Journal of Chromatography, 414 (1987) 55–63
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3425

ANALYSIS OF PENTACHLOROPHENOL IN WATER AND URINE BY ENRICHMENT WITH LIPIDEX® 5000

KOIDU NORÉN* and JAN SJÖVALL

Department of Physiological Chemistry, Karolinska Institutet, Box 60 400, S-104 01 Stockholm (Sweden)

(First received June 26th, 1986; revised manuscript received September 26th, 1986)

SUMMARY

A method using the lipophilic gel Lipidex® 5000 for sorption of pentachlorophenol (PCP) from water and urine is described. The procedure for water gives a simultaneous clean-up from lipophilic contaminants and also offers the possibility for determination of less polar compounds, such as 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (*p,p'*-DDT) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), collected in a separate fraction. The extracted PCP is derivatized to pentachlorophenyl acetate, which is determined by electron-capture gas chromatography. The average recovery of 0.01–0.15 ng PCP per ml water was 96% and of labelled *p,p'*-DDT (ca. 5 ng/ml) and TCDD (ca. 0.001 ng/ml) 95 and 92%, respectively. A similar method for enrichment of PCP was applied to acidified urine and to urine hydrolysed with hydrochloric acid or with digestive juice from *Helix pomatia*. Recoveries of 0.1–2.5 ng PCP per ml non-hydrolysed urine were on an average 92% and of 2.5–10 ng PCP per ml hydrolysed urine 96%. The analyses indicate that PCP in urine from non-occupationally exposed persons is originally conjugated and to some extent liberated when stored. The contamination of organic solvents and laboratory environments with PCP is discussed.

INTRODUCTION

Pentachlorophenol (PCP) has been widely used as a fungicide and herbicide. In Sweden, its use for slime control in the paper industry was stopped in 1972, and in 1977 all use of PCP was prohibited. However, PCP is still found as an environmental contaminant and in biological materials. Thus, simple methods are needed for monitoring of this pollutant.

The most common methods for analysis of PCP in water and urine involve liquid–liquid extraction, derivatization and usually also a purification step before quantification by electron-capture gas chromatography (GC) [1–10]. Derivatization has also been performed directly in the water sample without previous extraction [11]. Other techniques for separation of PCP from water include sorp-

0378-4347/87/$03.50

tion on anion-exchange resins [12,13] and on octadecylsilane-bonded silica [14]. Rostad et al. [15] compared the extraction efficiencies of four different bonded phases: *n*-octyl, *n*-octadecyl, cyclohexyl and phenyl. Good recoveries of PCP (100 μg/l) were obtained with all except the *n*-octyl phase. PCP in urine has been extracted with a polystyrene resin, Amberlite XAD-4 [16]. We have previously found that Lipidex® 1000, a lipophilic gel based on a hydroxypropylated cross-linked dextran matrix, can be used for enrichment of 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (*p,p'*-DDT) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) from water [17]. We have now studied the use of Lipidex® 5000, which is less polar than Lipidex 1000, for enrichment of PCP from water and urine. The method permits simultaneous clean-up and separation from less polar compounds. PCP was derivatized with acetic anhydride and analysed by GC. The derivative was identified by mass spectrometry (MS).

EXPERIMENTAL

Solvents

Glassware was washed in an ultrasonic bath and rinsed with redistilled ethanol. All chemicals used were of analytical-reagent grade. Acetone, hexane, methylene chloride, toluene and pyridine were redistilled. Water was deionized and purified with a Milli-Q® cartridge (Millipore, Bedford, MA, U.S.A.). Methanol was treated with sodium hydroxide before distillation. To 2.5 l of methanol, 100 g of sodium hydroxide were added and the flask was shaken occasionally. After 12–24 h the methanol was distilled twice. The product was controlled for interferences by the following procedure: 50 ml of solvent were reduced to ca. 0.5 ml. Internal standard [6 ng of α-hexachlorocyclohexane (α-HCH) in 1 ml of hexane] and 3 ml of hexane were added, and the mixture was gently shaken with 3 ml of 50% sulphuric acid. After centrifugation the hexane phase was reduced to 1 ml and treated with acetylating reagent as described below in the method sections.

