CHROM. 3786

A SENSITIVE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF PENTACHLOROPHENOL IN HUMAN BLOOD*

A. BEVENUE AND M. L. EMERSON
Department of Agricultural Biochemistry, University of Hawaii, Honolulu, Hawaii 96822 (U.S.A.)
AND
L. J. CASARETT** AND W. L. YAUGER, JR.**
Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96822 (U.S.A.)
(Received September 10th, 1968)

SUMMARY

A method for the determination of pentachlorophenol residues in human blood has been designed which involves the simultaneous application of acid pH, mild heat, and agitation of the sample with benzene extractant for the isolation of the pesticide from blood. It is applicable to quantities of one milliliter or less of blood and the detectability limits of the pesticide are in the low parts per billion range.

INTRODUCTION

Pentachlorophenol and/or its sodium salt is extensively used as a wood preservative in areas where insect infestation is a problem, as a contact herbicide in agricultural areas, as a household treatment for termites, and as a disinfectant and mildew retardant. Pentachlorophenol (PCP) is a significant problem in occupational and industrial usage and, to a degree, a hazard to the public; the magnitude of the problem is manifested by the frequency of acute intoxications and occurrence of fatalities from this material. Examples recently reported are industrial intoxications in Canada[1] where one case was fatal; and in Texas[2] where two cases were fatal. Incidents of public intoxication of children from the chemical have been reported in England[3] and, most recently, in the United States[4] where two cases were fatal.

Despite the frequency of acute intoxication for some years, data have been sparse on the subacute or chronic effects of PCP. In Hawaii, where the use of the chemical is heavy, a program has been in progress to search for possible effects of chronic exposure. One facet of the study has included a periodic sampling of the State's general population, including a detailed study of a group occupationally exposed to the chemical[5].

To evaluate chronic effects and to understand more fully the kinetics of human

* Published with the approval of the Director of the Hawaii Agricultural Experiment Station, University of Hawaii, as Journal Series No. 996.
** Community Pesticides Study, Hawaii.

excretion of PCP, a sensitive method was needed for the analysis of this compound in blood. The analytical procedure developed for PCP in urine[6], although sensitive and yielding excellent recoveries, was not applicable to blood analysis, and the available methods for the analysis of this chemical in blood lacked specificity, sensitivity, reproducibility, or convenience[7]. The proposed method described below is relatively rapid, sensitive, and reliable.

APPARATUS AND MATERIALS

Gas chromatographs

Aerograph Model 204-B electron capture detector; 1/8 in. × 5 ft. spiral borosilicate glass column packed with 3 % SE-30 silicone on Chromosorb W, acid-washed (AW) and treated with dimethyldichlorosilane (DMCS), 80–100 mesh; column temperature 160°; injection temperature 200°; detector temperature 200°; nitrogen carrier gas, flow rate 30 ml/min.

F & M Model 810, electron capture detector; 1/4 in. × 4 ft. spiral borosilicate glass column packed with a mixture of 5 % QF-1 silicone (10,000 cS) and 3.3 % DC-200 silicone (12,500 cS) on Chromosorb W (AW, DMCS), High Performance grade, 80–100 mesh; column temperature 170°, injection temperature 200°, detector temperature 200°; argon–methane 90:10) carrier gas, flow rate 75 ml/min.

Both instruments were equipped with Leeds and Northrup Speedomax H recorders, 1 mV full scale, chart speed 0.5 in. per min.

Thin-layer chromatography apparatus

Desaga-Brinkmann apparatus (Brinkmann Instruments Inc., Westbury, N.Y.), including 20 × 20 cm glass plates and the Model S-11 applicator.

Infrared spectrophotometer

Perkin-Elmer Model No. 337, 4X-Beam condenser.

Reagents

Sulfuric acid, reagent grade, 0.1 *N* solution.
Benzene, Mallinckrodt Nanograde.
Heptane, Matheson Coleman and Bell, reagent grade, redistilled.
Hexane, Mallinckrodt Nanograde.
Aluminum Oxide G (E. Merck AG, Darmstadt, Germany).
Rhodamine B, 1 % solution in ethanol.
Diazomethane solution; prepared as previously described[6].

