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MULTI-RESIDUE METHOD FOR THE DETERMINATION OF CHLORINATED PHENOL METABOLITES IN URINE

THOMAS R. EDGERTON, ROBERT F. MOSEMAN, RALPH E. LINDER and LYNN H. WRIGHT

Environmental Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711 (U.S.A.)

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SUMMARY

Electron-capture–gas chromatographic (EC–GC) methods for the determination of chlorinated phenol metabolites of hexachlorobenzene (HCB) and pentachlorophenol (PCP) in urine are presented. After extraction the sample was reacted with diazomethane to produce the methyl ether of each metabolite prior to determination by EC–GC. An acid alumina column was used for cleanup and separation of methylated phenols into groups. Average recoveries of greater than 80% were obtained from urine fortified with known amounts of the phenol metabolites under investigation. A level of 1 ppb* was established as minimum detection limit for each phenol metabolite. Previously unreported urinary metabolites of HCB and PCP were found as a result of a rat feeding study. Levels of chlorinated phenol residues from (a) human general population and (b) a worker occupationally exposed to PCP are also included.

INTRODUCTION

The identification of metabolites of hexachlorobenzene (HCB) and pentachlorophenol (PCP) has been, because of their occurrence in biological and environmental samples, the subject of many studies. Liu and Sweeny[1] found PCP and more polar derivatives of HCB in rat urine. Mehendale *et al.*[2] found PCP, pentachlorobenzene and an isomer of tetrachlorobenzene as urinary metabolites of HCB in rats. Koss *et al.*[3] identified PCP, pentachlorothiophenol, tetrachlorohydroquinone and a tetrachlorothiophenol isomer as metabolites of HCB in rats. Engst *et al.*[4] identified 2,3,4,6- and /or 2,3,5,6-tetrachlorophenol, 2,4,6- and 2,3,4-trichlorophenol and pentachlorobenzene as urinary metabolites of HCB. Recently Renner and Schuster[5] reported a new urinary metabolite of HCB in rats as 2,4,5-trichlorophenol.

The metabolites of PCP were investigated by Jakobson and Yllner[6] who determined tetrachlorohydroquinone to be a urinary metabolite of PCP in the mouse.

* Throughout this article the American billion (10^9) is meant.

Ahlborg *et al.*[7] identified tetrachlorohydroquinone as a metabolite of PCP in rats and mice and also reported tetrachlorohydroquinone as a metabolite in workers exposed to PCP.

This paper describes quantitative residue methods and confirmation procedures for several chlorinated phenol metabolites of HCB and PCP in urine. The developed method was applied to human general population urine and urine from a worker occupationally exposed to PCP.

EXPERIMENTAL

Animals

Adult female Sherman rats, 3–4 months of age and weighing 215–275 g were distributed into the following treatment groups: group 1, 4 rats on 575 ppm cornstarch in chow; group 2, 6 rats on 100 ppm HCB; group 3, 6 rats on 100 ppm PCP.

The HCB and PCP diets were prepared by mixing a quantity of HCB or PCP with a small amount of cornstarch (resulting in a starch concentration of about 575 ppm in the final mix) in a mortar, then making the appropriate dilution with ground chow and mixing in an electric mixer.

Rats were housed 2 per cage, identified with ear tags, and provided their respective diets and water *ad libitum*. Animals were weighed weekly and food consumption measured at least 2 days each week. Individual urine samples were collected overnight after 30 days and after 107 days. Rats were fed plain chow during urine collection to avoid non-ingested parent compound in the collected urine.

No clinical signs of toxicity were observed. Weight gain and food consumption were comparable in all rats. Average HCB or PCP ingestion over the first 30 days was 6.5 mg/kg/day for both compounds.

*Apparatus**

Tracor, 220, gas chromatograph equipped with a nickel-63 electron-capture detector, was operated in the pulsed linearized mode. Borosilicate glass columns (1.8 × 4 mm I.D.) were packed with 80–100 mesh Gas-Chrom Q coated with 5% OV-210, 3% OV-1, 3% Silar 10-C, 4% SE-30–6% OV-210 or 1.5% OV-17–1.95% QF-1. A 5% DEGS coated on 80–100 mesh Gas-Chrom P was also used. The OV-1, SE-30–OV-210, and OV-17–QF-1 columns were operated at 170° with a 5% methane in argon carrier gas flow-rate of 60 ml/min. The OV-210 column was operated at 160° with a 5% methane in argon flow-rate of 40 ml/min. The DEGS column was operated at 170° with a 5% methane in argon flow-rate of 80 ml/min. Detector, inlet and transfer line temperatures were 300, 235, and 220°, respectively.