Reagents

Acetic anhydride, formic acid, hydrochloric acid, sulphuric acid and sodium bisulphite (Merck, Darmstadt, F.R.G.) were used as supplied. Acetylating reagent was prepared just before use by mixing 1.0 ml of pyridine and 0.4 ml of acetic anhydride. Purified water was used for preparation of 0.01 *M* potassium carbonate. *Helix pomatia* digestive juice (containing β-glucuronidase and sulphatase) was from Pointet Girard, France. Immediately before use, 0.3 ml were added to 5 ml of 0.2 *M* sodium acetate buffer (pH 4.5), and the solution was passed through a column (4 mm I.D.) containing a 2-cm layer of Sepralyte® (Analytichem International, Harbor City, CA, U.S.A.) prewashed with 5 ml of methanol and 5 ml of water.

PCP was from Riedel-de Haen (Seelze-Hannover, F.R.G.). Pentachlorophenyl acetate was prepared according to Rudling [1]. α-HCH (K & K Lab., New York, NY, U.S.A.), 6 ng/ml in hexane, was used as an internal standard for water and non-hydrolysed urine samples. For hydrolysed urine, a concentration of 12 ng/ml was used. Radioactively labelled *p,p'*-DDT ([^{14}C]-*p,p'*-DDT), 30

mCi/mmol, was from the Radiochemical Centre (Amersham, U.K.) and radioactively labelled TCDD ([^{3}H]TCDD), 50 Ci/mmol, was from KOR (Cambridge, MA, U.S.A.). Radioactivity was determined in an LKB 1211 Minibeta liquid scintillation counter. Insta-Gel® (Packard Instrument, Downers Grove, IL, U.S.A.) was used as scintillation liquid.

Column chromatography

Chromatography was performed in glass columns (0.5 or 1.0 cm I.D.) with a sintered glass disc and a PTFE stopcock. Silica gel 60 (Merck), 70–230 mesh, was heated for at least 48 h at 130°C and stored at this temperature. Lipidex 5000 (Packard Instrument) was washed with aqueous ethanol and ethanol at 70°C [18] and stored in methanol at 4°C. Immediately before use it was washed with methanol on a Büchner funnel. The solvent was removed by suction, and 1 or 2 g of the moist substance was transferred to a chromatographic column (1 cm I.D.). The gel was washed with 10 ml of 20% aqueous methanol and 10 ml of water. It was stirred with a glass rod and gently pressed to give a homogeneous firm packing.

Gas chromatography and gas chromatography–mass spectrometry

A Pye Unicam GCV instrument was used with a ^{63}Ni electron-capture detector and a fused-silica capillary column (25 m × 0.32 mm I.D.) coated with OV 1701, 0.25 μm film thickness (Orion, Helsinki, Finland). The temperatures of injector, column and detector were 200, 180 and 290°C, respectively. An all-glass falling-needle injection system was used.

GC–MS was performed on a VG 7070E instrument (Manchester, U.K.) using selected-ion monitoring. A fused-silica column (25 m × 0.32 mm I.D.) coated with a 0.25-μm layer of cross-linked methyl silicone (Quadrex, New Haven, CT, U.S.A.) was used at 170°C with an all-glass falling-needle injector. The column outlet extended into the ion source, which was at 250°C. Helium was the carrier gas. Samples were ionized by electron impact at 70 eV, the accelerating voltage was 6 kV and the resolution 1000. For pentachlorophenyl acetate the ions at m/z 305.86 (M^+) and 263.85 ($M^+ - C_2H_2O$) were monitored and for α-HCH the ion at m/z 217.92 ($M^+ - 2Cl$).

