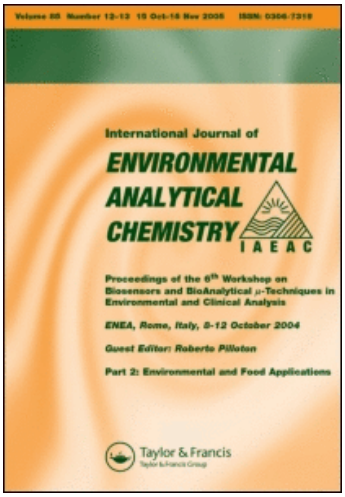

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DETERMINATION OF PENTACHLOROPHENOL (PCP) IN SAMPLES OF THE ENVIRONMENTAL SPECIMEN BANK USING ISOTOPE DILUTION

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Within the program of the Environmental Specimen Bank a quick and efficient method for determination of pentachlorophenol (PCP) in various environmental matrices has been developed. The method includes alkaline hydrolysis of bound PCP, acidification, simultaneous steam distillation and extraction in one glass apparatus. After clean-up and derivatization with acetic anhydride the samples were analyzed by gas chromatography/mass spectrometry. Concentrations were calculated using ^{13}C-labeled PCP as the internal standard. Validation was carried out with various environmental samples (soil, fish, conifer needles, kale).

The method can be used for various biological samples without any modification. The extracts are free of matrix components (lipids, chloropyll, terpenes, etc.) and other contaminants, which results in clear chromatograms with few peaks; therefore, correct integration is facilitated. Although the recoveries of PCP are in the range of 50–90%, due to losses during the several method steps, these losses can be corrected with the ^{13}C-labeled internal standard, resulting in high precision (1.5–2.2% standard deviation).

Keywords: Pentachlorophenol; environmental matrices; steam distillation; acetylation; isotope dilution

INTRODUCTION

Environmental Specimen Banking in Germany is a systematic and efficient program of environmental monitoring in which concentrations of hazardous chemical substances are measured in suitable environmental specimens. This enables the determination of environmental effects as well as establishment of the cor-

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relation between effects and concentration. The spectrum of the observed organochlorine compounds covers a range of such substances as polychlorinated biphenyls, organochlorine insecticides, and polychlorinated dioxins, as already has been reported.[1–3] Based on the knowledge of environmental levels,[4–8] transport, distribution and transformation of pentachlorophenol,[9–12] as well as its effects on organisms in the environment,[13–14] it is also important to determine the PCP levels in the samples stored in the environmental specimen bank. Therefore, the aim of this study was to introduce a suitable, fast and efficient analytical method for determination of pentachlorophenol in environmental matrices within the boundaries of this program.

Most of the common analytical methods use acidification of the sample to convert PCP to its non-ionized form, liquid-liquid extraction into an organic solvent and a subsequent clean-up procedure.[4,14,15] Other procedures are based on the relatively high volatility of pentachlorophenol, which allow the use of a steam distillation and extraction procedure.[7,16,17] The main idea of the presented study was also to develop a modified procedure[18] for simultaneous distillation and extraction of pentachlorophenol from environmental matrices.

MATERIALS AND METHODS

Sample Collection

Samples of sediments, soil, fishes (dab—*Limanda limanda*, bream muscles—*Abramis brama*, and cod—*Gadus morkua*), pine needles (*Picea abies*) and kale (*Brassica* var. *sabellica*) were taken from the Environmental Specimen Bank of the Institute of Ecological Chemistry in Neuherberg (Germany). All samples were homogenized and stored in a specimen tank above liquid nitrogen until analysis.

Chemicals

Nonane and standards of ^{13}C-pentachlorophenol and pentachlorotoluene were purchased from Promochem, Germany; standards of pentachlorophenol and pentachlorophenol acetate are from Dr. Ehrenstorfer, Augsburg; Germany; n-hexane PESTANAL was obtained from Riedel de Haen, Seelze, Germany, potassium carbonate, potassium hydroxide, sodium chloride, sulfuric acid, silicone defoamer and acetic anhydride, p.a. grade, were purchased from Merck, Darmstadt, Germany.

Standard Solutions

Stock solutions of the following compounds were prepared: pentachlorophenol and pentachlorotoluene at a concentration of 1000 μg/ml, ^{13}C-pentachlorophenol and pentachlorophenol acetate at a concentration of 100 μg/ml.

