Analysis of Pentachlorophenol Residues in Soil, Water, and Fish

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Pentachlorophenol (PCP) is extracted from soil and fish with 0.1*M* potassium hydroxide. After adjusting the pH to below 7, the PCP is extracted with toluene, methylated with diazomethane, and analyzed by electron-capture gas chromatography. As little as 0.5 p.p.b. of PCP can be detected in soil or fish. In 1 liter of water it is possible to determine 0.01 p.p.b. Recovery of added PCP is in the range of 80 to 100%. A method for the preparation of PCP trimethylsilyl ether is described, and this derivative is used for qualitative test. A further identification of PCP is made with a mass spectrometer.

The increased use of PCP and its sodium salt in industry and agriculture involves a risk of pollution in lakes and streams. It is known that this substance is accumulated in fish, and the mean survival time LD_{50} is about one hour at one p.p.m., depending on species, pH and temperature of the water (Goodnight, 1942). Less well known is the effect of PCP at a level sublethal for fish, but lethal for lower steps in the food chain. This creates a need for more sensitive and simple methods of determining PCP. A review of the literature on PCP, covering its use, properties, toxicology, and analytical methods, has been published (Bevenue and Beckman, 1967).

PCP has been steam-distilled from plants and soil, and determined as the sodium salt by UV absorption (Hilton, 1966). Other spectrophotometrical procedures are available for detecting residues of PCP in water (Goto *et al.*, 1963; Uede *et al.*, 1962) and in fish (Tsuda and Kariya, 1963). More sensitive methods employing electron-capture gas chromatography are available for determination of PCP in urine (Bevenue *et al.*, 1966) and in fruits (Cheng and Kilgore, 1966). Usually, the PCP is converted to its methyl ether for gas chromatography determinations (Kanazawa, 1963; Stanley, 1966). Phenols can also be converted to their trimethylsilyl ethers for gas chromatographic separations (Langer *et al.*, 1958).

The present paper describes simple procedures for the extraction of PCP in soil, water, and fish. The methods are adapted for gas chromatographic determinations and the analyses are sensitive, especially in the case of water.

EQUIPMENT AND MATERIALS

Gas Chromatograph. Aerograph Hy-Fi 550 B. Electrometer 500 and ECD.

Recorder. Speedomax H. Recorder speed, 15 inches per hour.

Column. 120- × 0.18-cm. I.D. borosilicate glass, containing 4% SF 96 and 8% QF 1 in the proportions of 2 to 3 on silanized Gaschrom P, 100- to 120-mesh.

Temperatures: injection block, 195° C.; oven about 155° C.; detector, 195° C. The part of the detector outside the oven was heated separately and the temperature was measured inside the detector cylinder. The nitrogen carrier gas passed through a copper column 140 × 1.5 cm., heated to 160° C., and then through a molecular sieve with the same dimensions. Gas flow rate was 25 ml. per minute.

Combined Mass Spectrometer and Gas Chromatograph. LKB 9000. Emission temperature was 250° C. and electron energy 70 e.v.

Reagents. Nitrosomethylurea (Amstutz, 1943) was used as the precursor for the preparation of diazomethane by Arnt (1943). He also describes a simplified method using the same precursor, which gives a somewhat impure product, but is nevertheless quite serviceable for the analysis. This simplified method has the advantage that it renders the hazardous distillation of diazomethane unnecessary.

PROCEDURE

Extraction of Samples. Two to 5 grams of soil is shaken for 2 hours with 0.1*M* potassium hydroxide, 5 ml. for each gram of soil. A portion of the alkaline solution is centrifuged and then separated. About 0.03 gram of boric acid is added for each gram of alkaline solution, and the pH adjusted to between 6.5 and 7.0. The water solution is extracted with toluene in the proportion of 3 to 1 by shaking for 30 minutes.

A 1-liter sample of water is acidified with sulfuric acid to a pH of 3 to 5 and extracted with 10 ml. of toluene in a 1.3-liter bottle. The bottle is placed in a horizontal position on a set of powered rollers and made to rotate about its longitudinal axis for 30 minutes. Distilled water is then added so that an aliquot of the toluene phase can be easily removed.

A sample weighing 2 to 20 grams, either a portion of fish meat or a whole specimen, in the case of small fish, is cut into slices and refluxed with 0.1*M* potassium hydroxide for 20 minutes in a 1-liter flask with a condenser. Ten milliliters of potassium hydroxide is added for each gram of fish. When the flask is cold, boiling stones are added and 500 ml. of 0.5*M* sulfuric acid is poured through the condenser. The flask is adapted for distillation. Two hundred milliliters of the distillate is acidified with sulfuric acid and extracted with 5 ml. of toluene. To determine whether the distillation is complete, an additional fraction of 10 ml. is taken up in a separate flask. This distillate is acidified and extracted in the same way as the main part.