Method for water

The water sample (100 ml) was mixed with 2 ml of formic acid and passed through a 1-g bed of Lipidex 5000 at a flow-rate of 60 drops/min. The flask was rinsed with a total of 10 ml of 20% aqueous methanol, which was passed through the column followed by 5 ml of 50% aqueous methanol. Aqueous solvent in the void volume was then expelled with a stream of dry nitrogen. Then 5 ml of hexane were transferred to the column. When ca. 1 ml had passed through the column, the flow was stopped and the gel was allowed to swell for 10 min. Elution was then continued with an additional 30 ml of hexane. PCP was finally eluted with 10 ml of acetone. Toluene (0.5 ml) was added to this fraction, and the solvent was evaporated under reduced pressure at 30°C to ca. 0.5 ml. A 1-ml volume of

internal standard (6 ng/ml α-HCH) and 50 μl of acetylating reagent were added. The flask was tightly stoppered and placed in an ultrasonic bath. After 15 min the mixture was cooled, transferred to a tube and washed with 3 ml of 0.01 *M* potassium carbonate. After centrifugation the hexane layer was removed and analysed by GC.

In recovery experiments, water samples were fortified with 1–15 ng of PCP in 10–150 μl of acetone and [^{14}C]-*p,p'*-DDT (ca. 50 ng) in 10 μl of toluene or [^{3}H]TCDD (ca. 0.1 ng) in 10 μl isooctane. The hexane eluate was collected in a graduated glass cylinder (glass stopper). The volume of the hexane was measured, the cylinder was shaken and 1.0 ml of the hexane phase was taken for determination of radioactivity.

Method for urine

For non-hydrolysed urine samples, 2 ml of urine were mixed with 0.5 ml of formic acid and 2 ml of water. Alternatively, 10 ml of urine, 0.5 ml of formic acid and 5 ml of water were used.

For hydrolysed urine samples, two different methods were used. In method A, 2 ml of urine were mixed with 100 mg of sodium bisulphite and 0.5 ml of concentrated hydrochloric acid in a glass tube with PTFE-lined screw cap; the tube was carefully sealed and heated in a boiling water-bath for 1 h. In method B, 2 ml of urine were mixed with 2 ml of the purified solution of *Helix pomatia* digestive juice and incubated at 62°C for 1 h; the sample was cooled and 0.5 ml of formic acid were added.

After the treatments above, the cooled samples were passed through a 2-g bed of Lipidex 5000 followed by 50 ml of water, 10 ml of 20%, 10 ml of 50% and 25 ml of 70% aqueous methanol. Nitrogen was blown through the column for a short period and elution was continued with 5+30 ml of hexane as described for water and 15 ml of acetone. Then 1 ml of internal standard containing 6 and 12 ng of α-HCH was added to non-hydrolysed and hydrolysed samples, respectively. For derivatization, 150 μl of acetylating reagent were used. Occasionally, adsorption chromatography was used for additional clean-up. Silica gel (0.6 g) was transferred to a glass column (0.5 cm I.D.) containing 3 ml of hexane. The solvent was drained and the gel was washed with 7 ml of hexane. The hexane phase of the acetylated sample was reduced to 0.2 ml by a gentle stream of nitrogen and transferred to the column. The column was rinsed with 0.5 ml of hexane. Pentachlorophenyl acetate and the internal standard were eluted with 5 ml of 25% methylene chloride in hexane. The eluate was concentrated and analysed by GC.

Method for air

Lipidex 5000 (5 g) was transferred to a chromatography column (2 cm I.D.). The outlet of the column was connected to a flask containing isooctane and ended in the solvent. A flow of air through the column, ca. 5 ml/min, was obtained by suction. After different times of sampling, the column was eluted with 50 ml of hexane and 50 ml of methanol. The methanol fraction was reduced to ca. 0.5 ml. Internal standard (6 ng of α-HCH in 1 ml of hexane) and 3 ml of hexane were added, and the mixture was shaken with 3 ml of 50% sulphuric acid. After centrifugation the hexane phase was concentrated and acetylated.