Cleaning of Glass Ware

All glass ware was rinsed with water, 0.5 M KOH, distilled water and heated up to 400°C over night. Before use it was rinsed with n-hexane.

Apparatus

The gas chromatograph/mass spectrometer system was a Fisons 8000 equipped with a GC 8000, an autosampler AS800 and a quadrupole-MS MD800.

Separation Conditions

A DB-5 fused silica capillary column (30 m length, 0.33 mm internal diameter, and 0.25 μm film thickness) with phenyl-methyl bonded phase was used for gas-chromatographic separation. A column temperature of 60°C was held for 2 minutes, then a temperature gradient of 10°C/min was applied up to 250°C. This final temperature was held for 5 minutes. Carrier gas: Helium, column head pressure 50 kPa, injector temperature 250°C, detector interface temperature 250°C. All injections were made in the splitless mode.

The following ions (m/z) in SIM modus were traced: 264 and 266 for pentachlorotoluene as the recovery standard, 266 and 268 for pentachlorophenol acetate as well as 272 and 274 for ^{13}C-pentachlorophenol acetate as the internal standard.

Hydrolysis

The homogenized samples (5–20 g wet weight) delivered directly from the specimen tank were placed in a 500 ml round-bottomed flask. The sample was spiked with 10 μl of ^{13}C-pentachlorophenol (1 μg/ml). Then 50 ml ethanol, 100 ml of 2 mol/l KOH, 100 ml of bidistilled water, 10 g NaCl and some droplets of silicone defoamer were added. A glass column for simultaneous distillation and extraction[18] (Figure 1) was attached to the flask. For hydrolysis of PCP conjugates and to remove the ethanol the sample was heated for nearly 1 hour.

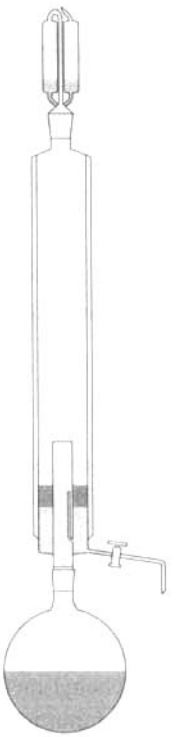

FIGURE 1 Distillation apparatus

Extraction

The hydrolyzed sample was acidified with 100 ml 1.5 mol/l sulfuric acid. The flask was replaced below the glass apparatus (Figure 1) and 15 ml of n-hexane were added. The isolation of the pentachlorophenol was accomplished by simultaneous distillation and extraction with steam and n-hexane as a keeper in 2 h. After extraction, the organic layer was transfered through the side valve into a 250 ml separatory funnel.

Clean-up

The n-hexane layer was shaken for 5 minutes in a separatory funnel with 100 ml of 0.2 M potassium carbonate and 5 g NaCl. After phase separation, the aqueous layer was transferred into an Erlenmayer flask. The organic layer was shaken again with 50 ml potassium carbonate and 2.5 g NaCl and afterwards discarded.

Derivatization

The water layers were combined in the separatory funnel, treated with 2 ml of acetic anhydride and 10 ml of n-hexane and shaken vigorously. After 30 minutes the water layer was removed, the organic layer was dried over anhydrous sodium sulfate and added to a 25 ml conical tube. After adding 5 ml n-hexane to the water layer, it was shaken again and the water layer was discarded. The organic layer was dried and collected in the 25 ml conical tube. 150 μl nonane was added and the solution was concentrated by means of a rotary evaporator to approximately 200 μl. After that, the solution was further concentrated under a gentle stream of nitrogen to nearly 100 μl. 10 μl of the recovery standard (pentachlorotoluene - 10 μg/ml) was added and the extract was transferred to a glass microvial.

Calculation of Concentration and Recovery

Concentrations were calculated using the ^{13}C-PCP as internal standard. Recovery of each sample was calculated with the ^{13}C-PCP internal standard and pentachlorotoluene as recovery standard.

Optimization

Hydrolysis

It was investigated whether previous alkaline hydrolysis is necessary, or if the acidic distillation is sufficient to extract all bonded and free PCP. Therefore, samples were analyzed with the method described above, with and without alkaline hydrolysis.

