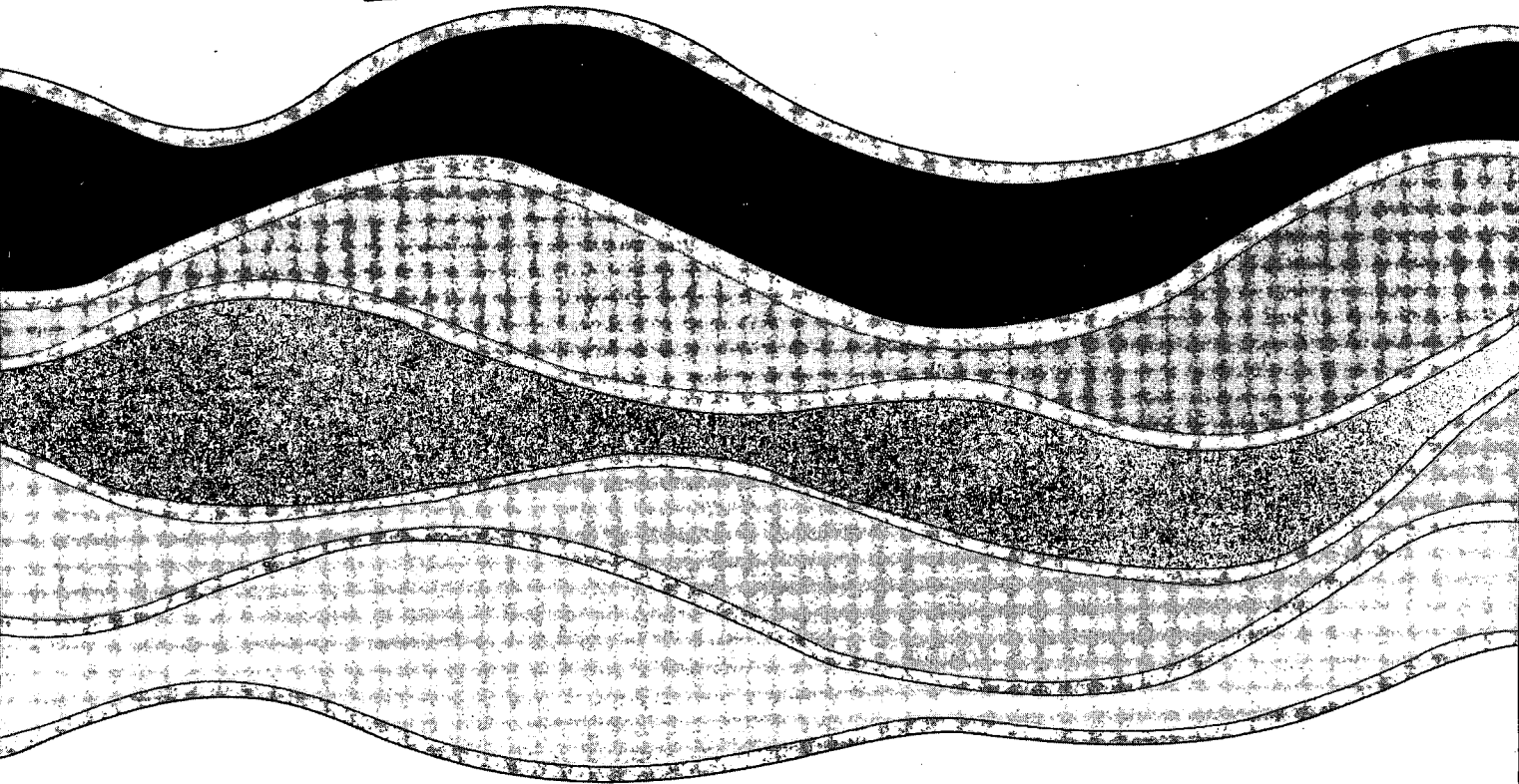


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**ON-LINE EXTRACTION AND  
DERIVATIZATION OF  
PENTACHLOROPHENOL AND RELATED  
COMPOUNDS FROM SOILS USING A  
SUPERCRITICAL FLUID EXTRACTION  
SYSTEM**