TABLE I

RECOVERIES OF PCP, [^{14}C]DDT AND [^{3}H]TCDD ADDED TO WATER

Compound	*n*	Amount added (ng/ml)	Recovery★ (%)	
			Average	Range
PCP	2	0.150	92	92–93
	1	0.100	100	
	11	0.050	97	92–100
	6	0.010	94	90–100
[^{14}C]DDT	4	0.5	95	90–98
[^{3}H]TCDD	8	0.001	92	86–100

★Recoveries of PCP in the acetone fraction and of [^{14}C]DDT and [^{3}H]TCDD in the hexane fraction from Lipidex 5000.

RESULTS AND DISCUSSION

Recovery experiments were performed by addition of different amounts of PCP and [^{14}C]*p,p'*-DDT or [^{3}H]TCDD to deionized, distilled water, water purified with a Milli-Q cartridge, tap water and water from a lake. Two samples, one with and the other without added compounds, were run in parallel. The recoveries were 90–100% for 0.01–0.15 ppb PCP added (Table I). An advantage of the method is that the least polar compounds are removed into the hexane fraction. The method can be used for separation and determination of the more non-polar organochlorine compounds, as indicated by the recoveries for labelled *p,p'*-DDT and TCDD, 95 and 92%, respectively (Table I). The overall blank value of the analysis was 0.001–0.002 ng PCP per ml. The uncorrected levels of PCP found in some water samples are shown in Table II.

The average recoveries of 0.1–2.5 ng PCP per ml urine were 94% with no hydrolysis of the sample and 96% of 2.5–10 ng/ml for samples hydrolysed with hydrochloric acid and *Helix pomatia* digestive juice (Table III). The overall blank value for a 2-ml sample corresponded to 0.2 ng PCP per ml urine.

Lipidex 5000 is a hydrophobic gel, which swells in organic solvents and repels

TABLE II

LEVELS OF PCP IN SOME WATER SAMPLES

Water sample	PCP (ng/ml)
Municipal drinking water from tap	0.005
Redistilled	0.003
Deionized, cartridge-purified★	0.001
Redistilled, cartridge-purified★, outlet through latex tubing	0.011
Hand-pumped from well	0.003
Stored in a plastic bucket for 18 h	0.013
Lake	0.003

★Milli-Q from Millipore.

TABLE III

RECOVERIES OF PCP ADDED TO URINE

Pretreatment of sample	PCP added (ng/ml of urine)	*n*	Recovery (%)	
			Average	Range
Non-hydrolysed	0.1★	3	94	90–100
	0.5	3	86	80–94
	1.25	2	102	96–109
	2.5	5	94	85–100
Hydrolysed				
Hydrochloric acid	2.5	1	100	
	5.0	5	96	90–100
	10.0	5	95	87–100
Helix pomatia	2.5	2	98	96–100
	5.0	5	94	88–101
	10.0	5	97	87–100

★10 ml urine analysed.

and contracts in water. The elution with 20 and 50% methanol gives a gradual change of the gel character and enables elution with the non-polar hexane. In analyses of water, the column can be reused after regeneration by elution with 10 ml of 20% methanol and 10 ml of water. In analyses of urine, the amount of Lipidex was increased and an elution with 70% methanol was added to obtain sufficiently clean extracts for the GC analysis.

PCP has been analysed directly by GC [16,19], but because of the low response it is usually derivatized to a compound with better chromatographic properties. The acetylation procedure using ultrasonic agitation gave a quantitative yield in 15 min. The mixture was washed with 0.01 *M* potassium carbonate to reduce the amount of reagent before the GC analysis. Since the sample was injected with a falling-needle technique, any remaining pyridine did not give rise to a broad solvent peak. The enzymic hydrolysis yielded somewhat cleaner extracts than the hydrolysis with hydrochloric acid. An unknown peak, derived from hydrochloric acid, was observed in the chromatogram after acidic hydrolysis (Fig. 1).

