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Pentachlorophenol Residues in Human Adipose Tissue

Takeshi Ohe

Kyoto City Institute of Public Health, Higashi-Takada-cho, Mibu, Nakakyo-ku, Kyoto, 604, Japan

Pentachlorophenol (PCP) is widely used as a bactericide, herbicide and insecticide in agriculture and a preservative of wood and various products which are prone to microbiological attacks in industry and households. Considerable works have been performed for evaluating the toxicity of the compound for aquatic and mammalian animals (BEVENUE and BECKMAN 1967, STRUFE 1968). HOBEN et al. (1976) recently reported the inhaltion toxicity of PCP for rats. The LD_{50} for inhalted aerosol of PCP was much lower than the ingested and intraperitoneal dose.

BRAUN and SAUERHOFF (1976) studied the pharmacokinetic profile of PCP in monkeys. After a single oral dose of 10 mg/kg PCP, the half-life value for elimination of PCP from plasma was 84 h for females and 72 h for males while the half-life value for excretion was 92 h and 41 h for females and males, respectively. This result demonstrates that orally administered PCP is extensively absorbed.

PCP is rather stable in environment and accumulates in biological systems. Many investigaters identified PCP residue not only in the samples from environment such as sewage influent, river water and fish but also in human blood, urea and adipose tissue (STRUFE 1968, SHAFIK 1973, ZITKO et al. 1974, CHAU and COBURN 1974).

These results indicate that the distribution of PCP in our environment may be quite widespread and humans are continuously exposed to low levels of PCP.

This paper reports the residue level of PCP in human adipose tissue of the general population in Japan.

MATERIALS AND METHODS

Reagents

Potassium carbonate solution, -0.1M. Prepare fresh daily and wash with hexane; Sodium sulfate, -Wash with hexane; Silica gel, -Kieselgel 60 (70 - 230 mesh ASTM). Prepare according to the method by HOLDEN and MARSDEN (1969); Pentachlorophenol, -Pesticide residue grade; Pentachlorophenol acetate, -mp 149.5°C. Prepare according to the method by CHAU and COBURN (1974); 2,3,4,6-Tetrachlorophenol, -Technical grade.

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Sampling Collection

Samples of human adipose tissue were obtained through medical examiners at hospitals in Kyoto and Osaka city in 1974. The tissue samples were deposited in glass jars, previously acid washed and with residue free acetone and hexane. The caps were lined with aluminum foil. Samples were immediately frozen and kept until analyzed.

Extraction of Adipose Tissue

The adipose tissue was cut up into small pieces. A 2 g sample was extracted three times with 30 ml portions of hexane for every 5 min in a homogenizer and centrifuged to separate the two layers. The combined extracts were filtered through sodium sulfate and concentrated to 10 ml below 50°C under a gentle stream of N_2. A 1 ml aliquot was evaporated in a preweighed flask to determine the lipid content.

Cleanup and Determination

Two ml of hexane extract (approximately 500 mg as lipid content) was prepared into a test tube and was adjusted to 5 ml with hexane. Three ml of K_2CO_3 solution (0.1M) was added to the tube. The mixture was mixed on a thermo mixer for about 1 min and centrifuged to separate the two layers. The K_2CO_3 layer was transfered into another test tube. The hexane layer was partitioned with another 2 ml of K_2CO_3 solution. To the combined K_2CO_3 solution, 0.3 ml acetic anhydride and 3 ml hexane were added and kept for about 20 min at room temperature with occasional shaking. The aqueous layer was repeatedly extracted with another 3 ml hexane and the hexane layer was combined with the first hexane fraction. The combined hexane fraction was washed twice with 3 ml portions of water, dried over sodium sulfate, and concentrated to about 1 ml on a hot plate (below 50°C) with the aid of a stream of N_2. The concentrate was applied to the silica gel dry column (2 g). The column was eluted with 8 ml of hexane and then 8 ml of 15 % ether in hexane. The 15 % ether eluate was collected and examined by gas chromatography with electron-capture detection (EC-GC).

Quantitation was accomplished through the comparison of the peak height measurement obtained from the sample and the standard. The gas chromatography used was Shimazu GC 5AP. The operative conditions for PCP analysis as follows: column dimensions, 2 m x 3 mm (i.d.) glass; column packing, 1.5 % OV-17 Chromosorb W (AW-DMCS) 80/100 mesh: column temp., 180°; detector temp., 210°; N_2 flow rate, 1.0 kg/cm^2.