**H.B. Lee, T.E. Peart and R.L. Hong-You**

**NWRI Contribution No. 92-01**

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**ON-LINE EXTRACTION AND DERIVATIZATION OF PENTA  
CHLOROPHENOL AND RELATED COMPOUNDS FROM SOILS USING A  
SUPERCRITICAL FLUID EXTRACTION SYSTEM**

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NWRI Contribution No. 92-01

## **MANAGEMENT PERSPECTIVE**

Pentachlorophenol and related compounds are considered as priority pollutants by many environmental regulatory agencies. Although methodology exists for the determination of such compounds, most of them are time consuming and require a lot of solvent and chemicals. A supercritical fluid extraction method has been developed to replace the existing procedures for the determination of chlorophenols from soil. The quick sample turnaround time and the minimal amount of chemicals used represent a breakthrough in efficiency and environmental friendliness. With the incorporation of robotics, this SFE method has the potential to be fully automated in the laboratory.

## **SOMMAIRE À L'INTENTION DE LA DIRECTION**

De nombreux organismes de réglementation en matière d'environnement jugent que le pentachlorophénol et les composés connexes sont des polluants d'intérêt prioritaire. Bien qu'il existe des méthodes pour doser ces composés, la plupart de celles-ci exigent beaucoup de temps et une grande quantité de solvants et de produits chimiques. On a mis au point une méthode d'extraction par fluide supercritique afin de remplacer les procédés existants de dosage des chlorophénols contenus dans le sol. Le délai d'exécution rapide et la faible quantité de produits chimiques utilisés représentent une percée au niveau de l'efficacité et du respect de l'environnement. Avec l'introduction de la robotique, cette méthode peut être entièrement automatisée en laboratoire.

## ABSTRACT

An on-line supercritical fluid extraction (SFE) and derivatization procedure for the determination of pentachlorophenol (PCP) and related compounds from soil samples is described. Phenols are extracted from soil and acetylated in-situ with supercritical carbon dioxide in the presence of triethylamine and acetic anhydride at a temperature of 80°C. Quantitative recovery of di-, tri-, tetra- and penta- chlorophenols was obtained by a 10-minute extraction with carbon dioxide at 37.2 MPa (365 bar, 0.8 g/mL density) from soil samples fortified to 0.5 and 5  $\mu\text{g/g}$  levels. In a comparison study, the SFE and the steam distillation methods both produced very similar results for PCP and other chlorophenols in a reference sample. When this method is applied to some contaminated soil samples collected in a wood treatment plant, results for chlorophenols in a sample can be obtained in a short 90 minutes.

## RÉSUMÉ

Le présent article décrit une méthode en direct d'extraction par fluides supercritiques et de préparation de dérivés en vue du dosage du pentachlorophénol (PCP) et de composés connexes dans des échantillons de sol. Les phénols sont extraits du sol et acétylés directement avec du dioxyde de carbone supercritique en présence de triéthylamine et d'anhydride acétique, à une température de 80 °C. Une récupération quantitative des di-, tri-, tétra- et pentachlorophénols a été effectuée par une extraction de 10 minutes au dioxyde de carbone, à 37,2 MPa (365 bars, densité de 0,8 g/mL), à partir d'échantillons de sol enrichis à 0,5 et 5 µg/g. Dans une étude comparative, l'extraction par fluide supercritique et la distillation par entraînement à la vapeur ont donné des résultats similaires pour le PCP et d'autres chlorophénols contenus dans un échantillon de référence. Lorsque cette méthode est appliquée à certains échantillons de sol contaminé prélevés dans une usine de traitement du bois, il est possible de déterminer, en 90 minutes, les teneurs de chlorophénols dans un échantillon.

