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Determination of Pentachlorophenol in Urine

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Pentachlorophenol (PCP) and its sodium salt (Na PCP) are included among the most versatile pesticides, due to their efficiency in destroying a great variety of undesirable animal or plant species, and also for their solubilities in organic solvents or water. They are the second most-used pesticides in the U.S.A., being employed as insecticide (termicide), fungicide, algicide, molluscicide and bactericide. Approximately 80% of the produced PCP is used as a wood preservative as well as in other activities associated with the forest products industry.

PCP can be harmful to humans, especially to occupationally exposed workers as has been demonstrated in cases where death has occured due to intense exposure (BERGNER et al. 1965 ; CHAPMAN & ROBSON 1965; GOMES & CABALLERO 1965; MASSON et al. 1965; TRUHAUT et al. 1952).

Laboratory control of PCP-exposed workers is usually done by determining PCP in urine. Generally, the analysis involves an initial PCP extraction by organic solvents, followed by derivatization and identification using electron-capture gas chromatography. Derivatization is generally done by methylation using an ethereal diazomethane solution. This procedure was introduced by BEVENUE et al. (1966). The major problem involved in this analysis is related to the methylating reagent, which must be handled with extreme care, due to its toxicity and potential explosiveness. Furthemore the solutions are unstable and their preparation requires care due to their carcinogenic and skin and respiratory irritant properties (BEVENUE & BECKMAN 1967).

Another process of derivatization, which was described by RUDLING (1970) to search PCP in water and fishes, is based upon the formation of an acetylated product by treating PCP with acetic anhydride. Such a method was simplified by ERNEY (1978), to analyse for PCP in milk. This is an extremely simple and fast process. ERNEY's method was adapted to determine PCP in the urine of exposed workers due to a need to monitor these exposed workers in a PCP and Na PCP synthesis factory.

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MATERIALS AND METHODS

Gas Chromatography: A gas chromatograph equipped with a ^{63}Ni electron-capture detector was used. Inlet and detector temperatures were 240 C; nitrogen flow rate was 40 mL/min.

Gas Chromatographic Columns: A 1,8 m x 4 mm I.D. glass column packed with 10% DC-200 on 100-200 mesh Chromosorb W(AW) was used. Oven temperature was 220 C.

A 1,2 m x 2 mm I.D. glass column packed with 3% OV-17 + 5% OV-210 on 100-200 mesh Chromosorb W(AW) was used. Oven temperature was 180 C.

Reagents and Solutions:

(a) Pentachlorophenol - 99.9% (Carlo Erba)
(b) Solvents - redistilled benzene and hexane
(c) Solutions - 1 N HCI; 0.1 M potassium carbonate; saturated sodium sulfate.
The water used to prepare solutions and the potassium carbonate solution was decontamined through hexane extractions.
(d) Acetic anhydride: 97% (Merck).
Purification of acetic anhydride was performed by distillations in an all-glass system.

Preparation of PCP Acetate:

PCP Acetate was prepared according to CHAU & COBURN (1974). The melting point of the substance was 150 C (149.5-150.5).

Urine Samples:

The urine samples were colected at the end of a week of work from two groups:

I) Control Group: 27 urine samples of non-occupationally exposed workers.
II) Exposed Group: (a) 9 urine samples of PCP-occupationally exposed workers in synthesis factory in full time activity.
(b) 12 urine samples of workers from the same factory, collected in a period in which the synthesis of PCP was being reduced and they were working in other activities in the same industry.

Extraction

Transfer 20 mL urine to a 125-mL separatory funnel. Add 1 N HCI just to pH near 2. Extract twice with 20 mL benzene. Use saturated Na_2SO_4 to break any emulsion. Extract

combined benzene layers twice with 50 mL 0.1 M K_2CO_3 solution and discard organic phase. Combine aqueous portions in 250-mL separatory funnel. Add 1 mL acetic anhydride. Invert funnel , open stopcock, mix solution until pressure is minimal. Repeat funnel inversions at 1 min intervals until separatory funnel can be shaken without pressure buildup. Add 20 mL hexane and shake vigorously. Remove hexane and analyse by gas chromatography (GLC). For low levels of PCP (< 0.05 pg) evaporate combined hexane extracts to 5 mL before GLC analysis.

Analysis of urinary creatinine was performed in all samples (method of DJURIC 1967) to permit better results expression.

RESULTS

To determine the recovery of PCP from urine, a triplicate sample of the pooled urine was fortified with 0.2; 0.5 and 0.8 µg PCP/mL sample. To prepare the fortified samples, PCP was dissolved in a 0.01 N NaOH solution. The average recoveries of PCP from the analysed samples were 89; 88 and 87% , respectively, with a mean value of 88%. Sensivity of the electron-capture detector to the PCP derivative is approximately 10 pg. Representative gas chromatograms for PCP acetate reference standard in hexane, control of reagents and extract from urine sample of GROUP II.a are shown in figure 1. Table 1 and 2 show, respectively, the results obtained from the control group and exposed group (a and b).

