Bull. Environm. Contam. Toxicol. 25, 824-827 (1980)

Simplified Monitoring Procedures for Benzo(a)pyrene, Hexachlorobenzene and Pentachlorophenol in Water

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Normal methods for quantitative analysis of trace organic pollutants in marine waters necessitate large volumes of sample as well as expensive extraction solvents (GUERRERO et al. 1976, ISENSEE et al. 1976, SCHIMMEL et al. 1978). Thus, these methods cannot be applied to routine pollutant monitoring in small-volume laboratory test systems, such as bioassay and bioaccumulation measurements. Our experiments on the uptake and depuration of benzo-(a)pyrene (BaP), hexachlorobenzene (HCB), and pentachlorophenol (PCP) in marine organisms required analysis of these compounds on small aliquots of water from the experimental systems. Simplified methods of water analysis were developed which require only a 5.0 mL sample to measure concentrations of pollutants in the tank ranging from 0.2 ppb to 200 ppb (ng/g). The use of these simplified monitoring methods is not only applicable to the title compounds but can be extended to other compounds in their respective categories, such as substituted benzenes and phenols as well as polynuclear aromatics. These methods can also be applied to other aquatic toxicity studies and microbial studies since the volume required for analysis is small. In addition, these simplified monitoring procedures can be applied to the analysis of waste-water effluents or to other situations where concentrations are in the ppb range.

EXPERIMENTAL

Reagents. The extraction solvents, petroleum ether and diethyl ether, were obtained from Burdick and Jackson and isooctane was obtained from Mallinckrodt. HCB, BaP and PCP were obtained from Aldrich and were used without further purification. The vials used were soaked in Micro cleaning solution overnight, rinsed with distilled water, petroleum ether (PE) and benzene, dried at 250°C overnight and then rinsed with PE before use.

Distilled water used as a carrier solvent was extracted with PE. UV grade CH_3CN and MeOH used as carrier solvent were obtained from Burdick and Jackson.

Extraction Procedures. For all compounds, a 5 mL aliquot of the water was placed in a 2 or 3 dram vial with a teflon-lined cap.

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0007-4861/80/0025-0824 $01.00

Samples for BaP analysis were extracted twice with 1.0 mL of petroleum ether and the extracts were combined in a second vial. The solvent was evaporated with a slow stream of dry nitrogen filtered through molecular sieve. The residue was dissolved in 200 µL of MeOH and 50-100 µL were analyzed by high pressure liquid chromatography (HPLC).

For HCB, samples were extracted once with 1.0 mL of isooctane and the extract was directly analyzed by gas chromatography with an electron capture detector (GC-ECD). Generally, 2-10 µL were injected.

For PCP, samples were first acidified with 2-3 drops 6N H_2SO_4 delivered by Pasteur pipet and then extracted twice with 1.0 mL of petroleum ether/diethyl ether (1:1). The combined extracts were placed in a second vial and the solvent was evaporated as described above. The residue was dissolved in 200 µL of CH_3CN and 5-100 µL were analyzed by HPLC.

Apparatus. Benzo(a)pyrene was analyzed using a HPLC unit equipped with a spectrofluorometer. A Water's C-18 µbondapak 4.0 x 300 mm column was used. The solvent was 10% H_2O/MeOH at a flow rate of 1.5 mL/min. The spectrofluorometer was set with an excitation wavelength of 365 nm and an emission wavelength of 403 nm.

Hexachlorobenzene was analyzed using a gas chromatograph equipped with a ^{63}Ni electron-capture detector. A 6' glass column (4 mm i.d.) packed with 5% QF-1 on 100-120 mesh Supelcoport was used at a temperature of 160°C (EDGERTON et al. 1979). The injector port and detector temperatures were 250°C and 300°C, respectively. The carrier gas was 5% methane/argon at a flow rate of 30 mL/min.

Pentachlorophenol was analyzed using a HPLC equipped with a variable UV detector set at 254 nm. The column was a Water's C-18 µbondapak 4.0 x 300 mm. The carrier solvent was 60% CH_3CN/H_2O with 1% HOAc; the flow rate was 2.0 mL/min.

