Gas Chromatographic Determination of Pentachlorophenol in Human Blood and Urine

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The Hawaii Community Studies on Pesticides has used methods developed by BEVENUE et al. (1966,1968) to determine pentachlorophenol(PCP) residues in human blood and urine. The PCP-blood method has also been applied to the determination of 2,4-D and dicamba in human blood and urine (RIVERS et al. 1970).

The modified PCP analytical procedure described in this paper represents an efficient combination of features taken from the procedures previously used, plus the use of a mechanical mixing technique developed by THOMPSON (1970) for extracting organochlorine pesticides from human serum samples. The procedure retains the accuracy of the procedures previously used, requires less time and equipment to perform, and applies to both human blood and urine.

Experimental

Apparatus. Extractions were carried out in 16X125 mm culture tubes fitted with screw caps with teflon-lined rubber liners. Samples were mixed on a Roto-Rack™, a rotary mixing device available from Fisher Scientific Company.

Gas chromatographic determinations were made on a MicroTek MT 220 gas chromatograph under conditions previously described(RIVERS et al. 1970).

Extraction and Methylation. Two ml of human blood plasma or urine were combined with 6.0 ml of benzene and 2 drops of conc. H_2SO_4 in a culture tube. The tube was sealed and rotated on the "Roto-Rack" for two hours at 50 r.p.m. After mixing, the sample was centrifuged briefly to bring about phase separation, and 3.0 ml (representing 1.0 ml of sample) of the benzene (top) layer were transferred to a 10-ml volumetric flask. Diazomethane solution (0.2 ml), prepared as previously described (RIVERS et al. 1970), was added to the extract, and the mixture was allowed to stand for 15 min. Excess diazomethane was removed by gently bubbling dry nitrogen through the extract until the yellow color disappeared, and the extract was diluted to 10.0 ml with isooctane.

Gas Chromatography. The methylated extract was diluted as necessary, and 2 to 8 ul were injected into the gas chromatograph. Following each extract injection, an amount of PCP standard (as

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its methyl ether) which gave a response of similar peak height (±10%) was injected, and results were calculated by direct comparison of peak height.

Results and Discussion

Results of a recovery study to determine the accuracy and reproducibility of the method over the range of PCP levels encountered in samples in this laboratory are presented in Table 1.

TABLE 1

Recovery of PCP from samples fortified before extraction.

Sample	PCP found (p.p.m.)	PCP added (p.p.m.)	Average[a] (p.p.m.)	Recovery (%)	Std. Dev. (%)
Blood	0.19	0.10	0.28	90	±3.1
Plasma		0.50	0.67	96	±3.2
		5.00	4.66	89	±1.2
		50.0	45.8	91	±6.7
Urine	0.01	0.10	0.10	90	±2.7
		0.50	0.48	94	±4.0
		5.00	4.82	96	±2.1
		50.0	49.5	99	±1.2

[a]Average of three values.

PCP levels in pooled blood plasma and urine samples were determined by the methods of BEVENUE et al.(1966,1968). Aliquots of the samples were then fortified with PCP at various levels (see Table 1), and each fortified aliquot was analyzed in triplicate. Recoveries of PCP ranged from 89% to 96% for blood plasma and from 90% to 99% for urine. Standard deviation from the mean value was less than ±5% for each type of sample, and the limit of detectability for the method is 0.01 p.p.m.

A comparison of PCP levels found in routine blood samples by the method of BEVENUE et al.(1968) and by the method detailed in this paper is given in Table 2. As expected, the results obtained

TABLE 2

Comparison of PCP levels found in routine blood plasma samples by two methods.

Sample	PCP found (p.p.m.)	
	Method of BEVENUE et al.(1968)	Present Method
B1	47.3	45.4
B2	5.18	5.32
B3	0.68	0.68
B4	0.17	0.19
B5	0.12	0.14
B6	0.08	0.07
B7	0.07	0.07

by these methods are in good agreement since the accuracy and reproducibility of the two methods are essentially similar. It follows that past data obtained using the old procedures can be compared directly with data obtained using the new procedure without the need to apply a derived factor to make the comparison valid.

No attempt was made to confirm the identity of PCP as detected by this procedure. The presence of PCP in blood and urine samples obtained from residents of Hawaii has long been established and need not be re-investigated. For investigators beginning PCP analyses, the confirmation can be done using thin layer chromatography and IR spectrophotography as reported by BEVENUE et al.(1966,1968).

No interferring responses were encountered in any of the samples analyzed. However, as previously reported (RIVERS et al. 1970), the use of the diazomethane solution in amounts greater than those indicated in the procedure can lead to interferences due to impurities in the reagent.

The real contribution of this procedure to the author's laboratory, beyond its accuracy and precision, is the significant decrease in time and equipment required to perform each analysis. Time-consuming transfers, centrifugations and concentration steps have been eliminated along with the equipment needs involved. The rate at which samples can be analyzed is now limited only by the time required for the GLC analysis.

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