DISCUSSION

Periodic laboratory control of PCP exposed workers , requires a sensible, fast and simple method. In our first approach, we tried the method described by BARTHEL et al. (1969) for the PCP analysis, using a special column of DEGS/H_3PO_4, without the need of a previous derivatization of the compound. However, we failed to obtain good results , because the column showed a very short life. The most used method, also recomended by the Manual of Analytical Methods (EPA 1977) suggests derivatization with diazomethane; such a method introduces some difficulties when it is used on a routine basis, due to problem in obtaining and using the methylating reagent. The method described by ERNEY (1978) to determine PCP in milk by gas chromatography of its acetylated derivative, seemed to us adequate and showed a series of advantages when compared to the others: (a) the PCP extraction from a benzene solution by K_2CO_3 solution is very selective, since this procedure separates the acid compounds from the organochlorine insecticides and PCBs; (b) the further extraction of the formed PCP Ac with hexane, makes possible its separation from the herbicides such as 2,4-D and 2,4,5-T, which remain in alkaline solution; (c) it doesn't

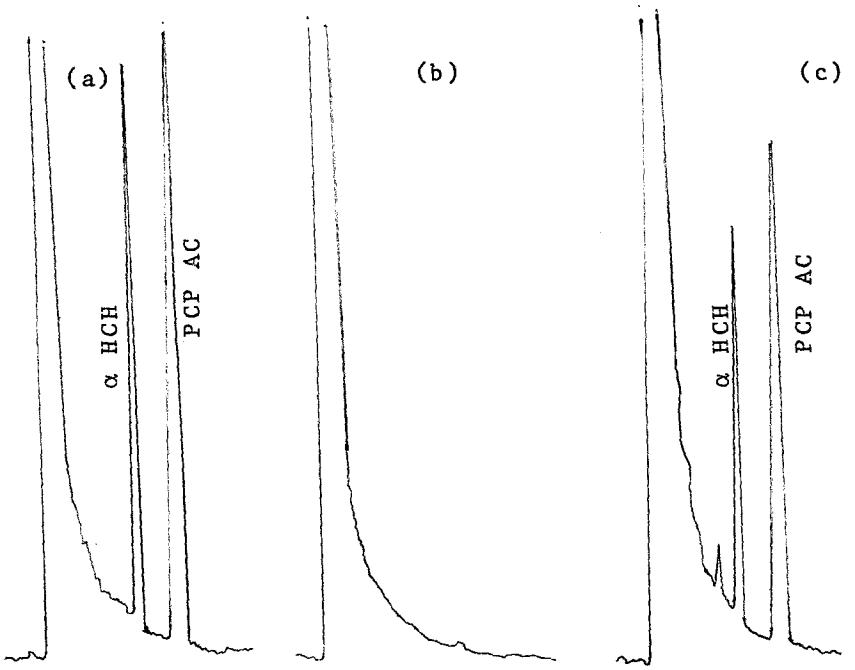

Figure 1. Gas chromatogram of (a) 0.75 ng PCP acetate reference standard (b) control of reagents (c) hexane extract from urine sample of the GROUP II-a, in column 10% DC-200.

Table 1. PCP contents in urine samples from workers of the GROUP I.

Sample nº	µg PCP/g of creatinine	PCP ppb	Sample nº	µg PCP/g of creatinine	PCP ppb
1	7.3	9.6	15	nd	nd
2	1.5	7.5	16	3.2	4.8
3	1.1	9.6	17	5.1	5.0
4	nd*	nd	18	3.4	12
5	nd	nd	19	6.4	9.6
6	2.9	4.8	20	nd	nd
7	6.8	8.4	21	9.7	15
8	nd	nd	22	6.9	6.7
9	3.1	8.3	23	9.6	8.4
10	3.0	4.8	24	6.6	6.3
11	8.5	15	25	3.2	6.3
12	12	17	26	10	16
13	11	15	27	12	34
14	nd	nd			
TOTAL					
mean				5.7	8.8
range				nd- 12	nd - 34
SD				3.4	6.7

*nd = not detected, < 10 pg PCP Ac

Table 2. PCP contents in urine samples from workers of GROUP II

II-a			II-b		
Sample nº	µg PCP/g of creatinine	PCP ppb	Sample	µg PCP/g of creatinine	PCP ppb
1	630	1100	1	86	120
2	2700	3400	2	110	190
3	520	960	3	100	70
4	200	340	4	21	40
5	1100	1300	5	160	240
6	150	420	6	98	190
7	210	410	7	47	120
8	770	1500	8	130	300
9	620	1300	9	23	32
			10	130	400
			11	25	39
			12	82	92
TOTAL					
mean	760	1200		83	150
range	150-2700	340-3400		25-160	32-400
SD	700	940		46	110

require purification in column, recomended by several authors, to eliminate interferences; (d) possibility of formation of the acetyl derivative in the aqueous alkali extract.

It was necessary to decontaminate every material used in the PCP analysis by electron-capture gas chromatography , because of the possible presence of this compound and other chlorophenols. The proposed method for glass decontamination showed excellent results. However, we didn't accomplish a perfect purification of the ecetic anhydride even after several distillations, as shown in figure 1 (b). CHAU & COBURN (1974) call special attention on this interference of the acetic anhydride, which has a similar retention time in relation to PCP Ac. For this reason it was necessary to extract the sample concurrently with a control of reagents.

PCP Ac identification was based upon the relative retention of the α HCH. Quantification was done through the comparison of the peak height measurement obtained from sample and standard. All samples were also analysed in OV-17 column.

The values found in the control group were from non-detectable to 34 ppb. It is common knowledge the presence of PCP in urine. The possible sources of human exposure were well quoted by DIETRIK (1977). The values found in the group of occupationally exposed workers showed individual variations. The II-a group showed results significantly higher than those of the group II-b, as it was expected, for different work conditions in which the samples were collected. Several authors also found great individual differences among the group of studied workers (CASARETT et al. 1969; BEVENUE 1967; BEGLEY et al. 1977). We didn't find a correlation between the time of exposure in the factory or age, with urinary levels of PCP.

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