## INTRODUCTION

Abnormal discoloration of wood, commonly referred as sapstain, is caused by fungi which derive nourishment from wood cells. Other than by kiln-drying, sapstain and mold on the surface of lumber can be prevented by treatment of wood with anti-sapstain chemicals. Due to their effectiveness, pentachlorophenol (PCP) and its derivatives are the most widely used anti-sapstain chemicals in Western Canada in the last 50 years. Recently, the application of PCP by the sawmilling and forestry industries has become an environmental concern since PCP is toxic to fish and mammals and technical grades of PCP are known to contain the highly toxic chlorinated dibenzo-*p*-dioxins and furans. In response to these concerns, the use of PCP as a wood preservative in British Columbia has mostly been phased out. However, this chemical is still being used in wood treatment plants in other parts of Canada for special applications.

PCP in soils or sediments can be traditionally determined by solvent extraction techniques (e.g. Soxhlet) [1] or by a steam distillation approach [2,3]. In both cases, the extraction process takes a few hours or longer. In the case of solvent extraction, a large amount of solvent must be used and a great deal of coextractives are produced. The latter often create a problem in the subsequent cleanup and chromatographic analysis. If the extracted PCP is to be analyzed by gas chromatography in the form of an acetyl, methyl or pentafluorobenzyl derivative, extra time is required for the additional derivatization step.

Supercritical fluid extraction (SFE) has been proven to be a more efficient alternative than existing solvent extraction techniques for most solid samples. It has been successfully applied to the determination of PCBs [4], chlorobenzenes [5], PAHs [4,6], dioxins [7], resin and fatty acids [8] in sediment and other matrices and the list is growing rapidly. Because of the non-polar nature of supercritical carbon dioxide, extraction recovery of polar parameters is low unless a modifier such as methanol is added to the

system. Supercritical fluid extraction of free pentachlorophenol from a soil sample has also been reported [9]. More recently, the possibility of combined supercritical fluid extraction and derivatization of polar compounds has been explored [10]. This latter approach further reduces sample preparation time and at the same time enhances the extractability of polar compounds since derivatives are in general less polar than their parent compounds. Our work on resin and fatty acids [8] demonstrated that this one-step technique can be applied to the rapid screening of the acids in sediment samples. In this report, we shall describe a rapid and quantitative method using an on-line extraction/derivatization technique for the determination of PCP and related compounds in soils contaminated by the wood preserving chemical.

## EXPERIMENTAL

All chlorophenol standards were supplied by Supelco. Acetic anhydride and triethylamine were purchased from Aldrich Chemicals. The anhydride was triple-distilled and the fraction of b.p. 138-140°C was collected and used. Distilled-in-glass solvents were supplied by Burdick and Jackson. Carbon dioxide (SFE grade) with a helium head pressure of 10.5 MPa was obtained from Scott Specialty Gases and Linde.

Stock solutions of individual chlorophenols at 1000  $\mu\text{g}/\text{mL}$  were prepared in acetone. Mixtures of the 14 chlorophenols (Table I) at 10 and 50  $\mu\text{g}/\text{mL}$  were prepared for the spiking of soil samples and the preparation of calibration standard. A mixture of 2,4-dibromophenol and 2,4,6-tribromophenol at 10  $\mu\text{g}/\text{mL}$ , also in acetone, was prepared as a surrogate standard.

For consistency, all samples were prepared in the following manner prior to extraction. Two layers of GFC filters cut to the diameter of the extraction thimble were placed on top of the bottom thimble cap to minimize contamination and plugging of the frit. The 7 mL thimble was then filled with 200 mg of Celite followed by 1 g of sample,



which was previously mixed and ground. Fifty  $\mu\text{L}$  of the above surrogate solution and 30  $\mu\text{L}$  of triethylamine were spiked to the soil sample. If the soil was completely dry, 50  $\mu\text{L}$  of water (equivalent to a 5% moisture content) was also added directly to the sample. The thimble was shaken on a vortex mixer for 15 seconds after addition of each liquid. The sample was topped by another 200 mg of Celite and 30  $\mu\text{L}$  of acetic anhydride were added before it was subject to supercritical carbon dioxide extraction. In the case of recovery experiments, samples were prepared as described above except that the surrogate solution also contained a known amount of the 14 chlorophenols.