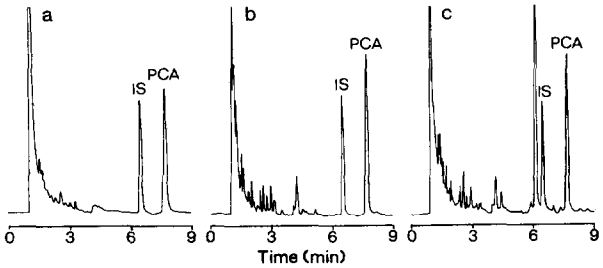

Fig. 1. GC analyses of a reference mixture corresponding to 10 ng PCP per ml (a) and a urine sample hydrolysed with digestive juice from *Helix pomatia* (b) or hydrochloric acid (c). I.S. = internal standard; PCA = pentachlorophenyl acetate.

TABLE IV

RELEASE OF PENTACHLOROPHENOL IN URINE DURING STORAGE

Time of storage (h)	PCP (ng/ml of urine)★		
	A	B	C
0	0.2	0.1	0.3
4 (24°C)	0.5	0.3	0.7
24 (4°C)	0.8		
Hydrolysed sample	16.4	9.4	13.6

★Corrected for blank values.

In 1979 Edgerton and Moseman [10] pointed out the importance of hydrolysis in analyses of PCP in urine. In the absence of hydrolysis they found less than 25% of the amount determined after hydrolysis. We noticed some release of PCP when the sample was stored at room temperature (Table IV). This was also observed on repeated thawing of frozen samples. Analyses immediately after collection with and without hydrolysis indicated that PCP was present almost exclusively as conjugates in our samples (urine from persons with no occupational exposure) (Table IV)

Twelve samples from persons with no known exposure to PCP were analysed after hydrolysis with hydrochloric acid [10] or digestive juice from *Helix pomatia* [24]. The two methods gave congruent results (Table V). In two samples sub-

TABLE V

LEVELS OF PCP IN URINE OF SUBJECTS WITH NO OCCUPATIONAL EXPOSURE

N.D. = not determined.

Subject	Sex	Age (years)	Concentration of PCP (ng/ml)	
			Acid hydrolysis	Enzyme hydrolysis
1	F	26	2.0	2.3
2★	F	40	3.8	3.5
			8.5	8.0
			5.6	6.4
3	F	34	N.D.	4.1
4	F	40	8.2	8.5
5★	F	40	13.7	13.8
			17.4	18.1
6	F	42	21.9	21.0
7	F	50	N.D.	6.2
8	M	48	10.7	9.9
9	F	33	6.0	6.8
10	M	57	29.0	29.9
11	M	35	N.D.	7.7
12	M	43	12.2	12.0

★Samples collected at ca. two-week intervals.

TABLE VI

LEVELS OF PCP IN URINE FROM PERSONS NON-OCCUPATIONALLY EXPOSED

Country	Year of publication	Number of individuals	PCP (μg/l)		Ref.
			Average	Range	
Japan	1965	20	—	10-50	20
U.K.	1970	6	—	2-11	21
U.S.A.	1967	117	40	< 1-1840	22
U.S.A.	1967	173	44	3-570	22
U.S.A. (FL)	1976	60	20	9-80	7
U.S.A.	1976	34	—	1-193	23
U.S.A.	1979	4	—	20-80	16
U.S.A.	1980	12	15	4-74★	10
F.R.G.	1978	—	20★★	2-80★★	8
Switzerland	1979	12	13.5	6-23★	9
Sweden	1986	12	11	2-30	This study

★Hydrolysed with hydrochloric acid.
★★μg/day.

jected to clean-up on silica gel, pentachlorophenyl acetate was identified and the levels were confirmed by selected-ion monitoring GC–MS.

The levels of PCP in this small Swedish group are not high compared with levels in the general population from some other countries (Table VI). During the analyses we found several sources of contamination. Many of the solvents of analytical-reagent grade contained PCP. Four different batches of methanol from one producer contained 1–10 μg PCP per l, which was verified by GC–MS. The method used for purification of methanol eliminated this contaminant. Also crepe paper and latex tubes were shown to contain PCP. By a simple enrichment procedure, PCP could be detected in the laboratory air. The increase of PCP with time of sampling is shown in Fig. 2. The experiment indicates a constant, low level of PCP in the air.

