A Method for the Determination of Pentachlorophenol in Human Urine in Picogram Quantities

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Pentachlorophenol (PCP), together with its salts, is used as a contact herbicide and defoliant, as a control against microbiological attack in the manufacture of cellulosic products, adhesives, and paints, as a fermentation inhibitor for non-edible materials, and as a control against termites, powder post beetles, and other wood-boring insects (1, 2, 3). This pesticide can be harmful to humans, especially to the occupationally exposed worker, and acute toxicity problems have occurred with the use of PCP formulations primarily through carelessness or failure to observe the manufacturers' precautionary instructions for its use (4, 5, 6). No chronic toxicity data of any consequence exist on the human individual that may have been exposed to PCP over a long period of time, either through occupational use or through occasional use in the home to control insect infestation. Any

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effort to establish a relationship between long-term exposure of the individual to this pesticide and any apparent adverse physiological effects will necessitate the acquisition of analytical data on PCP residues in the human system. Unfortunately, the present available methods of analysis for PCP residues in tissues, and related materials, are applicable only if PCP is present in relatively large amounts (microgram or milligram quantities), or if a large sample is available for analysis (7, 8, 9, 10). The sample size of the material and the detection limit of the analytical procedure will govern the positive or negative findings for any pesticide residue. For example, Gordon (4) suspected PCP poisoning in an autopsy case but failed to find any evidence of PCP residue in the tissue samples with the analytical procedures available at that time. In a subsequent autopsy case, in which PCP poisoning was also suspected, he used much larger quantities of tissues for analysis and obtained positive evidence for the presence of this pesticide in the tissues.

Tissue material cannot be readily obtained from the live individual, and blood and urine specimens are considered to be reasonable substitutes for many clinical and chemical analytical studies. However, it is impractical and often impossible to obtain sufficient amounts of blood and/or urine to utilize the aforementioned analytical methods for PCP. It has been suggested that 24-hour urine samples should be used for analysis. This would be applicable to hospitalized cases, but it would not be feasible for a cross section sampling of a given population. Therefore, a procedure was devised whereby twenty-five to fifty ml of urine was sufficient for the determination of any PCP residues in the picogram-nanogram range.

Experimental

Apparatus

Gas Chromatographs: MicroTek MT 220, electron capture detector, inlet temperature 180 C, column 145 C, detector 193 C; nitrogen flow rate 70 ml/min.

F&M Model 720, TC detector (190 ma), inlet temperature 250 C, column 175 C, detector 270 C; nitrogen flow rate 40 ml/min.

Dohrmann Microcoulometer C-200, Chloride Cell T-300.

Gas Chromatograph Columns: MicroTek and Dohrmann: 5% QF-1 on Gas Chrom Q (100/120 mesh); 10% DC-200 on Chromport XXX (60/80 mesh); 6' x 1/4" glass. F&M: 20% Dow-11 on Chromosorb P (60/80 mesh); 2' x 1/4" stainless steel.

Reagents: Sulfuric acid, redistilled petroleum ether (b. p. 30-60 C).

Diazomethane in ether was prepared from "Diazald" according to directions of the manufacturer (Aldrich Chemical Co., Inc., Milwaukee, Wisconsin), but modified by using 7 g instead of 21.5 g of Diazald and making the final volume of the diazomethane solution to 400 ml with ethyl ether.

Procedure: Twenty five ml urine was transferred to a 125-ml glass-stoppered erlenmeyer flask. Five ml concentrated sulfuric acid was added slowly to the flask, with constant mixing of the contents to avoid superheating the mixture. (If urine samples are stored under refrigeration prior to analysis, a precipitate may appear in the samples. Before using, concentrated sulfuric acid should be added to the entire amount of urine sample in the same ratio as described above, 5:25, to redissolve the precipitate.) Fifteen ml petroleum ether was added to the erlenmeyer flask and shaken for two minutes, using a Burrel Wrist Action shaker. The mixture was transferred to a 125-ml separatory funnel, two ml isopropyl alcohol was added to prevent the formation of an emulsion, and the lower aqueous phase was removed and transferred back to the original erlenmeyer flask. A second portion of 15 ml petroleum ether was added to the flask and the shaking and extraction procedures were repeated. The two petroleum ether fractions were combined and washed twice with distilled water. The ether was removed by evaporation with the aid of a stream of nitrogen at room temperature or, preferably, by allowing the solutions to stand overnight in beakers, at room temperature, in a vented area. Accelerated drying on a steam bath with a stream of air will create a water condensation problem with the residue, and may necessitate further manipulation steps to remove the

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water, thereby increasing the chances of losses of PCP residues by volatilization. A constant volume of diazomethane solution (5 ml) was added to each sample residue. After a period of 5 minutes, the solvent was removed with a gentle stream of nitrogen and a minimum of heat. The residue was dissolved in petroleum ether, transferred to a 25 ml volumetric flask, made to volume, and aliquots were removed from this solution for PCP analysis by gas chromatography.

Increments of a PCP ether solution were applied to a gas chromatograph equipped with an electron capture detector. The linear range, with an input attenuation of 10 and an output attenuation of 64 (MicroTek GC, full scale sensitivity 3.2×10^{-9} amperes), was between a minimum of 30 picograms and a maximum of about 400 picograms. The gas chromatographic records of the PCP residue in the urine samples were excellent. Background interference from other components of the urine was practically nil, because of the extreme sensitivity of the detector to the PCP ether, and because of the method of preparation of the urine extract. The retention time of the PCP ether on the QF-1 column was 1.5 min; on the DC-200 column 6 min. Additional confirmation was obtained with the F&M gas chromatograph, using a Dow-11 column, and also with the Dohrmann microcoulometer gas chromatograph, which is specific for the chloride component of the PCP molecule.