All extractions were performed by a Hewlett-Packard 7680A SFE module using supercritical carbon dioxide of a density of 0.8 g/mL (37.2 MPa) and a flow rate of 2.0 mL/min. Static and dynamic extractions of 5 minutes each were carried out and the extraction chamber temperature was maintained at 80°C during this time. An octadecylsilane (ODS) trap, used for the collection of sample extracts, was kept at 15°C and 45°C, during the extraction and rinsing stages, respectively. SFE extracts from the trap were eluted by hexane in two 1.2 mL fractions.

The derivatized extract was partitioned with 3 mL of 1% potassium carbonate solution by vortexing in a centrifuge tube for 1 minute. This step removed the acetic acid formed in the acetylation reaction and the excess acetic anhydride reagent: both of them could lead to chromatographic problems if the uncleaned extracts were analyzed. The hexane extract was then transferred to a short (3 cm) anhydrous sodium sulfate column and a 5 cm 5% deactivated silica gel column prepared in tandem using disposable Pasteur pipets for further cleanup. The columns were first eluted with 5 mL of hexane and this fraction was discarded. The acetyl derivatives of chlorophenols were removed from the column by elution with 10 mL of 1+1 petroleum ether (b.p. 30-60°C) and dichloromethane. This was followed by solvent exchange into 5 mL or other suitable volume of iso-octane.

For comparison of SFE results, steam distillation of soil samples was also performed. One gram of soil was stirred with fifty mL of a 1% solution of potassium carbonate for 10 minutes in a 500 mL round bottom flask. One mL of acetic anhydride was added and stirred for another 10 minutes. The mixture was steam distilled for one hour into 3 mL of hexane in the condenser according to the method developed by Veith and Kiwus [2]. The acetates were then cleaned up as described above except that the silica gel column cleanup was omitted. A commercial standard reference soil sample (SRS 103-100) supplied by Fisher Scientific Co. was used in the comparison study.

Chromatographic analysis was carried out with a Hewlett-Packard 5890 Series II GC equipped with an electron capture detector and a split-splitless injection port. One  $\mu\text{L}$  splitless injection was made by a HP 7673 autosampler onto a 25 m x 0.2 mm ID HP-5 fused silica column. The initial oven temperature was 70°C (0.75 min hold) and it was programmed to 120°C at 30°C/min and then to 200°C at 2°C/min. Splitless time was 0.75 min. Hydrogen was the carrier gas and the column head pressure was 105 kPa. Instrument control and data acquisition were achieved by a personal computer running the HP 3365 ChemStation software in the Microsoft Windows environment.

To calibrate the instrument, a concentrated mixture of the acetyl derivatives of chlorophenols was prepared by an aqueous acetylation of a known amount of chlorophenols according to established procedures [1,11]. Quantitation of chlorophenols in soil samples was performed by an external standard method, using appropriate dilutions of the above mixture with iso-octane.

## RESULTS AND DISCUSSION

### *SFE of PCP from soil*

PCP was extracted from soil using supercritical carbon dioxide at 31.0 MPa and 70° for 60 minutes [9]. In this case, a 10% moisture was added to the sample as a modifier. We obtained similar results at 37.2 MPa and 80°C for a 15-minute extraction.

Also, we found that the same approach applied to the extraction of the di-, tri-, and tetra-chlorophenols as well although the recovery of the less chlorinated phenols were low under such conditions. Since chlorophenols are routinely analyzed in the form of acetyl derivatives in our laboratories, the above SFE approach would require an off-line derivatization step. The disadvantage of having an extra step in the procedure can be eliminated if the extraction and derivatization steps can be combined into one.