Preparation of the PCP Methyl Ether. The diazomethane solution, about 25 mg. per ml. in ether, is added

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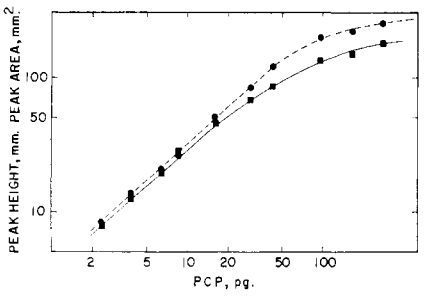

Figure 1. Linearity curve for PCP methyl ether

Peak height, mm. ————
Peak area, mm.² - - - - - - -

drop by drop to 1 ml. of toluene extract of PCP until a pale yellow color persists. After 1 hour in a hood, about 25% of the volume is evaporated off on a sand bath (100° C.) in a stream of purified nitrogen. The sample is then adjusted to the original volume (1 ml.). If less than 4 drops of the diazomethane solution are added, it is sufficient to store the reaction solution in a hood for one hour, and no evaporation is necessary.

Determination. PCP is recrystallized in benzene and a standard solution is prepared in toluene, 100 ng. per ml. The standard is methylated and diluted to 1, 5, 20, and 100 ng. per ml. Three- and 6-μl. aliquots of the solutions are injected into the gas chromatograph, and the peak heights and areas are measured. The mean values of three observations with the same volumes and concentrations are plotted on a log-log diagram (Figure 1). The linear range of the standard curve is only 2 to 50 pg., but the dilution faults will be small if the necessary dilutions are made by weighing. Five microliters of diazomethane-treated sample is injected and the peak height is compared with the standard curve. A suitable concentration and volume of standard solution are chosen and injected for gas chromatography. The peak heights are compared and the concentration of the sample is calculated, provided that only the linear range of the standard curve is used.

The PCP trimethylsilyl ether is prepared for a qualitative determination. Two milliliters of toluene extract of PCP, 0.5 ml. of trimethylchlorosilane (3% in toluene), and 1 ml. of hexamethyldisilazane (2% in toluene) are heated in a water bath (40° C.) for 30 minutes. A pinch of potassium carbonate is added and the mixture shaken for 2 minutes. The toluene solution is washed with an equal volume of water, dried with magnesium sulfate, and injected into the gas chromatograph. Retentions from the sample and a standard are compared.

RESULTS AND DISCUSSION

When the water is extracted with a small volume of toluene, 100 to 1, as described above, the pH must be 5 or less to obtain the best partition quotient. In the case of soil analysis, it is necessary to avoid pH values less than 6, because gel formation frequently interferes with extraction of PCP. The initial potassium hydroxide solution of PCP, therefore, is treated with boric acid to bring its pH to between 6.5 and 7.0. In this case, it is necessary to decrease the ratio of water to toluene (3 to 1) to get good partition.

This modified form of Stanley's methylation (1966)

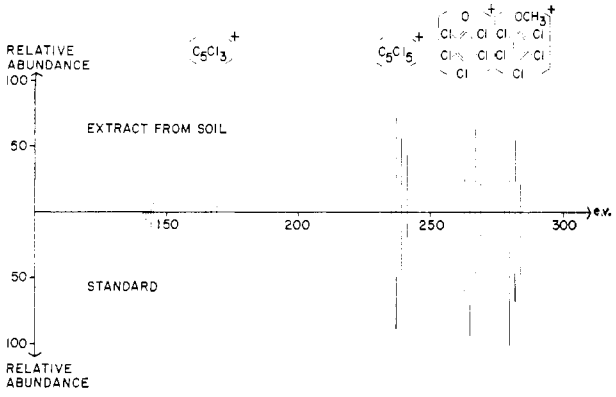

Figure 2. Mass spectrogram of PCP methyl ether from extracted soil and from a standard

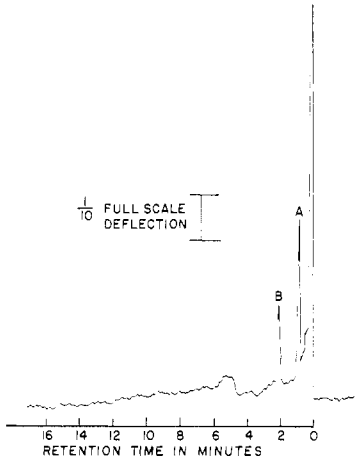

Figure 3. Gas chromatogram of TeP methyl ether (A) and PCP methyl ether (B). Peak B represents 4 pg. of PCP

is quantitative with just a few drops (100 μl.) of diazomethane solution in 1 ml. of toluene containing up to 200 ng. of PCP.