Analytical results were confirmed on a Finnigan Model 3200 quadrupole mass spectrometer equipped with a Model 9500 gas chromatograph and Model 6100 data system. Methane was used as the reagent gas for operation in the chemical-ionization mode with a source temperature of 120°, pressure 900 μm, 110 eV electron energy and 10 mA emission current.

For gas chromatography–mass spectrometry (GC–MS) a borosilicate glass

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* Use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Environmental Protection Agency.

column (1.2 m × 2 mm I.D.) was packed with 80–100 mesh Gas-Chrom Q coated with 5% OV-210. The column was operated at 90° isothermal for 1 min, then programmed at 4° per minute to a final temperature of 160°. Methane carrier gas flow-rate was 20 ml/min. Inlet, transfer line and ion source temperatures were 200, 250, and 120°, respectively.

Reagents and materials

Anhydrous, granular, sodium sulfate and sodium bisulfite were soxhlet extracted for 4 h with hexane and oven dried at 130°.

Acid alumina, Brockman Activity I (Fisher Scientific, Pittsburgh, Pa., U.S.A.), was dried for 24 h at 130° and stored in a desiccator.

Potassium hydroxide and hydrochloric acid were reagent grade.

N-Methyl-N′-nitro-N-nitrosoguanidine (Aldrich, Milwaukee, Wisc., U.S.A.), should be handled carefully since it is a known carcinogen.

All solvents were pesticide quality or equivalent.

2,3,4,6-Tetrachlorophenol, 2,3,5,6-tetrachlorophenol, pentachlorophenol, pentachlorothiophenol and 2,3,4,5-tetrachlorophenol, were obtained from Aldrich; tetrachloropyrocatechol from Pfaltz and Bauer (Flushing, N.Y., U.S.A.) and tetrachlorohydroquinone from K&K Labs. (Plainview, N.Y., U.S.A.) Pentachlorothiophenol, tetrachlorohydroquinone and tetrachloropyrocatechol were recrystallized prior to use.

The purity of HCB used for dietary feeding was greater than 99.5%. No apparent impurities were found by electron-capture–gas chromatography (EC–GC) analysis. PCP contained 0.8% 2,3,4,6-tetrachlorophenol as the only detectable impurity.

Methylating reagent

Potassium hydroxide (2.3 g) was dissolved in 2.3 ml of distilled water in a 125-ml Erlenmeyer flask and cooled to room temperature. A 25-ml volume of ethyl ether was then added and the flask was cooled in a refrigerator. The following step was carired out in a glove box or high-draft hood. N-Methyl-N′-nitro-N-nitrosoguanidine (1.5 g) was added in small portions to the flask with vigorous shaking. The ether layer was decanted into a scintillation vial and stored in a freezer[8].

Preparation of standard solutions

A standard of each phenol was prepared in hexane and stored at −15° in brown glass bottles. Urine fortifications were made from acetone dilutions of the seven mixed phenol standards. A volume containing 10 μg of each phenol was pipeted into separate 15-ml graduated centrifuge tubes. Methylation was accomplished by adding 5 ml of diazomethane reagent in a high-draft hood to each tube[9]. (Caution: Diazomethane is toxic and may be explosive under certain conditions.) The phenol standards were allowed to stand for 1 h before EC–GC determination. Nitrogen was bubbled through the individual standard solutions to remove any excess diazomethane prior to EC–GC analysis. For determination of elution patterns on an acid alumina column, a known amount of each phenol was methylated as a mixture and allowed to stand for 1 h. The methylated phenol mixture was concentrated to 0.2 to 0.3 ml under a gentle stream of nitrogen prior to being placed on the acid alumina column.

Preparation and elution of acid alumina column

A size 22-9 Chromaflex column (Kontes 420530) was loosely plugged with a small amount of glass wool. Acid alumina (4 g) was added in small increments with tapping. Anhydrous, granular Na_2SO_4 (1.6 g) was added on top of the alumina. A 30-ml volume of 40% benzene in hexane was used to wash the column free of interferences. After thoroughly air drying, the column was placed in an oven at 130° overnight prior to use.