Confirmation

Confirmation of PCP acetate derived from PCP in human adipose tissue samples was accomplished on

several EC-GC columns and a gas chromatography-mass spectrometer (GC-MS) comparing with the authentic sample. GC-MS instrumental conditions were as follows: Shimazu-LKB 9000 system was used with a 0.5 m x 3 mm (i.d.) glass column packed with 1.5 % OV-17 on Chromosorb W (AW-DMCS) 80/100 mesh. The operative conditions were; column temp., 140°; flow rate of helium, 25 ml/min; trap current, 70 μA; ionization voltage, 70 eV.

RESULTS AND DISCUSSION

The analyses were made with 25 samples of human adipose tissue, 13 males, 9 females and 3 unknown. The results are given in Table 1. No occupational contacts with PCP were recognizable with these subjects.

The PCP content ranged from nd to 0.57 ppm with a mean value of 0.14 ppm. The choice of the acetyl derivative for the rapid and precise determination of PCP in human adipose tissue was due to the possibility of formation of the acetyl derivative in the aqueous alkali extract (CHAU and COBURN 1974). The sensitivity of the electron-capture detection to PCP acetate was approximately 2.5 pg. A complete solvent blank was performed through the entire procedure with each set

TABLE 1

PCP contents in fat extracted from human adipose tissue

Sample No.	Sex	Age	PCP in fat (ppm)	Sample No.	Sex	Age	PCP in fat (ppm)
1	Male	50	0.41	14	Female	37	nd
2		31	tr.[a]	15		50	tr.
3		67	nd[b]	16		73	nd
4		68	0.02	17		-	0.16
5		53	0.01	18		-	0.28
6		69	nd	19		56	nd
7		-	0.10	20		40	0.01
8		-	0.57	21		50	nd
9		79	0.05	22		54	tr.
10		68	0.09	23	Unknown	-	0.53
11		63	nd	24		-	0.25
12		28	0.43	25		4	0.01
13		-	0.48				
	Total		mean	0.14			
			range	nd - 0.57			
			S.D.	0.04			

a) Levels less than twice background
b) Not detected

of samples and standard. The limit of detectability for PCP in adipose tissue was 5 ppb. Levels of PCP of less than twice background were designated as trace amounts in Table 1 and other values have been corrected for background. The method yielded recoveries in the range of 85 - 98 % with a sensitivity of 1 ppm on samples of fortified butter fat.

Several EC-GC columns were evaluated for the identification of PCP acetate derived from PCP in human fat samples. Table 2 lists relative retention times for PCP acetate and 2,3,4,6-tetrachlorophenol acetate compared to β-BHC on several selected column packings. An aliquot of the condensed PCP acetate fractions was subjected to GC-MS analysis (m/e=309).

A typical EC-GC elution pattern of PCP acetate derived from PCP in human adipose tissue is presented in Figure 1, together with that of a standard and solvent blank. Peak 2 corresponds to PCP acetate peak and peak 1 was tentatively assigned to 2,3,4,6-tetrachlorophenol acetate from the coincidence of the retention time with that of authentic sample. It is reported that 2,3,4,6-tetrachlorophenol is used as fungicide. Technical grade of PCP generally contains as impurities up to 13 % of other chlorophenols, of which isomeric 2,3,4,6-tetrachlorophenol constitute the principal part (MELNIKOV 1971).

Although PCP have been used in agriculture, industry and households, the origin of these levels of PCP in human adipose tissue has not well understood.

In the Agricultural Chemicals Control Law amended in 1963, PCP was designated as a designated pesticide (FUKUNAGA and TSUKANO 1969). In the standard of this amendment, pesticide are classified into four classes based on median tolerance limits (TLm) of their active ingredients for carp, Daphnia pulex, and Monia macrocopa, and PCP belongs to "Class D", which use are restricted because of their highly toxicity to fish. Since then, the use of PCP in agriculture has been decreased. On the other hand, the use of PCP in industry has been increased.

From recent experiments in various species, it is known that PCP is found as one of metabolites of

TABLE 2

Relative retention times of PCP acetate and 2,3,4,6-tetrachlorophenol acetate compared to β-BHC on various column packings

Liquid phase	Relative retention time		
	β-BHC	PCP-Ac	TeCP-Ac
1) OV-1	1.0	1.42	0.70
2) OV-17	1.0	0.85	0.43
3) QF-1-SE-30	1.0	1.16	0.73