### *On-line SFE/derivatization of chlorophenols*

It is well known that chlorophenols in water samples can readily be converted into stable acetyl derivative by an in-situ acetylation using acetic anhydride and a base such as a carbonate or bicarbonate [11]. Acetyl derivatives of chlorophenols with two or more chlorine atoms are sensitively detected by an electron capture detector and are more amenable to column cleanup than the free phenols. For these reasons as well as the fact that the acetyl derivatives are easily formed and stable under the SFE conditions, they are the most appropriate choice for this work.

Similar to the aqueous reaction, derivatization of chlorophenols under SFE conditions also required a base. Although the on-line acetylation of chlorophenols was working with an aqueous solution of potassium carbonate, quantitative derivatization of all phenols could only be achieved in the presence of triethylamine. Presumably, the inorganic base is less effective than the organic base since the former is less soluble in supercritical carbon dioxide and thus less available for the reaction. In order to have the highest recovery of the acetyl derivatives, approximately equal volumes of acetic anhydride and triethylamine should be used. A large excess (250  $\mu$ L or more) of the two reagents was found to be detrimental to the recovery of the derivatives. A chamber temperature of 80°C was chosen since, at this temperature, a 10-minute extraction was enough for the complete recovery of the chlorophenols in soil. Longer extraction time and a second extraction did not further improve the recovery. On the contrary, only 60 and 90% of the PCP could be recovered for the same period of time if the chamber temperature was set at 40 and 60°C, respectively.

### ***Method performance***

In order to evaluate the efficiency of the on-line SFE/derivatization procedure, the recovery of chlorophenols from clean soil samples fortified at different levels was determined. Basically, recoveries of 90% or above were obtained in the 0.5 and 5  $\mu\text{g/g}$  range for PCP and other chlorophenols (Table I). The results suggested that this method is also applicable to the quantitative determination of di-, tri- and tetra- chlorophenols if they are present in the soil samples.

The ruggedness of the SFE method was again tested with a standard reference soil sample (SRS 103-100) naturally contaminated by PCP. In a side-by-side comparison, our results for PCP and other chlorophenols generated by the on-line SFE/derivatization procedure for this sample are nearly identical to those obtained by the steam distillation procedure, indicating completeness of extraction and derivatization (Table II). Both techniques also showed similar degree of precision as indicated by the standard deviation in replicate determinations. It should also be noted that our PCP result for this reference sample (1483  $\mu\text{g/g}$ ) is more comparable to that obtained by the non-derivatized SFE approach (1361  $\mu\text{g/g}$ ) [9] than the rather ambiguous certified value ( $965 \pm 374$   $\mu\text{g/g}$ ) furnished by the supplier.

This new procedure is being evaluated for the determination of chlorophenols from contaminated soil samples collected in an Ontario site for the preservation of railroad ties and hydro poles. The texture of the samples varied from light color sandy type to dark color loamy soil. Other than PCP, which contributed 90% or more of the total chlorophenols by weight in nearly all cases, tetrachlorophenols and a few trichlorophenols were also detected in the samples analyzed. The levels of chlorophenols in these soil samples varied from ca. 100 ng/g for some trichlorophenols to over 1000  $\mu\text{g/g}$  for PCP, indicating the method is applicable to a wide range of concentrations. Again, the SFE results were in good agreement with the steam distillation results in the cases where both techniques were used for cross checking. If the surrogates (bromophenols) were less than 75% recovered, the extraction was repeated. Figure 1 is

an ECD chromatogram of a contaminated soil sample after SFE/derivatization and cleanup. The levels of 2,3,5-trichlorophenol, 2,3,5,6-, 2,3,4,6- and 2,3,4,5-tetrachlorophenol and PCP are 0.12, 0.98, 0.71, 0.55 and 57.8  $\mu\text{g/g}$ , respectively, in the sample. The entire analytical sequence (sample preparation, extraction, derivatization, cleanup, solvent replacement, GC analysis and report generation) required approximately 90 minutes. Based on a statistical analysis of the recovery data at low levels, a detection limit of 0.1  $\mu\text{g/g}$  was determined for all chlorophenols using a 1 g sample and a final volume of 5 mL.