A prepared column was removed from the oven just prior to use and allowed to cool to room temperature. The column was wetted with 7 ml of hexane. When the solvent layer reached the top of the Na_2SO_4, an aliquot of methylated sample or methylated standard phenol mixture in 0.2 to 0.3 ml was placed on top of the column. Quantitative transfer of the sample or standard was accomplished with three 0.5-ml rinsings with hexane. An additional 3.5-ml volume of hexane was added and the hexane fraction (5.0 ml) collected and discarded. 2,3,4,6-Tetrachlorophenol, 2,3,5,6-tetrachlorophenol, pentachlorophenol and pentachlorothiophenol were eluted with 20 ml of 10% benzene in hexane (fraction I). The remaining phenols, 2,3,4,5-tetrachlorophenol, tetrachloropyrocatechol and tetrachlorohydroquinone, were eluted with 20 ml of 40% benzene in hexane (fraction II). Fractions I and II were adjusted to an appropriate volume for injection into the gas chromatograph.

Analysis of urine

A 2-ml volume of urine was transferred into a 20 × 125 mm Teflon®-lined screw cap culture tube and 100 mg of sodium bisulfite was added. The urine was acidified by the addition of 0.5 ml of concentrated HCl. The tube was sealed and placed in a boiling water bath for 1 h with periodic shaking. After hydrolysis, an additional 100 mg amount of sodium bisulfite was added to the cooled urine sample and extracted twice for 1 h each on a mechanical rotator at 30 to 50 rpm using two 5-ml portions of benzene. The samples were centrifuged after each extraction and the extracts combined in an aluminium foil wrapped 15-ml centrifuge tube. The benzene extracts were concentrated to a volume of 0.3–0.5 ml in a water bath at 30° under a gentle stream of nitrogen and analyzed on a 5% DEGS column prior to derivatization with 5 ml of diazomethane reagent. The methylated extract was allowed to stand for 1 h. Prior to column cleanup, the methylated urine extract was concentrated to approximately 0.3 ml under a gentle stream of nitrogen. A 2-ml volume of hexane was added and reconcentrated to a volume of 0.3 ml.

RESULTS AND DISCUSSION

Table I lists relative retention data for the seven phenol methyl ethers under study on six different GC columns. A 5% OV-210 column was chosen for analysis of the chlorinated phenol metabolites because of its separation capabilities. The two tetrachlorophenols: 2,3,5,6 and 2,3,4,6 could be separated on a 5% DEGS column as the free phenol.

Recoveries of the seven phenolic metabolites of either HCB or PCP through an acid alumina column are listed in Table II. Fig. 1 illustrates the GC separation obtained on a 5% OV-210 column for the methyl ethers of the seven phenol methyl

TABLE I

RELATIVE RETENTION DATA FOR METHYLATED METABOLITES OF HCB AND PCP

Retention time relative to Aldrin.

Metabolite	*4% Se-30– 6% OV-210*	*1.5% OV-17– 1.95% QF1*	*5% OV-210*	*3% OV-1*	*5% DEGS**
2,3,4,6-Tetrachlorophenol	0.23	0.33	0.24	0.22	1.21
2,3,5,6-Tetrachlorophenol	0.23	0.33	0.24	0.22	1.13
2,3,4,5-Tetrachlorophenol	0.38	0.51	0.46	0.34	1.66
Pentachlorophenol	0.46	0.55	0.49	0.44	2.58
Tetrachloropyrocatechol	0.45	0.55	0.52	0.42	–
Tetrachlorohydroquinone	0.48	0.56	0.59	0.42	–
Pentachlorothiophenol	0.95	1.06	1.00	0.91	–

* Underivatized.

TABLE II

RECOVERIES OF METHYLATED METABOLITES OF HCB AND PCP FROM AN ACID ALUMINA COLUMN

Metabolite	*Amount added (μg)*	*Amount recovered (μg)*	*Recovery (%)*
2,3,4,6-Tetrachlorophenol*	5.00	4.650	93.0
	1.00	0.970	97.0
	0.10	0 092	92.0
2,3,5,6-Tetrachlorophenol*	5.00	4.723	94.5
	1.00	0.970	97.0
	0.10	0.093	93.0
Pentachlorophenol*	5.00	4.800	96.0
	1.00	0.930	93.0
	0.10	0.094	94.0
Pentachlorothiophenol*	5.00	4.850	97.0
	1.00	0.880	88.0
	0.10	0.091	91.0
2,3,4,5-Tetrachlorophenol**	5.00	4.720	94.4
	1.00	0.893	89.3
	0.10	0.091	91.0
Tetrachloropyrocatechol**	5.00	4.700	94.0
	1.00	0.950	95.0
	0.10	0.095	95.0
Tetrachlorohydroquinone**	5.00	4.850	97.0
	1.00	0.960	96.0
	0.10	0.091	91.0

* Fraction 1: 20 ml 10% benzene in hexane.
** Fraction 2: 20 ml 40% benzene in hexane.

ethers when injected as a mixture. Figs. 2 and 3 show this same mixture injected into the gas chromatograph after separation on the acid alumina column.