Unmethylated PCP injected into the gas chromatograph gave no peak with the column described here unless very heavy injections were made. This benefits the analysis, because injections of samples not treated with diazomethane indicate, to a certain degree, whether interfering substances are present. For gas chromatography of unmethylated PCP, a special technique is required (Kolloff *et al*, 1963; Smith *et al.*, 1964).

Hexamethyldisilazane was recommended as a reagent for preparation of trimethylsilyl ethers of phenols (Langer *et al.*, 1958). This method was tried on PCP without success. The preparation of the derivative described in this paper is not quantitative and can only be used for qualitative tests.

The identification of PCP can be made to some extent by gas chromatography of the methyl and trimethylsilyl ethers on different columns. The mass spectrometer gives a better qualitative determination, although it needs more substance. A soil sample was extracted and methylated using the described method and compared with a methylated standard of PCP. The spectrograms are in good agreement (Figure 2).

The gas chromatograms of some soil samples gave a peak with a retention of 0.40 in relation to the PCP methyl ether (Figure 3). This peak, examined with

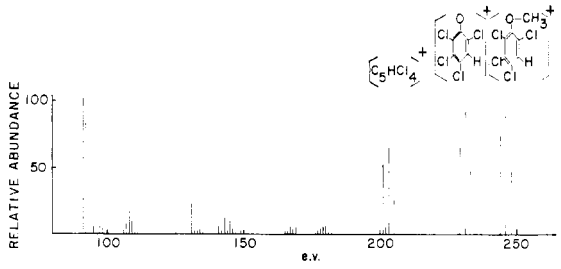

Figure 4. Mass spectrogram of TeP methyl ether extracted from soil

the mass spectrometer, proved to be the methyl ether of tetrachlorophenol (TeP), a by-product in the manufacture of PCP (Figure 4). Kanazawa (1963) and Akisada (1964, 1965) have described methods for the determination of PCP and TeP in technical products.

Methylated 2,4-dichlorophenoxyacetic acid (2,4-D) separates completely from the PCP methyl ether on the described column. Lindane also separates from the PCP methyl ether, and belongs to the type of substance discovered on a chromatogram before the methylation. Retentions are given in Table I.

Recoveries were performed on dry sieved sandy soil and unsieved garden soil, both types originally containing less than 0.1 p.p.b. of PCP. Two-gram portions of sandy soil and 5-gram portions of garden soil were fortified and analyzed. One-thousand-milliliter portions of tap water were fortified and analyzed. Untreated tap water contained less than 0.002 p.p.b. of PCP. Recovery studies of PCP in fish were made on plaice (*Pleuronectes platessa*) originally containing less than 0.1 p.p.b. of PCP. Four to 5 grams of fortified fish meat was used for each analysis (Table II). No further clean-up procedures were necessary before the chromatographic determinations.

The described methods were used for the analysis of soil samples taken near a lake in which fish had died in large numbers and PCP poisoning was suspected. One of the samples contained as much as 1.5% of PCP. One gram of this soil sample was added to a 3.5-liter aquarium with two guppies (*Lebistes reticulatus*). The water temperature was 22° C. and the pH 7.8. One of the fish, a male, died within 16 hours and the other, a female, 18 hours after the addition of the PCP-contaminated soil. The fish were analyzed by the described method. The male contained 105 p.p.m. and the female 110 p.p.m. (calculated on the whole wet fish). The water from the aquarium was analyzed 48 hours after the fish experiment and had a concentration of 3 p.p.m. PCP. Tsuda and Kariya (1963) have reported an accumulation of the same order in fish exposed to PCP-poisoned water during the same time as this experiment.

The above-mentioned procedure for analysis of fish was also useful for analysis of wood, and the method can probably be used for the determination of PCP in many classes of substances.

Table I. Relative Retentions on the Described Column

Extracts	Relative Retention[a]
PCP methyl ether	1.00
TeP methyl ether	0.40
2,4-D methyl ester	1.31
Lindane	1.30
PCP trimethylsilyl ether	1.86
TeP trimethylsilyl ether	1.21

[a] Retention time of about 2 minutes under the described conditions.

Table II. Recovery of PCP, Parts per Billion

Added	Found		Recovery, %
	Sandy soil		
2.05	1.98		96.6
2.05	2.09		102.0
2.05	1.78		86.8
2.05	1.57		76.6
2.05	1.92		93.7
2.05	1.64		80.0
		Mean	89.3
		Std. Dev.	9.7
	Garden soil		
2670	2560		95.9
3230	3020		93.5
	Water		
0.0535	0.0532		99.4
0.0535	0.0536		100.2
0.0670	0.0590		88.1
0.0540	0.0532		98.5
0.0540	0.0505		93.5
		Mean	95.9
		Std. Dev.	6.0
	Fish		
9.8	10.2		104.1
14.9	12.2		81.9
12.0	10.5		85.0
225	211		93.8
		Mean	91.2
		Std. Dev.	10.0

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