Rapid Analytical Methods To Measure Pentachlorophenol in Wood

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Pentachlorophenol (PCP) was measured in wood following extraction with methanol:0.1% acetic acid. The extract was derivatized with acetic anhydride in a biphasic solvent containing toluene and 0.1 M Na_2CO_3 (pH 11.4), and pentachlorophenyl acetate was quantitated by gas chromatography−mass spectrometry (GC−MS) using [$^{13}C_6$]PCP as an internal standard. A second detection method employed thin layer chromatography (TLC) after nitric acid-mediated oxidation of PCP to tetrachloro-1,4-benzoquinone. The third method was by colorimetry and required a liquid−liquid partitioning of PCP under acidic and alkaline pH against toluene. Then, PCP was coupled to 4-aminoantipyrine in the presence of sodium persulfate to form a dye which was measured at 580 nm. The limit of detection approached 50 ppb by GC−MS and 1 ppm by TLC when 100 mg of wood was used for analysis and 1 ppm for the colorimetric method when 1 g of wood was used for detection. The estimates of PCP were comparable for all three methods.

Keywords: *Chlorophenols; pentachlorophenol; contamination; off-flavor; rapid detection; wood*

INTRODUCTION

Pentachlorophenol (PCP) is a registered fungicide which is used as a preservative in wood products. PCP and other chlorinated phenol derivatives such as 2,4,6-trichlorophenol (TCP) and 2,3,4,6-tetrachlorophenol (TeCP) also may form in wood-based packaging material during bleaching processes (Chernikov et al., 1993). When present at elevated levels in nonhermetically sealed packaging material, these chlorophenols and their volatile chloroanisole derivatives, which are derived from fungal metabolism (Whitfield et al., 1989; Hill et al., 1995), can taint foods and beverages and impart pungent off-flavors (Whitfield and Last, 1986). Therefore, rapid screening methods are required to identify wood-based materials containing high levels of chlorophenols and avoid their use in packaging and transport. By selecting palettes and freight containers with acceptably low levels of chlorophenols, the incidence of food taintage can be significantly decreased.

In many instances, PCP is one of the predominant chlorophenols present in wood-based material and, thus, a good marker for overall chlorophenol contamination. There are numerous reports on analytical procedures to determine PCP in various products. The methods include colorimetry, UV and IR absorption, TLC, and gas and liquid chromatography (Mohler and Jacob, 1957; Ting and Quick, 1980; Shang-Zhi and Stanley, 1983; Butler and Dal Pont, 1992; Lee et al., 1987, 1989; Amon et al., 1989). Many of these methods involve tedious extractions, and some methods lack the necessary sensitivity and selectivity to reliably detect PCP. There is still a need to develop rapid screening methods to detect PCP in wood-based materials. In this study, we have established and validated two rapid screening methods to detect PCP in wood using either TLC or spectrophotometry and compared the estimates to a quantitative selective ion monitoring−gas chromatography−mass spectrometry (SIM−GC−MS) method. The results obtained by the colorimetric and TLC methods are in good agreement with the data obtained by SIM−GC−MS, which indicates that these methods are selective for PCP and can be used to rapidly monitor wood contamination.

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MATERIALS AND METHODS

Reagents. Pentachlorophenol was purchased from Fluka (Buchs, Switzerland). Isotopically labeled [$^{13}C_6$]PCP was obtained from Cambridge Isotopes Laboratories (Innerberg, Switzerland). TCP and TeCP were purchased from Supelco (Buchs, Switzerland). 4-Aminoantipyrine, 4,4′-methylenebis(*N*,*N*-dimethylaniline), and TCBQ were obtained from Aldrich (Buchs, Switzerland). ^{14}C-Radiolabeled PCP (10 mCi/mmol) was obtained from Sigma (Buchs, Switzerland). Thin layer chromatography plates HPTLC Fertigplatten Kieselgel 60F_{254} (10 × 10 cm, 0.25 mm) were from Merck (Geneva, Switzerland). Wood palette samples were obtained from transport delivery companies. All organic solvents and other chemicals were analytical grade unless specified.

Caution: Pentachlorophenol, 4-aminoantipyrine, and 4,4′-methylenebis(N,N-dimethylaniline) are toxic and should be handled appropriately. Nitric acid (65%) is highly pungent and a strong oxidizing agent. All manipulations should be performed in a well-ventilated hood with appropriate laboratory clothing and eye protective wear.