Table III lists recoveries of phenols from fortified urine. In the initial fortification studies with urine, difficulty was encountered with obtaining reproducible recoveries of pentachlorothiophenol, tetrachloropyrcatechol and tetrachlorohydroquinone. Recoveries for those three compounds averaged 11, 20 and 35%, respectively at the 1-ppm fortification level. An apparent auto-oxidation reaction took place

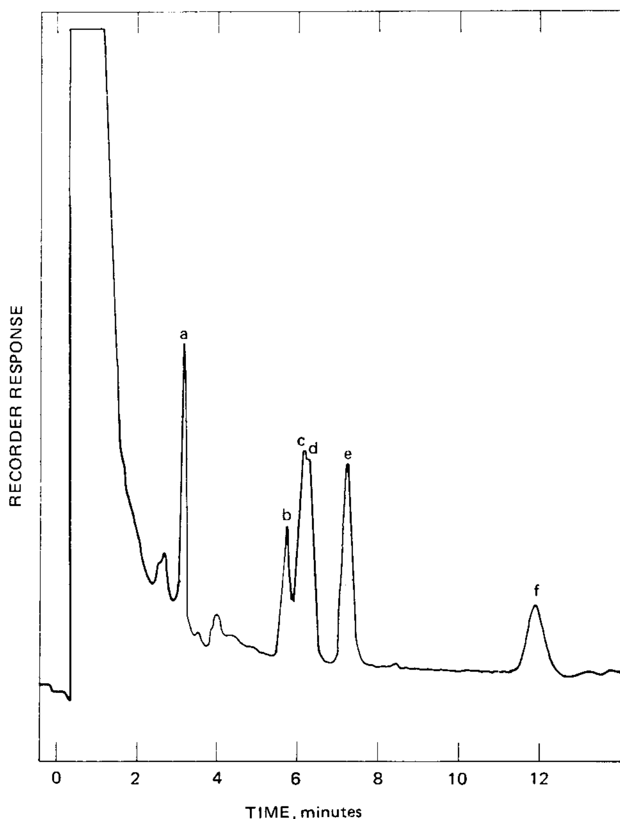


Fig. 1. Gas chromatogram of standard phenol methyl ether mixture. (a) 2,3,5,6- and 2,3,4,6-tetrachlorophenol; (b) 2,3,4,5-tetrachlorophenol; (c) pentachlorophenol; (d) tetrachloropyrocatechol; (e) tetrachlorohydroquinone; (f) pentachlorothiophenol. Column: 5% OV-210 on 80–100 mesh Gas-Chrom Q. Oven temperature 160°. 5% Methane in argon; flow-rate 40 ml/min.

thus reducing recoveries. Kirkland[10] observed that hydroxylated metabolites of Benomyl, 5-hydroxy-2-benzimidazolecarbamate and methyl-4-hydroxy-2-benzimidazolecarbamate were easily oxidized when unduly exposed to air and other oxidizing media. Bisulfite was added as a reducing agent. We also found that with the addition of bisulfite and prompt execution of the isolation procedures dramatic increase in recoveries were noted for pentachlorothiophenol, tetrachloropyrocatechol and tetrachlorohydroquinone. For this reason it was imperative that bisulfite was added to urine samples before and after hydrolysis of urine extracts.

In order to minimize the possible effect of photodecomposition, extracts of urine were collected in 15-ml centrifuge tubes which were adequately wrapped in aluminum foil.

It was determined in this work that the analysis of urine could not be interrupted prior to the methylation step. When urine samples were extracted and allowed to stand overnight before methylation, even with the addition of bisulfite, recoveries of pentachlorothiophenol, tetrachloropyrocatechol and tetrachlorohydroquinone were low and erratic.

