Journal of Chromatography, 346 (1985) 435–439
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 027

Note

Rapid and convenient separation of pentachlorophenol from human fat using silica Sep-Pak™ cartridges

G. A. S. ANSARI* and PAMELA Y. HENDRIX
Chemical Pathology Laboratory and Division of Biochemistry, The University of Texas Medical Branch, Galveston, TX 77550 (U.S.A.)
(Received July 2nd, 1985)

Pentachlorophenol (PCP) is widely used as an insecticide, fungicide, herbicide, and wood preservative. Due to its extensive usage, PCP has been found to be a ubiquitous environmental contaminant. Humans can be exposed to PCP mainly through inhaling contaminated air or consuming contaminated food and water, however it can also be absorbed through the skin. Since compounds such as hexachlorobenzene and γ-hexachlorocyclohexane can also be metabolized to PCP[1] this could further contribute to the magnitude of human exposure. Presently, it is not known to what extent these possible sources contribute to the total PCP body burden.

Previous investigators have shown a slow elimination of PCP from body tissue and significant levels have particularly been found in human adipose tissue. PCP residues were measured in human adipose tissue taken at autopsy in the United States (range 12–52 ppb*)[2], Canada (range 0–277 ppb)[3], and Japan (range 10–570 ppb)[4]. In fat samples analyzed[2–4], only 75–80% of the PCP was recovered and the procedures used were time consuming and cumbersome. In the present study, a simple and reproducible method was developed using a Sep-Pak™ silica cartridge as a clean-up step for the analysis of PCP in adipose tissue. The recovery of PCP from human adipose tissue was 92–97%.

EXPERIMENTAL

Materials

All solvents were pesticide grade (Burdick and Jackson, Muskegan, MI, U.S.A), [^{14}C]pentachlorophenol (PCP) (1.83 mCi/mmole) with a radiochemical purity of 91%, as determined by thin-layer chromatography, was provided by Dow Chemical, Midland, MI, U.S.A. Non-radioactive PCP (99+% purity, gold label) was purchased from Aldrich, Milwaukee, WI, U.S.A. Bio-beads SX2, 200–400 mesh was obtained from Bio-Rad Labs. (Richmond, CA, U.S.A.). C_{18} and Silica Sep-Pak™ cartridges were obtained from Waters Assoc., Milford, MA, U.S.A. Human fat samples obtained at autopsy were excised from the anterior abdominal wall and frozen at −80°C until analyzed.

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

0021-9673/85/$03.30

Gel permeation chromatography (GPC)

One gram of human fat was supplemented with ^{14}C-PCP (50 ppb, $3.4 \cdot 10^{-4}$ μCi), dissolved in 1 ml of cyclohexane and applied to a Bio-beads SX2 column (27 cm × 20 mm I.D.), pre-equilibriated with cyclohexane. The column was eluted with cyclohexane at a flow-rate of 4 ml/min. Fractions of 1 ml were collected and analyzed for fat and radioactivity as described later.

Sep-Pak chromatography

Fat (300–500 mg) containing ^{14}C-PCP (31 ppb, $2.1 \cdot 10^{-4}$ μCi) was dissolved in 1 ml of acetonitrile and applied to a C_{18} Sep-Pak cartridge. The sample was eluted with acetonitrile.

Silica Sep-Pak. Fat (300–500 mg) containing ^{14}C-PCP (100 ppb, $6.9 \cdot 10^{-4}$ μCi) was dissolved in 900 μl hexane and applied to a silica Sep-Pak cartridge. The column was eluted with various amounts of hexane and chloroform as shown in Figs. 2 and 3. The column was further eluted with tetrahydrofuran to determine the effect on fat recovery.

