A Qualitative Method for Detecting Hydroxylated Metabolites of Polychlorinated Biphenyls

by CARL A. BACHE and DONALD J. LISK
Pesticide Residue Laboratory, Department of Entomology
Cornell University, Ithaca, N.Y. 14850

Polychlorinated biphenyls (PCB's) are now known to be major environmental contaminants. PCB's can induce hepatic hydroxylating enzymes (RISEBROUGH et al. 1968; STREET et al. 1969; LINCER and PEAKALL 1970). Although knowledge of the metabolism of PCB's is speculative, it is possible that hydroxylated derivatives may be produced in biological systems. In the work reported a procedure is described to separate and detect an hydroxylated PCB from a mixture of PCB isomers.

Experimental

Two, 2',6,6'-tetrachloro-p,p'-biphenol (PCBOH) was used as a model compound. A mixture of this compound (0.2 µg/ml) and PCB isomers (Aroclor 1254, 2.44 µg/ml) was prepared in 50 ml of hexane. The solution was extracted successively with a 15 ml and a 10 ml portion of 2% sodium bicarbonate solution. The combined aqueous extracts were extracted with 10 ml of hexane. The pH of the aqueous solution was adjusted to 1 with hydrochloric acid and extracted with two, 50 ml portions of diethyl ether. The combined ether extracts were dried over anhydrous sodium sulfate for 20 minutes, decanted and 0.2 ml of water was then added to the solution. The solution was evaporated with air to 0.5 ml in a graduated centrifuge tube. The volume was adjusted to 4 ml with a solution of 10% methanol in diethyl ether. The solution was methylated with diazomethane by the procedure of SCHLENK and GELLERMAN (1960) to convert PCBOH to 2,2',6,6'-tetrachloro-4,4'-dimethoxybiphenyl for gas chromatography. After removal of excess diazomethane the solution was diluted to 10 ml with ether and finally to 50 ml with hexane. The solution was analyzed by electron affinity gas chromatography using a Varian Aerograph Model 705 gas chromatograph equipped with a nickel-63 detector. The column was glass, 1/8" inside diameter, 4 feet long and packed with 10% DC-200 on 100/120 mesh Gas Chrom Q. The temperatures of the column, flash heater and detector were 185°, 235° and 300°C and nitrogen (24 ml/min.) was the carrier gas.

Results and Discussion

The recoveries of PCBOH when six replicated solutions of the compound (0.2 µg/ml) in the presence of 2.4 ug/ml of Aroclor 1254 in hexane were carried through the procedure were 59.5, 63.7, 68.3, 83.3, 86.1 and 65.0. Phenols are notably volatile and loss of PCBOH during evaporation probably accounts for the recoveries

Bulletin of Environmental Contamination & Toxicology,
Vol. 9, No. 5 © 1973 by Springer-Verlag New York Inc.