Extraction

To optimize the extraction time and to estimate whether the extraction is complete, the distillation was terminated at 1, 2 and 4 h, the organic layer was removed, the glass column was cleaned, 15 ml n-hexane were added and the distillation was continued.

Clean-up

To determine whether clean-up is necessary, samples of pine needles were analyzed with the method described above and with the same method, but without clean-up. In this case the n-hexane (keeper) was not discarded after extraction

with 0.2 M K_2CO_3, and the same n-hexane was used for the back extraction of acetylated PCP.

Recovery

To optimize the recovery, several experiments were performed. During clean-up the n-hexane (keeper) was shaken three times with 100 ml 0.2 M K_2CO_3 and the 3 aqueous extracts were worked up separately. During derivatization the K_2CO_3 solution was shaken three times with 10 ml n-hexane and the 3 organic extracts were worked up separately.

Calibration

12 Samples of homogenized pine needles (10 g) were spiked with 10–120 ng ^{12}C-PCP and 50 ng ^{13}C-PCP. After three days the samples were analysed with the procedure described above. Standard deviation and recovery were calculated according to DIN 38402 (German industrial standard), detection and determination limits were expressed as 3- and 10-fold standard deviation of noise peaks, respectively.[19]

RESULTS AND DISCUSSION

The determination of PCP in the present study is based on the distinctive physico-chemical properties of this substance. It is soluble in most organic solvents, but its solubility in water depends on the pH. Additionaly, its relatively high volatility enables the use of a simultaneous steam distillation and extraction procedure, as was already reported.[7,17,18]

For determination of the total amount of PCP, the samples were heated with ethanol and potassium hydroxide before extraction to hydrolyze possible PCP-conjugates. Without hydrolysis the extracted amounts of PCP are obviously lower (Figure 2).

The simultaneous steam distillation and extraction procedure provides a simple, convenient way to isolate PCP from various environmental matrices without coextracts like lipids, chlorophyll, terpenes, etc. It is well known, that in solvent extracts a substantial number of coextracts are present. In such cases additional time consuming clean-up procedures are necessary.

Experiments with different extraction times (Figure 3) show a nearly complete extraction after 2 hours time. The extracted amounts of sample derived ^{12}C-PCP

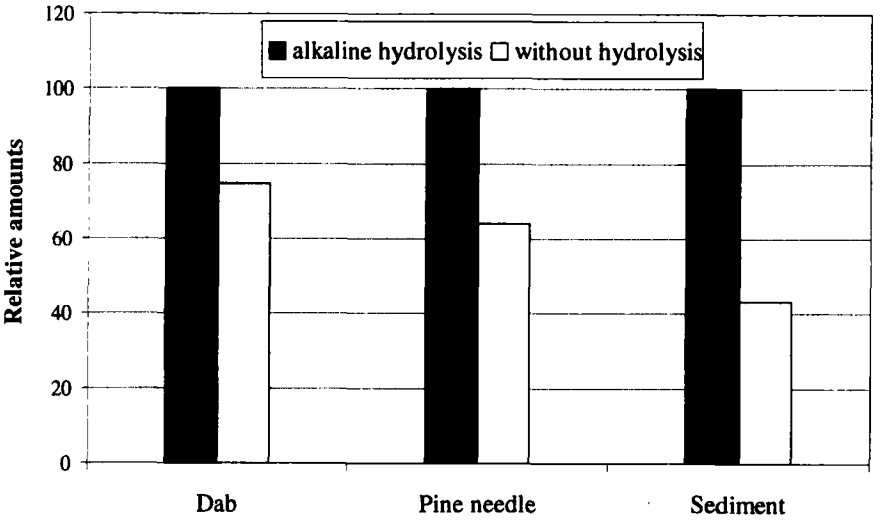

FIGURE 2 Normalized concentrations of Dab, Pine needles and sediment with and without alkaline hydrolysis. (Normalization is necessary due to highly different concentrations)

and spiked ^{13}C-PCP are very similar, with only slight differences in the soil sample. These two findings indicate a sufficient and correct extraction. Unfortunately, no biological reference material for PCP is available for further validation of the extraction procedure.