Fat and ^{14}C-PCP recoveries

Effluent from Sep-Pak cartridges was collected in 2-ml fractions and evaporated to dryness under vacuum at room temperature on a Searle vortex evaporator (Buchler Instruments, Fort Lee, NJ, U.S.A.). Vials were weighed to determine fat recovery and the residue was dissolved in hexane. An aliquot was removed, dissolved in Scintiverse (Fisher Scientific, Fair Lawn, NJ, U.S.A.) and radioactivity was measured on a Packard Mark III 6880 liquid scintillation counter (Searle Analytics, Des Plains, IL, U.S.A.). Counting efficiency was determined by external standardization.

RESULTS AND DISCUSSION

Using SX-2 column chromatography the recovery of ^{14}C-PCP was 86.5% (Table I). The representative profile of fat and ^{14}C-PCP recovery is shown in Fig. 1. Using silica gel Sep-Pak columns and eluting with hexane (10 ml) followed by chloroform (17 ml) gave a recovery of 92.3% ^{14}C-PCP in 8 ml of chloroform and had fat contamination $<1\%$. When an intermediate elution step (hexane–chloroform, 1:1, 5 ml) was added, the recovery of ^{14}C-PCP was increased to $\approx 97\%$. With this

TABLE I

RECOVERY OF ^{14}C-PCP FROM FAT BY CHROMATOGRAPHY

Mean of three determinations.

Column	*Fat recovery (%)*	*^{14}C-PCP recovery (%)*	*Fat contamination of ^{14}C-PCP (%)*
SX-2 bead	81 ± 3.8	86.5 ± 7.4	0.5 ± 0.1
Silica Sep-Pak*	100 ± 12.7	92.3 ± 10.3	0.8 ± 0.3
Silica Sep-Pak**	99.5 ± 4.2	96.8 ± 2.1	4.0 ± 0.5

* Column eluted with 10 ml hexane, 17 ml chloroform (Fig. 2).

** Column eluted with 10 ml hexane, 5 ml hexane–chloroform, 1:1 (v/v), 15 ml chloroform (Fig. 3).

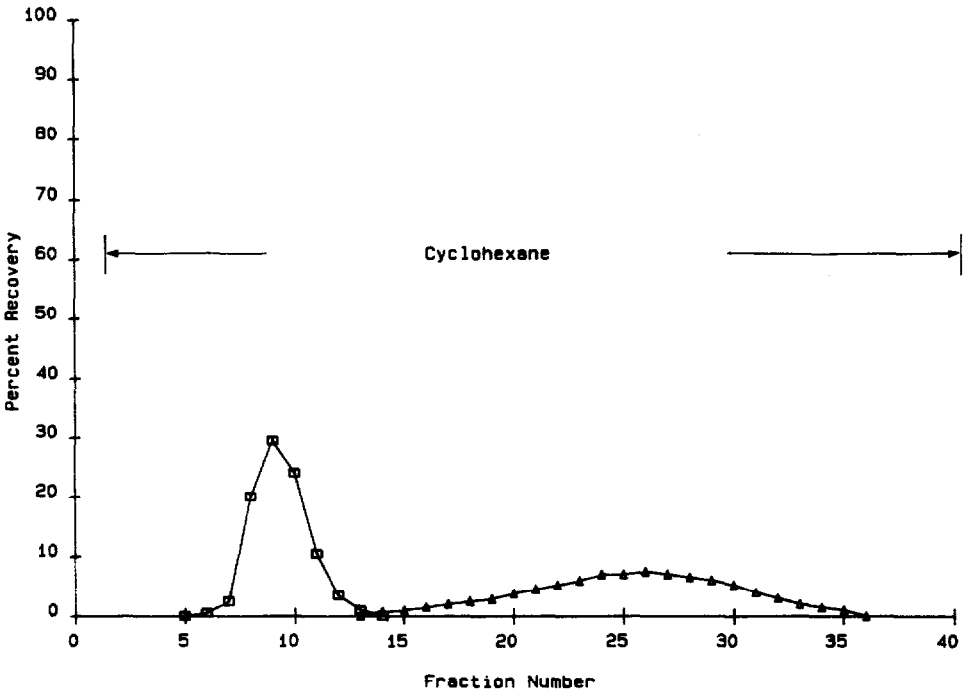

Fig. 1. Elution profile of human fat (□) and ^{14}C-PCP (△) using SX-2 column chromatography.

increase, fat contamination also increased to ≈4% (representative profiles are shown in Figs. 2 and 3).