TABLE III

RECOVERIES OF METABOLITES FROM FORTIFIED URINE

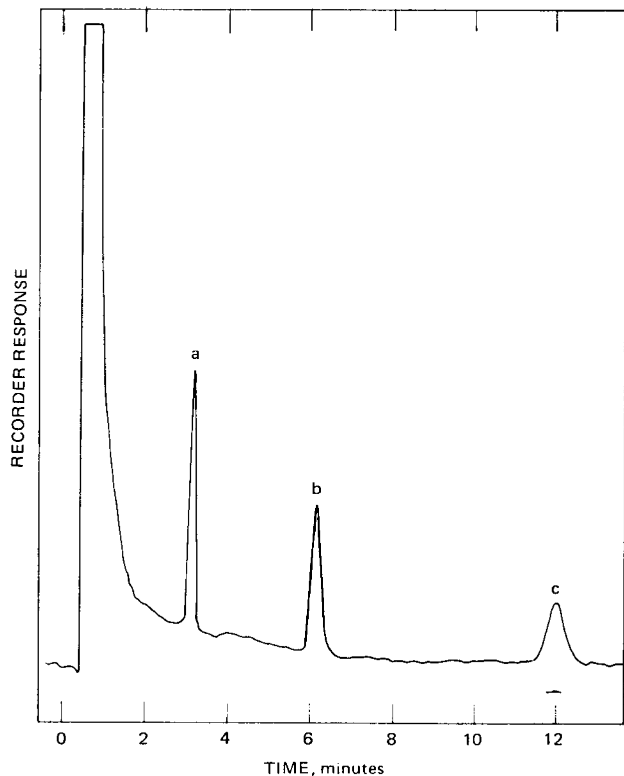
Four determinations for each measurement.

Metabolite	*ppm Added*	*Range (%)*	*Average Recovery (%)*	*Relative Standard Deviation (%)*
2,3,5,6-Tetrachlorophenol	1.0	89.3– 92.3	91.1	±1.3
	0.3	85.7– 92.3	88.8	±2.7
	0.1	82.0– 87.9	85.3	±2.5
	0.03	78.0– 85.6	82.3	±3.3
	0.01	79.1– 87.5	82.8	±3.6
2,3,4,6-Tetrachlorophenol	1.0	88.9– 92.4	90.9	±1.5
	0.3	86.1– 91.8	88.9	±2.4
	0.1	83.1– 88.3	86.0	±2.5
	0.03	80.8– 84.2	82.5	±1.7
	0.01	79.6– 86.8	82.6	±3.2
2,3,4,5-Tetrachlorophenol	1.0	89.3– 95.6	93.1	±2.7
	0.3	89.0– 94.3	91.8	±2.3
	0.1	86.0– 91.0	88.2	±2.1
	0.03	85.8– 90.3	87.4	±2.0
	0.01	82.8– 90.5	85.6	±3.4
Pentachlorophenol	1.0	95.2– 97.8	96.5	±1.1
	0.3	91.5– 95.2	93.4	±1.8
	0.1	86.0– 9.50	92.0	±4.1
	0.03	93.8–100.4	97.2	±2.8
	0.01	90.6– 96.3	93.2	±2.5
Tetrachloropyrocatechol	1.0	78.6– 81.4	80.1	±1.2
	0.3	78.7– 85.7	81.6	±2.9
	0.1	76.3– 83.0	79.8	±3.2
	0.03	59.1– 71.4	65.6	±5.4
	0.01	60.1– 69.7	63.7	±4.3
Tetrachlorohydroquinone	1.0	80.2– 82.7	81.5	±1.0
	0.3	77.0– 84.5	81.4	±3.2
	0.1	77.0– 84.0	80.9	±2.9
	0.03	75.6– 86.0	80.4	±4.6
	0.01	71.3– 77.3	74.6	±2.5
Pentachlorothiophenol	1.0	69.9– 73.6	71.9	±1.5
	0.3	69.3– 73.3	71.1	±1.7
	0.1	61.0– 73.0	66.4	±6.0
	0.03	49.8– 57.2	53.3	±3.3
	0.01	41.5– 51.3	47.3	±4.2

Figs. 4 and 5 illustrate chromatograms of urine samples from rats fed HCB or PCP. Adequate separation of the various phenols was accomplished using an acid alumina column.

Results of the analysis of urine from the HCB and PCP feeding studies and human urine are listed in Tables IV through VII. A minimum detection limit of 1 ppb was determined for the phenols in this study.