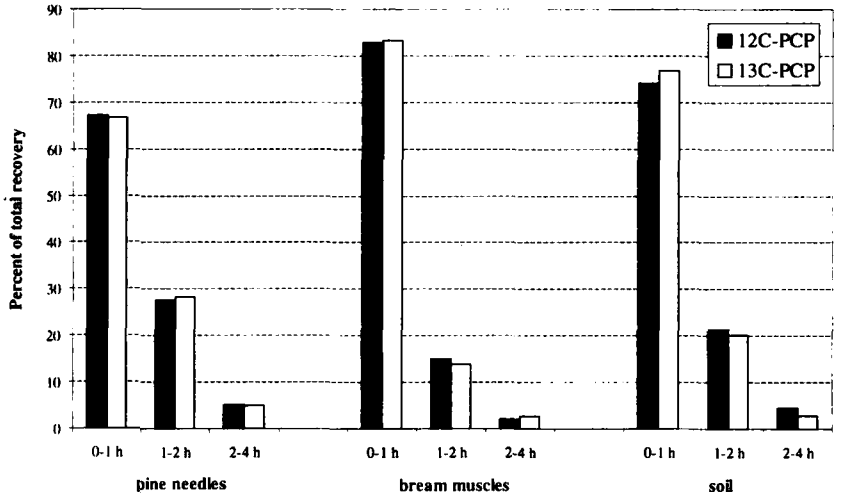

FIGURE 3 Extracted amounts of sample derived ^{12}C-PCP and spiked ^{13}C-PCP after 1 h, 2 h, and 4 h of extraction time in various environmental samples

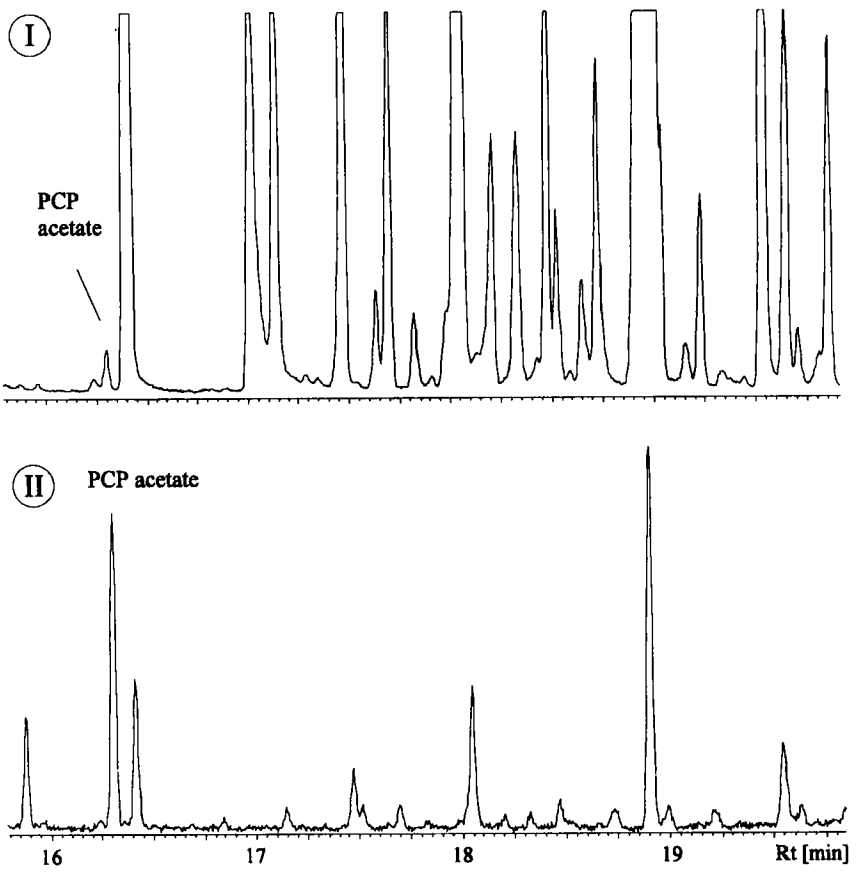

FIGURE 4 Total ion chromatograms of pine needle extracts without (I) and with (II) clean-up

Analytical interferences may become a problem, particularly at trace levels of PCP. These include chloronaphthalenes, p-methoxytetrachlorophenol, polychlorinated biphenyls, and pesticides.[14] Chromatograms from analysis of pine needles with and without clean-up in comparison (Figure 4) demonstrate the necessity and efficiency of the simple applied clean-up.