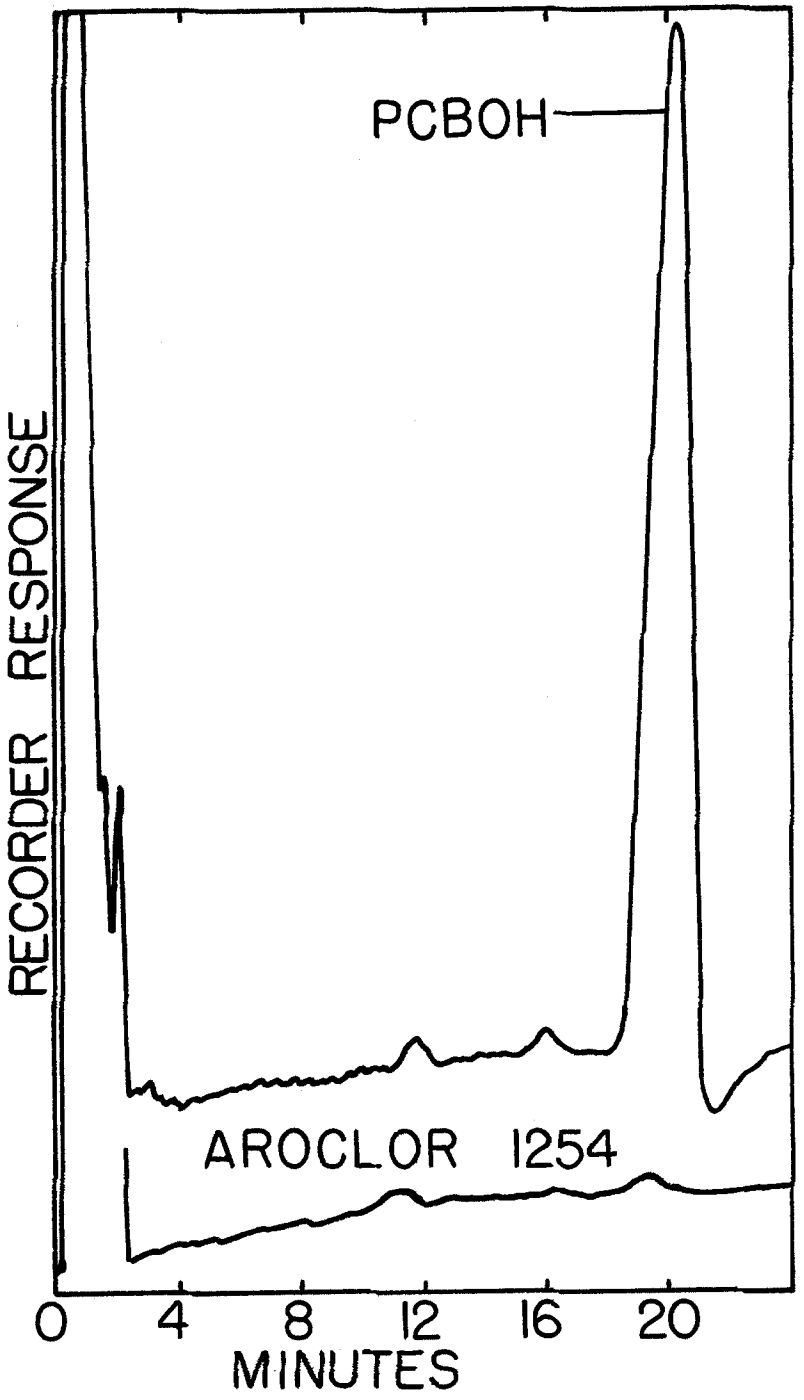

Figure 1. Gas chromatograms (above) of PCBOH (0.6 ng injected) after separation from Aroclor 1254 and (below) Aroclor 1254 (7.2 ng injected) alone carried through the procedure and eliminated.

being less than quantitative. The addition of 0.2 ml of water to the ether was found to reduce the extent of its loss by vaporization. Figure 1 shows chromatograms of PCBOH (as the methyl ether) after separation from Aroclor 1254 and that resulting after the procedure was applied to a solution of Aroclor 1254 alone. The concentrations of PCBOH and Aroclor 1254 were again 0.2 and 2.4 µg/ml, respectively. The retention time of PCBOH methyl ether was about 20.5 minutes. The chromatograms show that PCBOH can be separated from Aroclor 1254 without interference from the latter mixture of PCB isomers. The method may be useful in detecting the presence of possible hydroxylated metabolites of PCB's in biological fluids.

References

LINCER, J. L. and D. B. PEAKALL: Nature 228, 783 (1970).

RISEBROUGH, R. W., REICHE, P., HERMAN, S. G., PEAKALL, D. B. and M. N. KIRVEN: Nature 220, 1098 (1968).

SCHLENK, H. and J. L. GELLERMAN: Anal. Chem. 32, 1412 (1960).

STREET, J. C., URRY, F. M., WAGSTAFF, D. J. and A. D. BLAU: "Comparative effects of polychlorinated biphenyls and organochlorine pesticides in induction of hepatic microsomal enzyme." Presented at ACS meeting September 8-12, New York (1969).