In some experiments when ≈500 mg fat was used a small amount of radioactivity (<1%) was eluted in the hexane fraction. After elution with chloroform a small amount of fat (<2%) was recovered when the column was further eluted with tetrahydrofuran (4 ml).

Attempts were made to utilize the Sep-Pak C_{18} cartridge. Using a Sep-Pak C_{18} column and acetonitrile or methanol–water (9:1, v/v) fat and ^{14}C-PCP eluted together in 5 ml volume and therefore the C_{18} cartridge cannot be used for enrichment of PCP from fat.

The PCP enriched fraction obtained from silica gel Sep-Pak column chromatography can be analyzed either by capillary gas chromatography[5–6] or by high-performance liquid chromatography[7–10].

In summary, a procedure was developed by which PCP can be recovered from fat samples in high yields. This procedure is less cumbersome and less time consuming than procedures used earlier[2–4]. Recently, *in vitro*[11] and *in vivo*[12] studies have shown that PCP may exist in human fat as a fatty acid ester. The present method does not account for PCP-ester in the recovery. The effect of prior hydrolysis of fat followed by silica Sep-Pak separation has not been attempted.

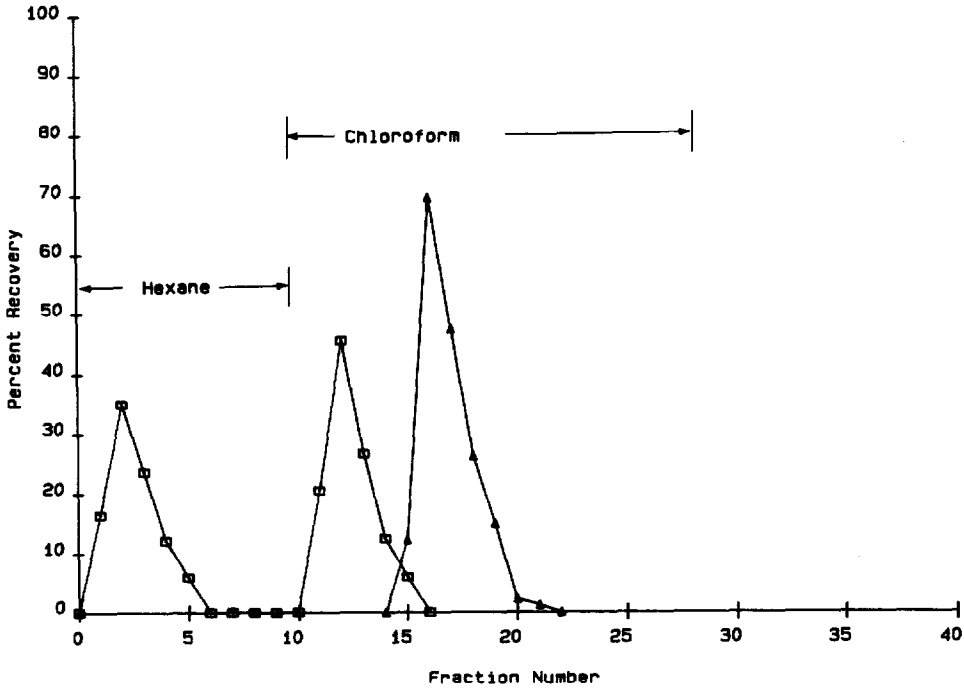

Fig. 2. Elution profile of human fat (□) and ^{14}C-PCP (△) using silica Sep-Pak column chromatography. Column eluted with hexane followed by chloroform.