Possible losses due to incomplete extraction of PCP with 0.2 M K_2CO_3 are negligible as are losses due to the extraction of the acetylated PCP with n-hexane (Figure 5).

One problem with PCP analysis is peak tailing of PCP in capillary columns resulting from its adsorption on the stationary phase. For reliable quantitation with an unequivocal identification it is, therefore, recommended to convert this substance to its derivatized form. Methods using derivatization with diazome-

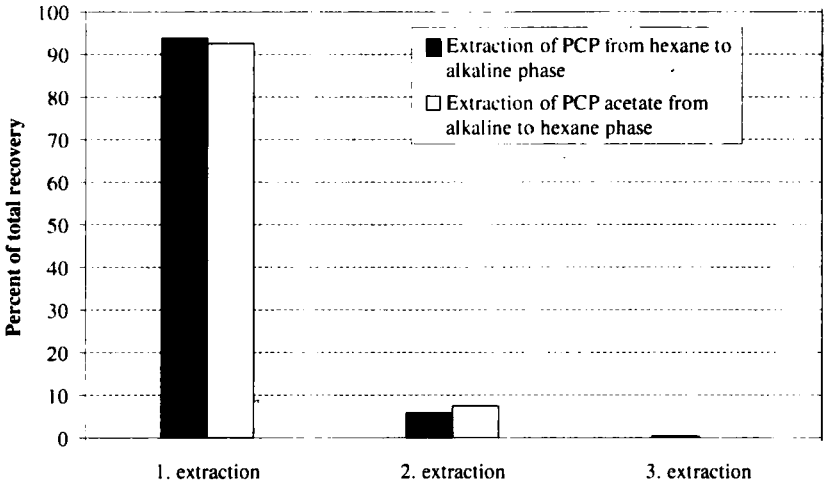

FIGURE 5 Extraction of PCP during clean-up with three 100 ml—portions of 0.2 M K_2CO_3 and PCP acetate after derivatization with three 10 ml—portions of n-hexane

thane, acetic anhydride,[17,20] methyl iodide[21] pentafluorobenzylbromide,[18,20] or trimethylsulfonium hydroxide[23] have been reported. Besides the potential risk from hazardous reagents such as diazomethane, most of these methods are time consuming and need additional clean-up after derivatization. These disadvantages can be avoided by the acetylation of PCP with acetic anhydride, as was used in this study. Acetylation is very suitable for the aqueous alkaline extract obtained from clean-up. During derivatization some losses of PCP may occur due to incomplete reaction or incomplete extraction of the acetylated product. But the recovery experiment proved that loss was negligible.

Calculation of PCP concentration was done by isotope dilution. Samples were spiked with ^{13}C labeled PCP, which acts chemically and physically in the same way as ^{12}C-PCP. Losses of ^{12}C-PCP during the analytical procedure by evaporation, incomplete derivatization, adsorption, etc. occur also for ^{13}C-PCP. MS-detection allows quantification of each component separately. With knowledge of the spiked amount of ^{13}C-PCP and the sample weight, exact calculations of ^{12}C-PCP concentration can be done, independent of recovery.

$$c(^{12}\text{C-PCP})[\text{ng/g}] = \frac{s(^{12}\text{C-PCP})*a(^{13}\text{C-PCP})[\text{ng}]}{s(^{13}\text{C-PCP})*F*w(\text{sample})[\text{g}]}$$

TABLE I Precision, recovery, detection and determination limits for the analysis of spiked pine needles (picea abies) (1–12 ng/g ^{12}C-PCP and 5 ng/g ^{13}C-PCP)

Number of samples	12
standard deviation	2.15%
recovery	60–80%
detection limit	40 pg/g
determination limit	120 pg/g

c = concentration of ^{12}C-PCP in the sample
s = signal
a = spiked amount of ^{13}C-PCP
w = sample weight
F = relation of signal response of 1 ng/μl ^{12}C-PCP to 1 ng/μl ^{13}C-PCP

Although high recoveries are not necessary for the accuracy of the result, they are important for the sensitivity and the detection limit of the method. Recovery of each sample can be calculated with the ^{13}C-PCP internal standard and a recovery standard (here: pentachlorotoluene (PCT)), which is added after evaporation of the solvent. Relations of signal response PCP-acetate/PCT are determined by standard measurements.