Pentachlorophenol (Eastman Kodak No. 3462) solutions

Stock solution preparation was 100 mg PCP in 100 ml 0.1 *N* sodium hydroxide. Standard solutions of methylated PCP were prepared at concentrations which would allow injections of 10–200 pg of PCP in 5 μl into the gas chromatograph. An aliquot of the stock solution was added to 1 ml of 0.1 *N* sulfuric acid and 5 ml of distilled water in a 30-ml separatory funnel; the mixture was extracted three times with 5-ml portions of benzene. The benzene fractions were combined in a centrifuge tube and evaporated to about 1 ml on a steam bath (40°) with the aid of a stream of filtered air. One milliliter of diazomethane solution was added to the concentrate and the mixture

was allowed to stand for 15 min; then it was aerated with a gentle stream of filtered air, at room temperature, to remove any excess diazomethane, and made to a suitable volume for analysis of the derived methylated pentachlorophenol by gas chromatography.

PREPARATION OF BLOOD SAMPLES

Exploratory studies with citrated whole blood, to which PCP was added, indicated no inherent problems that would inhibit the recovery or measurement of the PCP component with the proposed analytical procedure. Freshly drawn blood, stored in heparinized tubes, was analyzed within 24 h of the time of receipt. Additional blood samples were centrifuged immediately after receipt, the plasma fractions were removed and the red cell fractions were washed twice with physiological saline solution; the saline washes were added to the plasma fractions, and the plasma and red cell fractions were analyzed separately for PCP.

The sample (1–5 ml) was placed in a 125-ml glass-stoppered Erlenmeyer flask to which was added 20 ml of 0.1 *N* sulfuric acid, 12 ml of benzene and a 1-in. Teflon magnetic stirring bar. The flask was stoppered and the contents heated at 50° with constant stirring on a combination hot plate magnetic stirrer for 20 min. At the end of the heating period, the flask was removed from the heater and cooled immediately in an ice bath. The contents of the flask were transferred to a 40-ml centrifuge tube and centrifuged for 10 min; the upper benzene layer was removed by pipette and placed in a graduated test tube. The centrifugate was washed three times with 5-ml portions of benzene, with centrifugation between each wash, and the benzene layers were combined with the first benzene fraction. The benzene fraction was concentrated to about 1 ml on a steam bath (40°) with the aid of a stream of filtered air. One ml of diazomethane solution was added to the concentrate and the procedure continued as described above for the preparation of the standard PCP solutions.

ANALYTICAL PROCEDURES

Standard curves of the pentachlorophenol methyl ether were prepared from data obtained from the gas chromatograph. The curves were linear in the range of 10–200 pg. The volumes of the prepared blood samples were adjusted so that 2–10 μl of the solution would produce chromatographic responses within the linear range.

Thin-layer chromatography was used as a qualitative confirmatory procedure and also as a means for acquiring a sufficient amount of the suspect compound for conclusive identification by I.R. absorption analysis. The diazomethane-treated blood concentrates and PCP methyl ether standards were chromatographed on aluminum oxide G plates (250 μ thickness) by ascending chromatography, using heptane as the developing solvent. The developed plates were air-dried and sprayed with Rhodamine B solution; the blood samples and the PCP ether standards were compared with 3600 Å U.V. light.

The aluminum oxide in the area containing the fraction of the composite sample of the blood of occupationally exposed workers comparable to the R_F value of the PCP ether was scraped from the plate, extracted three times with 1-ml portions of hexane, and the hexane extract was concentrated to about 100 μl with a stream of

filtered air at room temperature. The concentrate was mixed with potassium bromide, made into a 1.5 mm pellet and compared with a similar preparation of a PCP methyl ether standard by I.R. spectrometry.