Extraction of PCP from Wood Palettes and Recovery Experiments. Wood from palettes was made into sawdust by filing. Analysis of PCP by the spectrophotometric method required 1 g of sawdust, while anaylses by the TLC and SIM−GC−MS methods required 100 mg of wood. In order to determine the recovey of PCP, spiking experiments were performed with radiolabeled [^{14}C]PCP. [^{14}C]PCP in methanol (10 μL, 37 nCi, 0.92 μg) was added to 1 g of sawdust in a test tube and allowed to evaporate for 0.5 h at room temperature. Methanol (20 mL) containing acetic acid (0.1%) was then added, and the PCP was extracted by incubation in a shaking water bath at 37 °C for 30 min. After centrifugation (4500*g* for 1 min), the methanol was retrieved and the sawdust was briefly washed with another 10 mL of methanol containing acetic acid. The extracts were pooled and evaporated under a stream of nitrogen gas at 40 °C. Radioactivity was measured by liquid scintillation counting using an LKB 1219 Rackbeta scintillation counter.

For the analyses of PCP by TLC or SIM−GC−MS, the amount of sawdust was decreased to 100 mg. The extraction of PCP was performed as above, except that 2 mL of methanol:acetic acid was used followed by washing with an additional 1 mL of methanol:acetic acid. The extracts were pooled into

S0021-8561(96)00596-1 CCC: $14.00

an Eppendorf tube (for TLC method) or a 5 mL glass test tube (for GC−MS method) and evaporated by nitrogen gas at 40 °C.

Analysis of PCP by Thin Layer Chromatography. The TLC analysis of PCP was adapted from a previously described method (Kovar et al., 1971; Ting and Quick, 1980) with modifications. The analysis was based upon the nitric acid-mediated oxidation of PCP to TCBQ (Crivello, 1980) which was then visualized by reaction with 4,4′-methylenebis(*N*,*N*-dimethylaniline) (Kovar et al., 1971). The dried sawdust extracts (100 mg) were treated with 20 μL of 65% nitric acid and incubated for 15 min at 37 °C. Methanol was then added (40 μL), and 20 μL of the extract was applied to the TLC plate. The chromatogram was developed in toluene and then air-dried. Detection of TCBQ was achieved by spraying the TLC plates with 2.5 g of tetramethyl-*p*-diaminodiphenylmethane containing 10 g of citric acid in 500 mL of distilled water followed by heating in an oven at 100 °C for 5 min. The TCBQ complex was visualized as a strong blue band with an R_f value of 0.9.

Spectrophotometric Analysis of PCP with 4-Aminoantipyrine. An acid/base solvent partition was required to remove components in the wood which interfered with the assay. The dried wood extract was dissolved in 3 mL of toluene followed by 3 mL of 1 mM HCl. After vortexing, the aqueous phase was removed (a centrifugation step may be required to separate the two phases). Then, 3 mL of 5 mM Na_2CO_3 (pH 11.4) was added to the organic phase and the mixture was vortexed vigorously. After centrifugation for 2 min (4500*g*), the aqueous phase containing the PCP was transferred to another tube. An additional 1 mL of alkaline carbonate buffer was added to the toluene fraction, vortexed, and centrifuged. The aqueous phases were combined, and residual toluene was removed from the aqueous phase by purging with nitrogen gas for 5 min at 40 °C. One milliliter of 100 mM NaH_2PO_4 (pH 5.8) was then added followed by 0.25 mL of 0.2% 4-aminoantipyrine in distilled water immediately followed by 0.5 mL of 8.4% sodium persulfate. The reaction mixture was vortexed briefly, and after a 2 min incubation at room temperature, the oxidized blue dye was extracted with 2 mL of toluene and measured at 580 nm. These were the optimal pH conditions for analysis of PCP. The effects of pH (using either sodium phosphate or sodium carbonate buffer), time, and reagent concentration were examined.

Analysis of PCP by Gas Chromatography−Mass Spectrometry. The sawdust extract (100 mg) was dissolved in 0.5 mL 100 mM Na_2CO_3 (pH 11.4) followed by the addition of acetic anhydride (10 μL) and 0.25 mL of toluene. The solution was shaken periodically over 30 min at room temperature. The toluene phase (centrifuged at 4500*g* for 5 min if phase separation is incomplete) was removed and analyzed by the GC−MS conditions described below.