## CONCLUSION

The method described here is suitable for the rapid yet quantitative and specific determination of chlorophenols in soil and sediment samples in the ng/g to  $\mu\text{g/g}$  range. This procedure is more efficient and has a wider application than the one reported for the SFE of free PCP from soil. The present SFE method is proven to be a reliable alternative to the established steam distillation procedure since they both produce similar results for real-life samples. The simple analytical procedure results in an extremely short sample turn around time and thus it is most valuable under an environmental emergency situation. It also stands out in environmental friendliness since it consumes much less solvents and chemicals than all existing methodologies involving the derivatization step.

## *Acknowledgement*

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**TABLE I. Precision and accuracy of the on-line SFE/derivatization procedure for the determination of chlorophenols in spiked samples. Mean and standard deviation of six replicate determinations.**

| Chlorophenol              | 0.5 $\mu\text{g/g}$ | 5.0 $\mu\text{g/g}$ |
|---------------------------|---------------------|---------------------|
| 2,6-Dichlorophenol        | 97 $\pm$ 7          | 93 $\pm$ 5          |
| 2,4-Dichlorophenol        | 92 $\pm$ 7          | 93 $\pm$ 6          |
| 3,5-Dichlorophenol        | 96 $\pm$ 8          | 97 $\pm$ 6          |
| 2,3-Dichlorophenol        | 87 $\pm$ 6          | 89 $\pm$ 5          |
| 3,4-Dichlorophenol        | 98 $\pm$ 6          | 89 $\pm$ 6          |
| 2,4,6-Trichlorophenol     | 93 $\pm$ 7          | 102 $\pm$ 5         |
| 2,3,6-Trichlorophenol     | 91 $\pm$ 6          | 97 $\pm$ 4          |
| 2,3,5-Trichlorophenol     | 101 $\pm$ 4         | 98 $\pm$ 4          |
| 2,4,5-Trichlorophenol     | 101 $\pm$ 6         | 101 $\pm$ 5         |
| 3,4,5-Trichlorophenol     | 94 $\pm$ 3          | 90 $\pm$ 4          |
| 2,3,5,6-Tetrachlorophenol | 90 $\pm$ 6          | 95 $\pm$ 5          |
| 2,3,4,6-Tetrachlorophenol | 101 $\pm$ 7         | 103 $\pm$ 5         |
| 2,3,4,5-Tetrachlorophenol | 104 $\pm$ 4         | 93 $\pm$ 4          |
| Pentachlorophenol         | 96 $\pm$ 6          | 102 $\pm$ 5         |

**TABLE II. Results of PCP and other chlorophenols ( $\mu\text{g/g}$ ) in a reference soil SRS 103-100 by SFE and steam distillation.**