RESULTS

The PCP standards, the fortified whole blood, and the freshly drawn blood from occupationally exposed individuals agreed closely in the several analytical characteristics examined. The retention times on the columns of 3 % SE-30 silicone and the QF1-DC200 silicone mixture were similar at 3.5 min and 6.3 min, respectively. All samples on thin-layer chromatograms, visualized with Rhodamine B and U.V. light, demonstrated R_F values of about 0.65. The comparative I.R. spectra were practically identical. The spectra for the PCP methyl ether standard and the material isolated from the composite blood sample of the occupationally exposed workers are shown in Fig. 1. All of the data provided convincing evidence that the material isolated from the human blood samples was pentachlorophenol. Ten replicate experiments gave a mean value of 0.81 p.p.m., with a standard deviation of 0.08. The analytical results of fortified bloods are shown in Table I; recoveries of PCP varied from 87 to 100 %, with a mean of 92 %. Pentachlorophenol residue data on the blood of individuals, most of whom were regularly and directly exposed to the chemical at work, are given in Table II. Apart from the expected variations of PCP found in the bloods, the lowest values were those obtained from samples of the bloods of office workers in the wood-treatment plants. Except for subject number 8, the plasma contained virtually all of the PCP in the blood; the cell fraction contained about 1 % of the compound after saline washing. It is noted that as little as 20 parts per billion (p.p.b.) were detected. Although no attempt was made to define the lower limit of

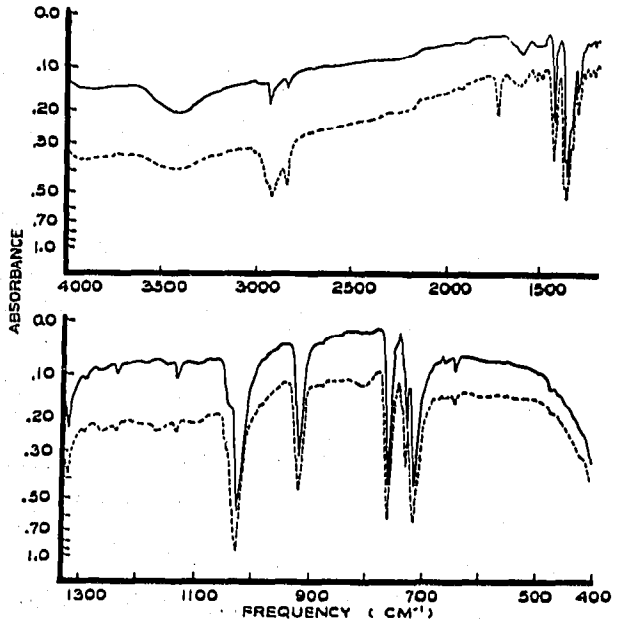

Fig. 1. I.R. spectra of pentachlorophenol methyl ether standard (———) compared with fraction isolated from blood of occupationally exposed workers (– – – –).

TABLE I

RECOVERY OF PENTACHLOROPHENOL FROM BLOOD

Sample[a]	*Amount PCP added* (μg)	*Amount PCP recovered* (μg)	% *recovered*
1	0.25	0.23	92
2	0.25	0.22	88
3	0.50	0.48	96
4	0.50	0.45	90
5	0.50	0.50	100
6	5.00	4.65	93
7	5.00	4.75	95
8	25.0	21.8	87
9	50.0	44.5	89

[a] 5.0 g blood sample.

TABLE II

PENTACHLOROPHENOL IN HUMAN BLOOD[a]

Subject	*Plasma*	*RBC*	*Whole blood*
1			0.34
2			0.84
3			0.87
4			2.45
5			2.57
6			3.95
7			6.01
8	0.99	0.08	
9	4.31	0.02	
10	4.91	0.03	
11	7.59	0.09	
12	8.26	0.04	
13	9.06	0.12	

[a] In p.p.m.

detection in this study, the value of 20 p.p.b. compares favorably with the limits of 3–10 p.p.b. found for the analytical method applied to urine[6].