$$r[\%] = \frac{s(^{13}\text{C-PCP})*a(\text{PCT})[\text{ng}]*100[\%]}{s(\text{PCT})*a(^{13}\text{C-PCP})[\text{ng}]*F*M}$$

r = recovery
s = signal
a = spiked amounts
F = relation of signal response of 1 ng/μl PCP-acetate to 1 ng/μl PCT
M = relation of molecular weight of PCP-acetate to ^{13}C-PCP

The results of the calibration experiment (Table I) show the good precision of the method. Recoveries are low and variable, but they are not important for the determination of PCP concentrations as shown above. Limits of detection and determination, expressed respectively as 3 and 10 times the standard deviation of blind samples, are low enough to obtain accurate results at trace levels.

These data were obtained from spiked samples and do not exactly reflect the extraction of contaminated environmental samples. Thus, additional analyses were carried out with different environmental matrices (Table II).

The analytical results for real samples equal the precision of spiked samples. The lower precision of soil is probably a result of low PCP concentrations.

TABLE II PCP concentrations (wet weight) and precision for the analysis of soil, fishes (dab, cod, bream), and kale.

matrix	*number of replicates*	*concentration [ng/g ww]*	*standard deviation [%]*
soil	4	0.15	5.62
dab (*Limanda limanda*)	6	2.99	1.97
cod (*Gadus morkua*)	6	1.35	2.08
bream muscles (*Abramis brama*)	4	2.02	1.60
kale (*Brassica* var. *sabellica*)	6	1.27	1.51

Dab was taken as a reference material and several analysis were carried out by different persons at different times (Figure 6). Even under these conditions the obtained results have a standard deviation of 3.5%.

CONCLUSION

The method described above was developed to analyze PCP in various samples of the Federal Environmental Specimen Bank. It can be seen clearly that the advantages of the method are outweight the disadvantages:

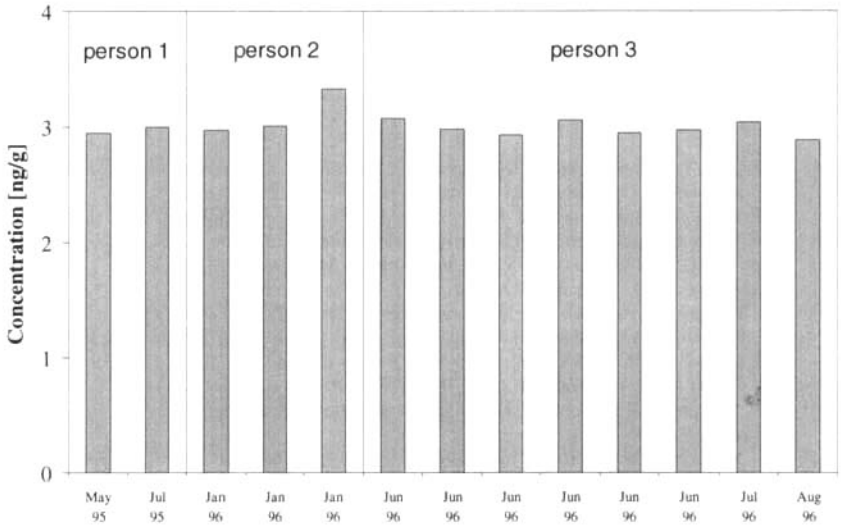

FIGURE 6 PCP concentrations of homogeneous dab samples analyzed by different persons at different times

disadvantages:

- for the separation of ^{12}C and ^{13}C-PCP MS-detection is necessary
- the ^{13}C-labeled PCP standard is relatively expensive
- for steam distillation a special glass apparatus is necessary

advantages:

- the method is suitable for various environmental samples without any modification
- variable sample amounts are possible
- no drying of the sample is necessary
- it provides clean extracts without coextracts like lipids or chlorophyll
- other analytical interferences can be separated by the simple clean-up
- MS-detection allows selective and sensitive analysis
- high precision can be obtained using isotope dilution with ^{13}C-PCP
- hazardous reagents can be avoided
- the method is fast with easy handling

The method is also suitable for other environmental matrices like earthworms, bladder kelp, mussel, poplar leaves, and sediment. Only the analysis of eggs causes handling difficulties. Although experiments indicate complete extraction of real samples, we could not prove that. Unfortunately, there is no biological reference material available with certified PCP amounts.

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