Duplicate standards and, when possible, duplicate urine samples were used to test the validity of the outlined procedure (see

Table I). Analyses were also made on urine samples fortified with PCP (see Table II). The PCP was dissolved in dilute (ca 0.01N) sodium hydroxide solution, and the resultant sodium pentachlorophenate was used for the preparation of the standard solutions and for the fortification of urine samples. The basic solutions were treated with sulfuric acid and prepared for analysis in the same manner as described above.

TABLE I

Reproducibility of Measurements of Pentachlorophenol in Urine

Picograms PCP in 5 ul Urine*	Mean
8.0 - 11.0	9.5
10.5 - 10.5	10.5
12.0 - 12.0	12.0
18.0 - 18.0	18.0
17.5 - 19.0	18.2
20.0 - 27.0	23.5
25.5 - 27.5	26.5
26.0 - 27.0	26.5
27.5 - 30.0	28.7
30.0 - 32.5	31.2
47.0 - 52.0	49.5
65.0 - 65.0	65.0
68.0 - 74.0	71.0
70.0 - 75.0	72.5
74.0 - 75.0	74.5
85.0 - 90.0	87.5
100.0 - 107.0	103.5
120.0 - 125.0	122.5
143.0 - 147.0	145.0
153.0 - 158.0	155.5
175.0 - 187.0	181.0
212.0 - 216.0	214.0

Standard Deviation ± 5.00%

* Duplicate measurements of PCP found in urine in a random sampling of the local male population.

TABLE II

Recovery of Pentachlorophenol from Urine

Amount PCP Found in Urine* (micrograms)	Amount PCP added (micrograms)	Amount PCP Recovered (micrograms)	% Recovery
0.275	0.50	0.625	80.6
0.360	0.50	0.810	94.2
0.540	0.50	0.990	95.2
0.600	0.50	1.000	90.9
0.625	0.50	1.125	100.0
0.900	0.50	1.255	89.6
1.125	0.50	1.440	88.6
1.175	0.50	1.575	94.0
		Average	91.6

* 25 ml samples

Mixtures of urine (25 ml) and sulfuric acid (2 ml) were boiled one hour under reflux conditions; PCP recovery was poor, because the phenol volatilized from the boiling mixture and deposited on the walls of the water-cooled condensers. Comparative PCP analyses were made of urine samples, prepared as described in the proposed procedure above, and of samples hydrolyzed one hour with sulfuric acid in a closed system using "cold finger" condensers. The hydrolyzed samples gave slightly higher PCP values, but the difference was not great enough to warrant using this more tedious procedure.

The identity of PCP isolated from the urine was confirmed by paper chromatography (11), using Whatman No. 1 paper impregnated with 10% paraffin oil and silver nitrate reagent, and developed with acetone-water (70:30). Ultraviolet examination of the paper revealed PCP ether spot areas, which were superimposable on

PCP ether standards spots with an Rf value of 0.21. However, because detection sensitivity had a lower limit of 0.2 micrograms, composite samples were necessary for confirmation. Thin layer chromatography, using silica gel and Rhodamine B, and developed in heptane-acetone (98:2), also confirmed the identity of PCP. Final confirmation was obtained by infrared absorption analysis of sample fractions from the gas chromatograph.

Discussion

The urine samples were treated with an excess amount of acid, to insure complete conversion of any pentachlorophenate in the urine to pentachlorophenol, to possibly eliminate any conjugated phenolic complex that might be present, and to avoid emulsion problems during the preparation of the sample extract. PCP is a relatively stable compound and, because of its chemical structure, coupling or substitution reactions common to many phenols should not occur.

The use of the PCP ether was advantageous for gas chromatography (12), because PCP, *per se*, chromatographs very poorly, if at all, unless specialized column techniques are employed (13, 14). PCP may be oxidized to chloranil (tetra-chloro-p-quinone) (15, 1) and misinterpretation of the gas chromatographic data is possible (16). However, the conditions of the designed method should not produce this compound and supplementary procedures confirmed the fact that PCP, *per se*, was isolated. The methyl ester of 2,4-D, if present as a contaminant, would cause misinterpretation (17), if only the QF-1 column were used; supplementary use of the DC-200 column would eliminate the doubt. The possibility of confusing the PCP ether with the methyl ester of DDA (bis[p-chlorophenyl]acetic acid) was eliminated because of the operating conditions of the gas chromatograph, i.e., the relatively low column temperature increased considerably the retention time of DDA as compared to the early emergence of the PCP ether from the column.

The infrared spectra of a standard PCP ether sample and the material extracted from the urine were prepared in an identical manner. A comparison of the spectra showed that the material extracted from the urine contained all of the major absorption bands observed in the standard spectra. The extracted material also showed absorption bands at 1500 cm^{-1}, 1800 cm^{-1}, 2800 cm^{-1}, and 3000 cm^{-1}. It is the opinion of the authors that these additional bands were derived from aliphatic contaminants.

No significance can be attached, at this time, to the amounts of PCP residue found in the urine of the individuals reported in Table I. This may be possible only after additional residue data have been obtained, together with complete and precise exposure histories of the individuals. It is not known whether the amounts of PCP residues thus far observed in the human system are capable of producing long-term harmful effects. Extensive clinical and toxicological data will be required before these factors can be given consideration. To date, the emphasis of the problem was on analytical methodology.

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