DISCUSSION

The identification of pentachlorophenol has been sufficiently well established in these studies to warrant the use of the method for the analysis of this compound in blood. Initial studies involving extraction of the blood samples with both polar and nonpolar solvents under variable pH (2 to 12) conditions, or refluxing the samples for variable periods of time at temperatures ranging from 50° to 100° at variable pH (2 – 12) followed by extraction with various solvents were unsuccessful; in all instances, little or no pentachlorophenol was recovered. The precise mechanism by which the described method was successful when others failed is not known. However, it is noted that the conditions of acid pH, heat, agitation, and the presence of the

benzene extractant were simultaneously involved in the proposed extraction procedure. Pentachlorophenol is a weak acid which readily combines with strong bases to give the corresponding water-soluble salt. The highly reactive hydroxyl group of the compound suggests a sufficiently strong bonding to blood components to make simple extraction procedures impossible.

Similar difficulties with other chlorinated pesticides have been reported. For example, DALE *et al.*[8] reported poor recoveries of several chlorinated insecticides from blood extracted with hexane; improved recoveries were experienced by pretreatment of the samples with heat[9]. DALE and coworkers suggested that some of the pesticide was bound to the lipoprotein components of the blood. Similar analytical difficulties have been noted for phenolic products in sweet corn and milk[10] and in pineapple[11]. An obverse situation is among the postulates suggested to explain the relatively low recoveries of dieldrin by hexane extraction in human and dog blood, *viz.*, that the dieldrin may bind to a hydroxy or amino group of the blood components[12]. From human urinary excretion data on PCP[5], it has been suggested that a binding of this type occurs in the blood. This postulate is partially supported by *in vitro* studies with bovine serum albumin[13].

Any detailed correlative analysis or interpretation of the PCP data obtained from the blood and urine of the subject cases is outside the scope of this paper and will be reported separately. The proposed method of analysis for PCP in blood, which involves a benzene–aqueous interface coupled with mild heat and low pH, is sufficiently sensitive, rapid, and reliable to provide data that will permit a better understanding of the biological behavior of concentrations of pentachlorophenol in humans.

REFERENCES

1 H. BERGNER, P. CONSTANTINIDIS AND J. H. MARTIN, *Can. Med. Assoc. J.*, 92 (1965) 448.
2 M. F. MASON, S. M. WALLACE, E. FOERSTER AND W. DRUMMOND, *J. Forensic Sci.*, 10 (1965) 136.
3 J. B. CHAPMAN AND P. ROBSON, *Lancet*, 1965, 1266.
4 J. E. SMITH, L. E. LOVELESS AND E. A. BELDEN, *Morbidity and Mortality Weekly Report, NCDC, PHS, USHEW, Atlanta, Ga.* 16 (October 7, 1967) 334.
5 A. BEVENUE, J. WILSON, L. J. CASARETT AND H. W. KLEMMER, *Bull. Environ. Contam. Toxicol.*, 2 (1967) 319.
6 A. BEVENUE, J. WILSON, E. F. POTTER, M. K. SONG, H. BECKMAN AND G. MALLETT, *Bull. Environ. Contam. Toxicol.*, 1 (1966) 257.
7 A. BEVENUE AND H. BECKMAN, in F. A. GUNTHER (Editor), *Residue Reviews*, Vol. 19, Springer-Verlag, New York, 1967, p. 83.
8 W. E. DALE, A. CURLEY AND C. CUETO, JR., *Life Sci.*, 5 (1966) 47.
9 C. CUETO, JR. AND F. J. BIROS, *Toxicol. Appl. Pharmacol.*, 10 (1967) 261.
10 M. C. BOWMAN AND M. BEROZA, *J. Agr. Food Chem.*, 16 (1968) 280.
11 A. BEVENUE, unpublished information.
12 J. ROBINSON, A. RICHARDSON AND J. M. DAVIES, *Arch. Environ. Health*, 15 (1967) 67.
13 J. R. GIGLIO AND J. MOURA GONCALVES, *Anais. Acad. Brasil Cienc.*, 35 (1963) 293.