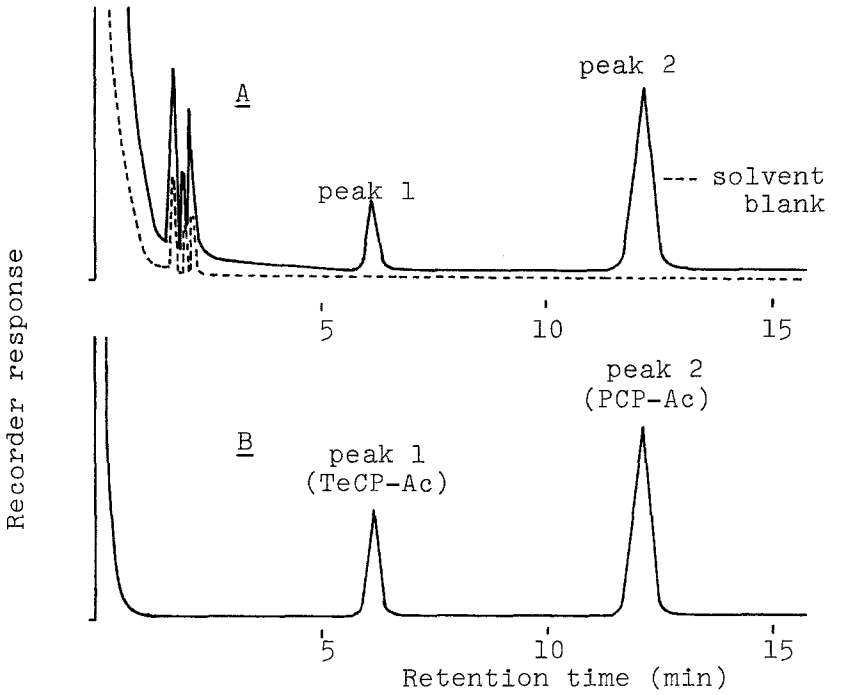

Figure 1. Gas chromatogram of a human adipose tissue sample
A. Human adipose tissue sample
B. Standard: Peak 1, 2,3,4,6-Tetrachlorophenol acetate, Peak 2, PCP acetate
EC-GC conditions; 1.5 % OV-17 on Chromosorb W (AW-DMCS) 2 m x 3 mm, column temp.: 180°, inj. temp.: 220°, detector temp.: 210°, carrier gas: N_2 1.0 kg/cm^2.

hexachlorobenzene (HCB) or γ-BHC (MEHENDALE et al. 1975, YANG et al. 1975, ENGST et al. 1976). Even if these results would apply to man, it doesn't likely that these metabolites affect the levels of PCP found in this study considering from the very low levels of HCB and γ-BHC in Japanese adipose tissue (MORITA et al. 1975, FUKANO and DOGUCHI 1977).

These PCP levels were slightly higher than those detected in human adipose tissues of the general population by SHAFIK (1973). As far as the levels obtained in this study are concerned, it can be concluded that humans are continuously exposed to low levels of PCP from the environment. However, no informations are available in the source and route of PCP residues. Although the present residue level

is not considered to be toxicologically significant, it seems to be necessary to pay attention to the source and route of PCP residue, and to investigate the prevention of the forthcoming development of PCP pollution.

REFERENCES

BEVENUE, A., and H. BECKMAN: Residue Reviews 19, 83 (1967).

BRAUN, W. H., and M. W. SAUERHOFF: Toxicol. Appl. Pharmacol. 38, 525 (1976).

CHAU, A. S. Y., and J. A. COBURN: J. Assoc. Offic. Anal. Chem. 57, 389 (1974).

ENGST, R., R. M. MACHOLZ, and M. KUJAWA: Bull. Environ. Contam. Toxicol. 16, 248 (1976).

FUKANO, S., and M. DOGUCHI: Bull. Environ. Contam. Toxicol. 17, 613 (1977).

FUKUNAGA, K., and Y. TSUKANO: Residue Reviews 26, 1 (1969).

HOBEN, H., J. S. A. CHING, and L. J. CASARETT: Bull. Environ. Contam. Toxicol. 15, 463 (1976).

HOLDEN, A. V., and K. MARSDEN: J. Chromatog. 44, 481 (1969).

MEHENDALE, H. M., M. FIELDS, and H. B. MATTHEWS: J. Agric. Food Chem. 23, 261 (1975).

MORITA, M., F. USHIO, T, NISHIZAWA, S. FUKANO, M. DOGUCHI, and S. MIURA: J. Food Hyg. Soc. Japan 16, 53 (1975).

SHAFIK, T. M.: Bull. Environ. Contam. Toxicol. 10, 57 (1973).

STRUFE, R.: Residue Reviews 24, 103 (1968).

YANG, R. S. H., F. COULSTON and L. GOLBERG: J. Assoc. Offic. Anal. Chem. 58, 1197 (1975).

ZITKO, V., O. HUTZINGER, and P. M. K. COHI: Bull. Environ. Contam. Toxicol. 12, 649 (1974).