The major metabolites from the HCB feeding study, as was also reported by Koss *et al.*[3] were tetrachlorohydroquinone, pentachlorothiophenol and PCP. Minor rat urinary metabolites isolated in our study were: 2,3,5,6-tetrachlorophenol, 2,3,4,5-tetrachlorophenol and tetrachloropyrocatechol. Separation of underivatized



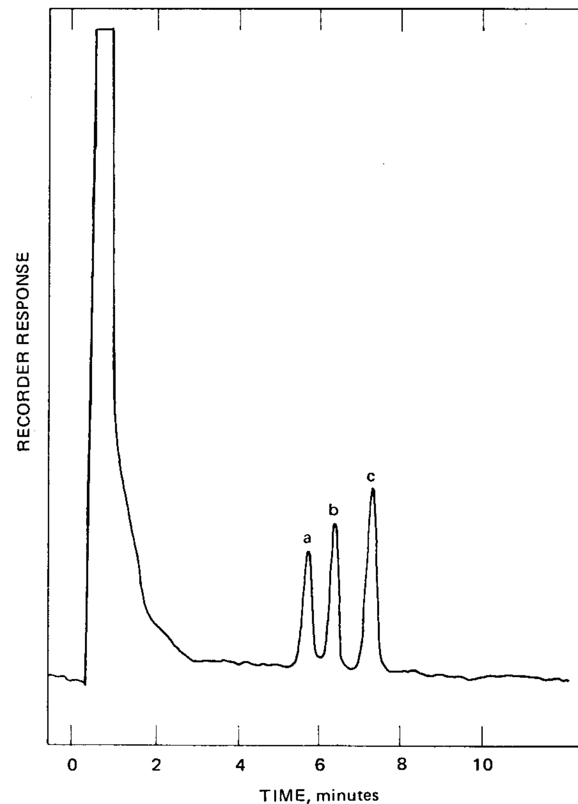


Fig. 2. Gas chromatogram of fraction I from acid alumina column of standard phenol methyl ether mixture. (a) 2,3,5,6- and 2,3,4,6-tetrachlorophenol; (b) pentachlorophenol; (c) pentachlorothiophenol. Column: 5% OV-210 on 80–100 mesh Gas-Chrom Q. Oven temperature 160°. 5% Methane in argon; flow-rate 40 ml/min.

Fig. 3. Gas chromatogram of fraction II from acid alumina column of standard phenol methyl ether mixture. (a) 2.3,4,5-Tetrachlorophenol; (b) tetrachloropyrocatechol; (c) tetrachlorohydroquinone. Column: 5% OV-210 on 80–100 mesh Gas-Chrom Q. Oven temperature 160°. 5% Methane in argon; flow-rate 40 ml/min.

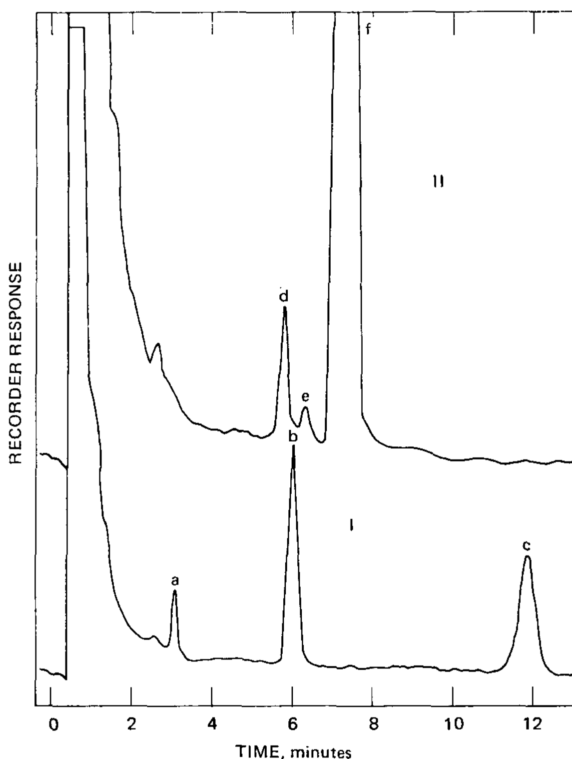


Fig. 4. Gas chromatograms of urine extract from a rat fed HCB. (I) Fraction I acid alumina column. (a) 2,3,5,6-Tetrachlorophenol, 74 ppb; (b) PCP, 405 ppb; (c) pentachlorothiophenol, 500 ppb. (II) Fraction II acid alumina column. (d) 2,3,4,5-Tetrachlorophenol, 20 ppb; (e) tetrachloropyrocatechol, 8 ppb; (f) tetrachlorohydroquinone, 436 ppb. Column: 5% OV-210 on 80–100 mesh Gas-Chrom Q. Oven temperature 160°. 5% Methane in argon; flow-rate 40 ml/min.

2,3,5,6-tetrachlorophenol from 2,3,4,6-tetrachlorophenol was accomplished on a 5% DEGS column. The three minor metabolites have not been previously reported in the literature.