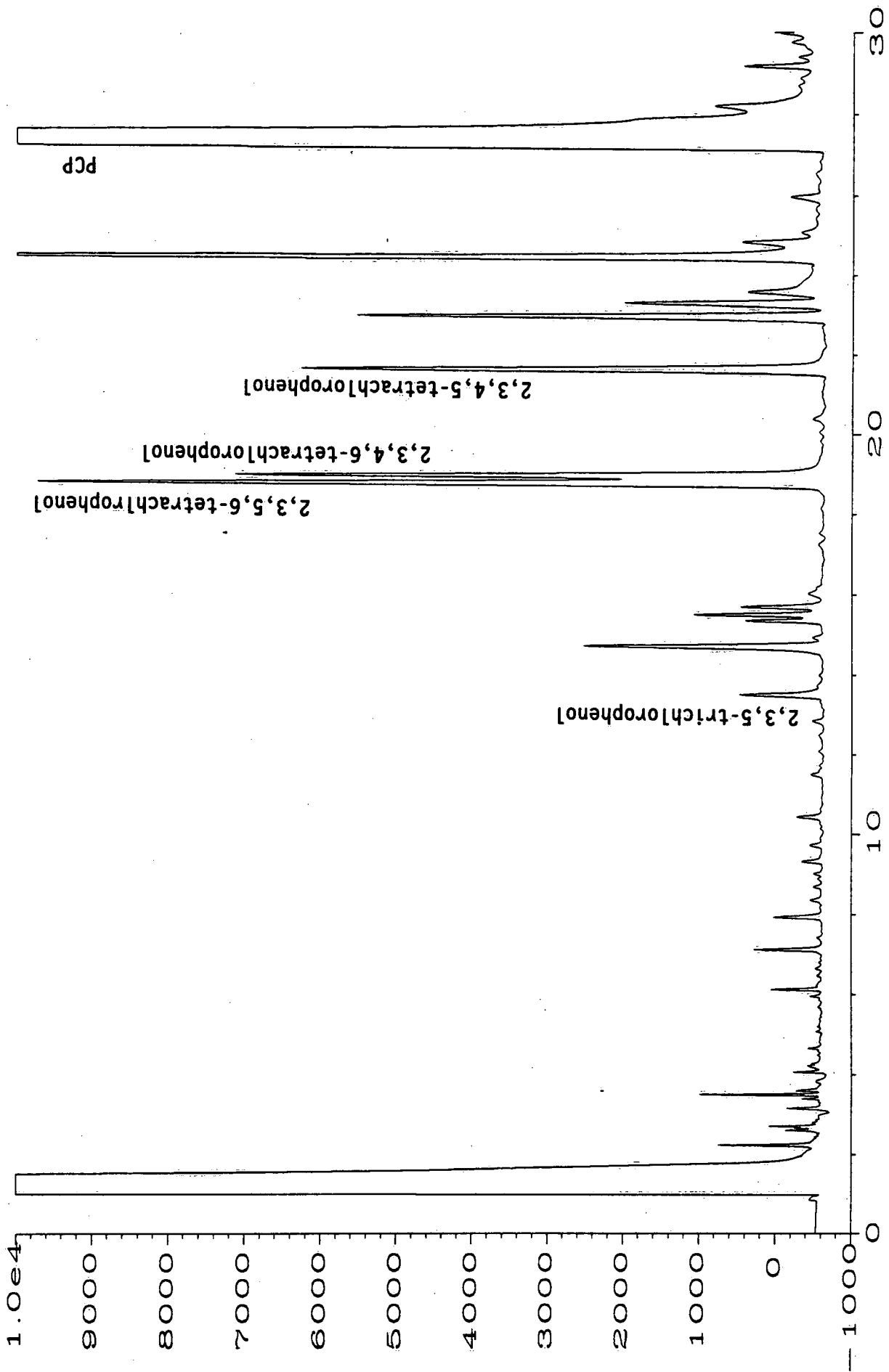
| Chlorophenol   | Steam Distillation*<br>(this work) | SFE**<br>(this work) | SFE*<br>(ref. 9) |
|----------------|------------------------------------|----------------------|------------------|
| 2,3,5-tri-     | $0.40 \pm 0.01$                    | $0.36 \pm 0.01$      | N.D.             |
| 2,3,5,6-tetra- | $14.4 \pm 0.4$                     | $13.9 \pm 0.3$       | N.D.             |
| 2,3,4,6-tetra- | $20.6 \pm 0.4$                     | $20.2 \pm 0.3$       | N.D.             |
| 2,3,4,5-tetra- | $1.9 \pm 0.1$                      | $1.8 \pm 0.1$        | N.D.             |
| PCP            | $1499 \pm 67$                      | $1483 \pm 93$        | 1361             |