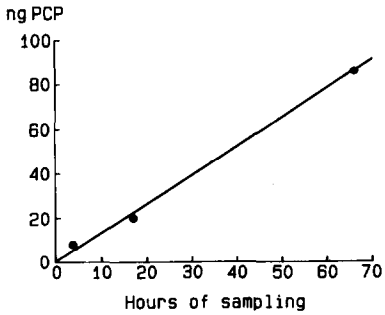

Fig. 2. Amounts of PCP sorbed from laboratory air passed through a bed of Lipidex 5000 at a rate of ca. 5 ml/min.

ACKNOWLEDGEMENTS

The technical assistance of Daisy Sjögård and Kerstin Sköld is gratefully acknowledged. This work was supported by the Swedish Council for Planning and Coordination of Research, the Swedish Medical Research Council (Grant No. 03X-219), the Swedish Society for Medical Research and Karolinska Institutet.

REFERENCES

1 L. Rudling, Water Res., 4 (1970) 533.
2 A.S.Y. Chau and J.A. Coburn, J. Assoc. Off. Anal. Chem., 57 (1974) 389.
3 C. Morgade, A. Barquet and C.D. Pfaffenberger, Bull. Environ. Contam. Toxicol., 24 (1980) 257.
4 H.-B. Lee, L.-D. Weng and A.S.Y. Chau, J. Assoc. Off. Anal. Chem., 67 (1984) 1086.
5 A. Bevenue, J.R. Wilson, E.F. Potter, M.K. Song, H. Beckman and G. Mallett, Bull. Environ. Contam. Toxicol., 1 (1966) 257.
6 J.B. Rivers, Bull. Environ. Contam. Toxicol., 8 (1972) 294.
7 R.C. Dougherty and K. Piotrowska, Proc. Natl. Acad. Sci. U.S.A., 73 (1976) 1777.
8 K. Grossler and K.H. Schaller, Z. Anal. Chem., 290 (1978) 111.
9 B. Zimmerli, T. Marschall and B. Marek, Mitt. Gebiete Lebensm. Hyg., 70 (1979) 443.
10 T.R. Edgerton and R.F. Moseman, J. Agric. Food Chem., 27 (1979) 197.
11 H.-B. Lee, L.-D. Weng and A.S.Y. Chau, J. Assoc. Off. Anal. Chem., 67 (1984) 789.
12 L. Renberg, Anal. Chem., 46 (1974) 459.
13 C.D. Chriswell, R.C. Chang and J.S. Fritz, Anal. Chem., 47 (1975) 1325.
14 L. Renberg and K. Lindström, J. Chromatogr., 214 (1981) 327.
15 C.E. Rostad, W.E. Pereira and S.M. Ratcliff, Anal. Chem., 56 (1984) 2856.
16 T.R. Edgerton, R.F. Moseman, E.M. Lores and L.H. Wright, Anal. Chem., 52 (1980) 1774.
17 B. Egestad, K. Norén and J. Sjövall, Lipidex® — An Alternative Sorbent for Extraction of Chlorinated Environmental Pollutants in Aqueous Media, presented at the Meeting on Solid Sorbents in Chemical Analysis, Stockholm, Nov. 18–19, 1985.
18 M. Axelson and J. Sjövall, J. Chromatogr., 126 (1976) 705.
19 D.A. Kalman, J. Chromatogr. Sci., 22 (1984) 452.
20 T. Akisada, Bunseki Kagaku, 14 (1965) 101.
21 M. Cranmer and J. Freal, Life Sci., 9 (1970) 121.
22 A. Bevenue, J. Wilson, L.J. Casarett and H.W. Klemmer, Bull. Environ. Contam. Toxicol., 2 (1967) 319.
23 F.W. Kutz, R.S. Murphy and S.C. Strassman, 104th Annual Meeting of the American Public Health Association, Miami Beach, FL, 1976, Abstract.
24 M. Axelson, B.-L. Sahlberg and J. Sjövall, J. Chromatogr., 224 (1981) 355.