The major metabolite isolated from the PCP feeding study was tetrachlorohydroquinone as was previously reported by Ahlborg *et al.*[7]. Minor metabolites identified in our work and not previously reported in the literature were: 2,3,4,5-tetrachlorophenol and tetrachloropyrocatechol. Because of its presence as an impurity in the PCP used in the feeding study, 2,3,4,6-tetrachlorophenol was not listed as a metabolite even though it was present in the urine samples analyzed. PCP was present at fairly high levels as would be expected.

PCP was identified in ten of the eleven urine samples from the human general population using the described analytical method. Levels ranged from 1 to 80 ppb. The presence of 2,3,4,6-tetrachlorophenol in the urine can be attributed to its presence as an impurity in preparations of PCP. The only measurable metabolites from the general population samples were tetrachlorohydroquinone and tetrachloropyrocatechol. The occupationally exposed worker contained a high level of PCP and measurable levels of tetrachlorohvdroquinone and tetrachloropyrocatechol.

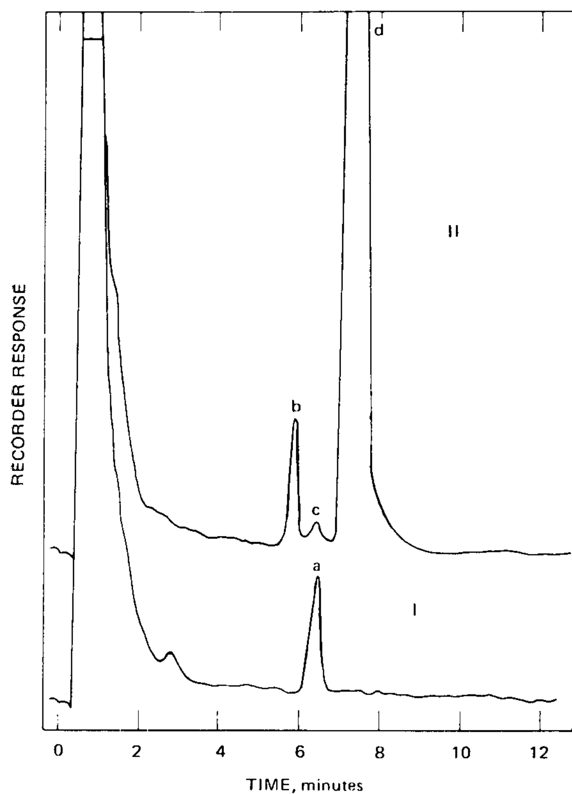


Fig. 5. Gas chromatograms of urine extract from a rat fed PCP. (I) Fraction I acid alumina column. (a) PCP, 12.3 ppm. (II) Fraction II acid alumina column. (b) 2,3,4,5-Tetrachlorophenol, 1.02 ppm; (c) tetrachloropyrocatechol, 0.18 ppm; (d) tetrachlorohydroquinone, 24 ppm. Column: 5% OV-210 on 80–100 mesh Gas-Chrom Q. Oven temperature 160°. 5% Methane in argon; flow-rate 40 ml/min.

TABLE IV

HEXACHLOROBENZENE METABOLITES IN RAT URINE

100 ppm in diet. Results in ppm.

Days on chow	*Sample No.*	*Penta-chloro-phenol*	*Tetra-chloro-hydro-quinone*	*Penta-chloro-thiophenol*	*Tetra-chloro-pyrocatechol*	*2,3,5,6-Tetra-chloro-phenol*	*2,3,4,5-Tetra-chloro-phenol*
30	2240	0.081	0.079	0.424	0.001	0.029	0.005
107	2240	0.405	0.182	0.500	0.003	0.074	0.008
30	2241	0.167	0.238	0.390	0.002	0.033	0.013
107	2241	0.420	0.413	0.303	0.007	0.074	0.015
30	2242	0.085	0.031	0.560	0.001	0.027	0.001
107	2242	0.355	0.339	0.136	0.004	0.048	0.010
30	2243	0.066	0.027	0.485	0.001	0.025	0.002
107	2243	0.189	0.090	0.200	0.002	0.055	0.004
30	2244	0.079	0.114	0.332	0.002	0.023	0.005
107	2244	0.412	0.170	0.197	0.002	0.044	0.008
30	2245	0.168	0.139	0.747	0.002	0.042	0.005
107	2245	0.471	0.349	0.363	0.007	0.085	0.012

TABLE V

PENTACHLOROPHENOL METABOLITES IN RAT URINE

100 ppm in diet. Results in ppm.