\* Replicate = 3

\*\* Replicate = 6

N.D. = no data

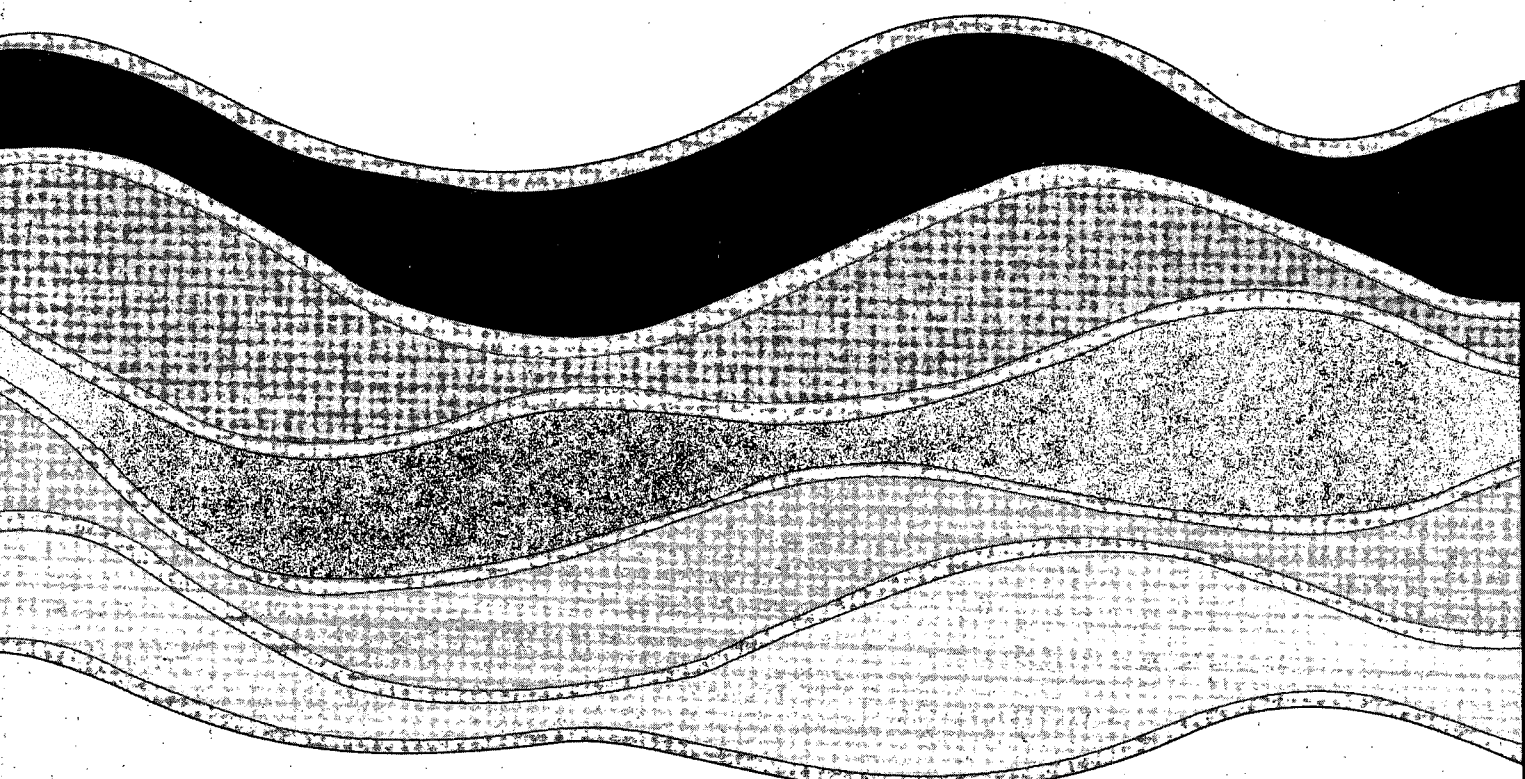




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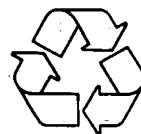


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