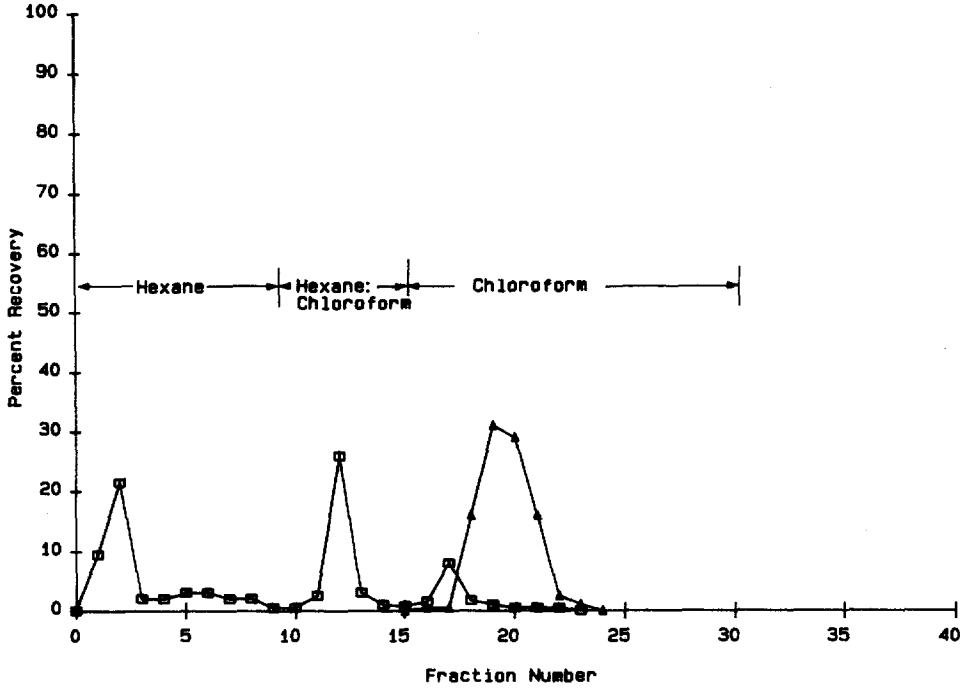

Fig. 3. Elution profile of human fat (□) and ^{14}C-PCP (△) using silica Sep-Pak column chromatography. Column eluted sequentially with hexane, hexane–chloroform (1:1, v/v) and chloroform.

ACKNOWLEDGEMENTS

The authors wish to thank Avis Morgan and Sandy Campanello for secretarial assistance. This work was supported by grant AM 27135 from the NIH.

REFERENCES

1 R. Engst, R. M. Macholz and M. Kujawa, *Bull. Environm. Contam. Toxicol.*, 16 (1976) 248.
2 T. M. Shafik, Bull. Environm. *Contam. Toxicol.*, 10 (1973) 57.
3 D. T. Williams, G. LaBel and E. Junkins, *J. Toxicol. Environm. Hlth.*, 13 (1984) 19.
4 T. Ohe, *Bull. Environm. Contam. Toxicol.*, 22 (1979) 287.
5 D. A. Kalman, *J. Chromatogr. Sci.*, 22 (1984) 452.
6 I. O. O. Korhonen, *J. Chromatogr.*, 303 (1984) 197.
7 N. G. Buckman, J. O. Hill, R. J. Magee and M. J. McCormick, *J. Chromatogr.*, 284 (1984) 441.
8 K. D. McMurtrey, A. E. Holcomb, A. U. Ekwenchi and N. C. Fawcett, *J. Liq. Chromatogr.*, 7 (1984) 953.
9 J. Nair, K. M. Munir and S. V. Bhide, *J. Liq. Chromatogr.*, 6 (1983) 2829.
10 H. A. McLeod and G. Laver, *J. Chromatogr.*, 244 (1982) 385.
11 E. G. Leighty and A. F. Fentiman, Jr., *Bull. Environm. Contam. Toxicol.*, 28 (1982) 329.
12 G. A. S. Ansari, S. G. Britt and E. S. Reynolds, *Bull. Environm. Contam. Toxicol.*, 34 (1985) 661.