Days on chow	Sample No.	Penta-chloro-phenol	Tetra-chloro-hydro-quinone	Penta-chlorothio-phenol	Tetra-chloro-pyro-catechol	2,3,5,5-Tetra-chloro-phenol	2,3,4,5-Tetra-chloro-phenol
30	2246	16.8	48.8	<0.001	0.16	<0.001	2.17
107	2246	12.3	24.0	<0.001	0.18	<0.001	1.02
30	2247	9.2	25.9	<0.001	0.15	<0.001	1.05
107	2247	10.8	28.4	<0.001	0.17	<0.001	0.93
30	2248	12.2	40.4	<0.001	0.15	<0.001	1.33
107	2248	6.6	10.8	<0.001	0.08	<0.001	0.36
30	2249	21.0	42.8	<0.001	0.11	<0.001	1.18
107	2249	10.1	20.4	<0.001	0.16	<0.001	1.00
30	2250	19.4	38.4	<0.001	0.40	<0.001	1.48
107	2250	15.3	29.7	<0.001	0.42	<0.001	1.31
30	2251	7.4	27.0	<0.001	0.31	<0.001	1.19
107	2251	8.1	14.3	<0.001	0.28	<0.001	0.87

TABLE VI

CONTROL URINE—PLAIN CHOW

Results in ppm.

Days on chow	Sample No.	Penta-chloro-phenol	Tetra-chloro-hydro-quinone	Penta-chlorothio-phenol	Tetra-chloro-pyro-catechol	2,3,5,6-Tetra-chloro-phenol	2,3,4,5-Tetra-chloro-phenol
30	2236	0.002	0.008	<0.001	0.004	<0.001	<0.001
107	2236	0.004	0.006	<0.001	0.002	<0.001	<0.001
30	2237	0.003	0.004	<0.001	0.002	<0.001	0.001
107	2237	0.002	0.012	<0.001	0.002	<0.001	0.004
30	2238	0.033	0.006	<0.001	<0.001	<0.001	0.015
107	2238	0.021	0.011	<0.001	0.003	<0.001	0.021
30	2239	0.041	0.037	<0.001	0.007	<0.001	0.011
107	2239	0.036	0.043	<0.001	0.009	<0.001	0.008

As can be seen from these results, pentachlorothiophenol in urine can be used as an indicator of possible exposure to HCB, PCP exposure would be indicated by a high level of PCP and the presence of tetrachlorohydroquinone and tetrachloropyrocatechol in the urine.

The phenolic metabolites as their methyl ethers were confirmed in urine extracts from the feeding study and human population by combined GC–MS. Chemical ionization using methane reagent gas produced fairly strong M + 1 quasi-molecular ion isotope clusters, beginning at *m/e* 245 for the three isomers of tetrachlorophenol, *m/e* 279 for PCP, *m/e* 295 for pentachlorothiophenol and *m/e* 275 for tetrachloropyrocatechol and tetrachlorohydroquinone. In addition, a fairly strong M + 1 quasi-molecular ion isotope cluster beginning at *m/e* 240 was tentatively identified as an

TABLE VII

HUMAN URINE

Results in ppm.

Sample No.	*Penta-chloro-phenol*	*Tetra-chloro-hydro quinone*	*Penta-chloro-thiophenol*	*Tetra-chloropyro-catechol*	*2,3,4,6-Tetra-chlorophenol*	*2,3,5,6-Tetra-chlorophenol*	*2,3,4,5-Tetra-chlorophenol*
1	0.006	<0.001	<0.001	<0.001	0.004	<0.001	<0.001
2	0.012	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
3	0.004	<0.001	<0.001	0.002	0.003	<0.001	<0.001
4	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
5	0.080	0.002	<0.001	0.001	0.013	<0.001	<0.001
6	0.004	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
7	0.015	0.003	<0.001	0.004	0.004	<0.001	<0.001
8	0.012	0.006	<0.001	0.005	0.002	<0.001	<0.001
9	0.009	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
10	0.038	0.008	<0.001	0.007	0.009	<0.001	<0.001
11	0.018	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
12*	3.60	0.024	<0.001	0.024	0.123	<0.001	0.005

* Occupationally exposed to PCP.

isomer of trichlorodihydroxybenzene from the PCP feeding study samples. The phenolic metabolites in the urine from the occupationally exposed worker were confirmed by GC–MS as tetrachloropyrocatechol and tetrachlorohydroquinone.

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