS

This investigation was supported by grants from the National Institutes of Health (ESO-152102) and the U.S. Department of the Interior, the Office of Water Research and Technology (14-34-0001-9096), and by Union Carbide subcontract 7182, Oak Ridge National Laboratory.

LITERATURE CITED

1. **Acheson, M. A., R. M. Harrison, R. Perry, and R. A. Wellings.** 1976. Factors affecting the extraction and analysis of polynuclear aromatic hydrocarbon in water. Water Res. **10**:207-212.
2. **Andelman, J. B., and M. J. Suess.** 1970. Polynuclear aromatic hydrocarbons in the water environment. Bull. W.H.O. **43**:479-508.
3. **Bedford, J. W.** 1974. The use of polyurethane foam plugs for extraction of polychlorinated biphenyls (PCB's) from natural waters. Bull. Environ. Contam. Toxicol. **12**:622-625.
4. **Gilmour, C. M., F. Merryfield, F. Burgess, L. Purkerson, and K. Carswell.** 1961. Persulfate oxidizable carbon and BOD as a measure of organic pollution in water. 15th Ind. Waste Conf. Purdue Univ. **106**:143.
5. **Haque, R., D. W. Schmedding, and V. H. Freed.** 1974. Aqueous solubility, adsorption and vapor behavior of polychlorinated biphenyl Aroclor 1254. Environ. Sci. Technol. **8**:139-142.
6. **Harvey, G. R., and E. G. Steinhauer.** 1976. Transport pathways of polychlorinated biphenyls in Atlantic water. J. Mar. Res. **34**:561-575.
7. **Herbes, S. E.** 1977. Partitioning of polycyclic aromatic hydrocarbons between dissolved and particulate phases in natural waters. Water Res. **11**:493-496.
8. **Kurtz, D. A.** 1977. Adsorption of PCB's and DDT on membrane filters—a new analysis method. Bull. Environ. Contam. Toxicol. **17**:391-398.
9. **Leoni, V., G. Puccetti, and A. Grella.** 1975. Preliminary results on the use of Tenax^{R} for the extraction of pesticides and polynuclear aromatic hydrocarbons from surface and drinking waters for analytical purposes. J. Chromatogr. **106**:119-124.
10. **Osterroht, C.** 1974. Development of a method for extraction and determination of non-polar, dissolved organic substances in sea water. J. Chromatogr. **101**:289-298.
11. **Risebrough, R. W., P. Rieche, D. B. Peakal, S. G. Herman, and M. N. Kirven.** 1968. Polychlorinated biphenyls in the global ecosystem. Nature (London) **220**:1098-1102.
12. **Saxena, J., J. Kozuchowski, and D. K. Basu.** 1977. Monitoring of polynuclear aromatic hydrocarbons in water. I. Extraction and recovery of benzo(a) pyrene

with porous polyurethane foam. Environ. Sci. Technol. 11:682–684.

13. **Sayler, G. S., L. C. Lund, M. P. Shiaris, T. W. Sherrill, and R. E. Perkins.** 1979. Comparative effects of Aroclor 1254 (polychlorinated biphenyls) and phenanthrene on glucose uptake by freshwater microbial populations. Appl. Environ. Microbiol. **37**:878–883.
14. **Suess, M. J.** 1976. The environmental load and cycle of polycyclic aromatic hydrocarbons. Sci. Total Environ. **6**:239–250.
15. **Vick, R. D., J. J. Richard, H. J. Svec, and G. A. Junk.** 1977. Problems with Tenax-Gc for environmental sampling. Chemosphere **6**:303–308.
16. **Wodzinski, R. S., and M. J. Johnson.** 1968. Yields of bacterial cells from hydrocarbons. Appl. Microbiol. **16**: 1886–1891.