RESULTS AND DISCUSSION

These simplified methods of analysis are modifications of existing methods for the water analysis of BaP (GUERRERO et al. 1976), HCB (ISENSEE et al. 1976), and PCP (SCHIMMEL et al. 1978). However, these modifications have greatly reduced the cost of glassware and solvents required; the time required to perform the analyses is also significantly reduced. These simplified methods were successfully applied to the analysis of water samples taken as part of bioaccumulation experiments conducted in our labs.

Known amounts of BaP, PCP and HCB were dissolved in CH_3CN and added to 5 mL aliquots of instant ocean to test recovery and precision. CH_3CN was used as a solvent to avoid any pre-partition effects. All methods showed excellent precision and gave high

recoveries of spiked samples. The simplified methods gave recoveries comparable to the longer methods of analysis (Table 1).

TABLE 1

Comparison of Simplified and Longer Methods
Values Measured for the Longer Methods are in Parentheses

	Sample Volume (mL)	Recovery (%)	Detection Limit (ppb)
BaP	5 (1,000)	87 (80)	0.04 (0.001)
HCB	5 (1,000)	80 (74)	0.10 (0.005)
PCP	5 (500)	91 (84)	40 (0.2)

Analysis of spiked samples having BaP concentrations (Table 2) ranging from 0.04 ppb to 2.0 ppb gave recoveries greater than 80%. Ten replicate samples (1.0 ppb) gave a relative standard deviation of 6.4%.

Analysis of HCB concentrations (Table 2) greater than 0.5 ppb gave recoveries greater than 75%. Recoveries were lower below 0.5 ppb. Ten replicate samples (1.0 ppb) gave a relative standard deviation of 3.2%.

The results of analyses of PCP concentrations ranging from 40 ppb to 200 ppb are shown in Table 2. Recoveries of greater than 80% were achieved when concentrations were greater than 80 ppb. Ten replicate samples (200 ppb) gave a relative standard deviation of 3.6%.

Minimum amounts necessary to give peak heights twice the baseline noise were determined for benzo(a)pyrene, hexachlorobenzene and pentachlorophenol to be 0.2, $5x10^{-6}$ and 50 ng, respectively. However, considering recovery and reproducibility, the practical detection limits of the simplified methods were 0.04 ppb (BaP), 0.1 ppb (HCB) and 40 ppb (PCP).

The simplified methods of analysis described above have several advantages. The volume of water sample needed is small. The amount of extraction solvent has been reduced to 1-2% that of methods applied to larger samples. The time for preparation and analysis of samples allows for analysis of 8-12 samples/hour. Although the lower limit for these procedures is higher than one would expect in open ocean samples, for monitoring routine laboratory experiments and low level toxicity studies, these methods are excellent.

TABLE 2. Recovery of Benzo(a)pyrene, Hexachlorobenzene and Pentachlorophenol from Spiked Instant Ocean Samples

	Amount Added to 5 mL Instant Ocean (ng)	Concentration (ppb)	Recovery (%)	N
BaP:	0.2	0.04	82	3
	1.0	0.20	88	3
	2.0	0.40	87	3
	5.0	1.0	90	3
	10.0	2.0	85	3
		Mean±SD	87±3	
HCB:	0.5	0.10	50	3
	1.0	0.20	62	3
	2.5	0.50	77	3
	5.0	1.0	82	3
		Mean±SD	80±4*	
PCP:	200	40	74	2
	400	80	105	2
	600	120	96	2
	800	160	91	2
	1000	200	91	2
		Mean±SD	91±11	

*Based on 0.5 and 1.0 ppb concentrations.

ACKNOWLEDGEMENTS

We thank Drs. G. Neff and E. Atlas for helpful comments on this manuscript and Water's Associates, Milford, MA, for solvent conditions for PCP analysis. This work was supported by the National Science Foundation, Grant No. OCE77-25663.

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