SIM−GC−MS analyses were performed with a Hewlett Packard 5890 gas chromatograph and a 5972 mass selective detector. The column was an HP-5 MS 5% phenylmethyl silicone column (30 m × 0.25 mm, 0.25 μm film thickness). The carrier gas was helium with a constant flow rate of 30 cm/s. The oven program was set at 80 °C for 1 min to 220 °C at 15 °C/min, resting for 1 min, and then to 300 °C at 25 °C/min and resting for 1 min. The injector port temperature was 280 °C, and injection (1 μL) was in the splitless mode with an HP 7673 automatic injector. The internal standard [$^{13}C_6$]PCP was added to 100 mg of the sawdust in 10 μL of methanol solution (1 μg, 10 ppm) for the heavily contaminated samples and at 0.1 μg/100 mg of sawdust (1 ppm) for weakly contaminated wood samples. Detection was performed in the selected ion monitoring mode using *m*/*z* 266 as the target ion for PCP acetate and *m*/*z* 274 for [$^{13}C_6$]PCP acetate. This ion was chosen rather than *m*/*z* 272 (M + 6) for [$^{13}C_6$]PCP acetate because of the "isotope cluster" mass ion overlap of the five chlorine atoms with the unlabeled PCP.

RESULTS AND DISCUSSION

Solvent Extraction of PCP from Sawdust. PCP is often purified from wood-based material by soxhlet extraction or steam distillation from an acidified aqueous extract. Both of these methods provide relatively clean sample preparations; however, the time required is long, and the methods are not suitable for conducting multiple analyses. Therefore, several extraction methods were evaluated including liquid−liquid solvent partitioning and purification by solid phase resins (silica, C-18 adsorbents, XAD-2, etc.) which could be used to rapidly isolate PCP from sawdust. The most efficient method for recovering PCP with a minimum of interfering material was a solvent extraction with methanol containing 0.1% acetic acid. This extract could be directly assayed for PCP by the TLC method and by SIM−GC−MS following chemical derivatization; however, an additional step, a liquid−liquid solvent partition against toluene, was required to remove substances which interfere with the spectrophotometric assay.

Table 1. Recovery of [^{14}C]PCP Spiked in Sawdust Samples

	% recovery of [^{14}C]PCP in sawdust[a]			
	sawdust 1	sawdust 2	sawdust 3	sawdust 4
methanol:acetic acid extract	80.0	89.7	75.7	83.5
5 mM Na_2CO_3, pH 11.4	54.0	44.8	49.7	48.2

[a] [^{14}C]PCP (37 nCi, 0.92 μg) was added to 1 g of sawdust and extracted with 20 mL of methanol containing 0.1% acetic acid. After evaporation, the samples were resuspended in 3 mL of toluene, and radioactivity was measured by liquid scintillation counting to determine percent recovery and counted again following acid/base partitioning in alkaline 5 mM Na_2CO_3 (pH 11.4) phase.

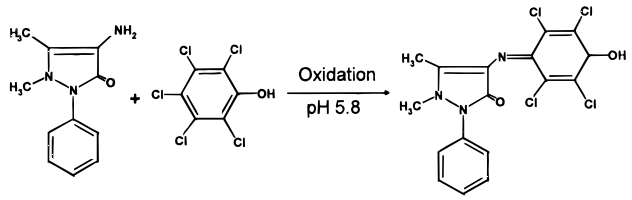


Figure 1. Coupling of PCP with 4-aminoantipyrine in the presence of sodium persulfate.

The recovery of ^{14}C-labeled PCP added to sawdust at the level of 1.0 ppm following extraction with acidified methanol and an acid/base solvent partition against toluene is presented in Table 1. On average, 80−85% of the radioactivity is recovered in the acidic methanol extract, of which approximately 60% is recovered following alkaline extraction to yield an overall recovery of 49 ± 3.8%. The recovery of PCP following solvent partitioning under alkaline pH can be increased to over 65% by performing an additional extraction of the toluene phase with 3 mL of 5 mM Na_2CO_3 (pH 11.4).

Quantification of PCP by Spectrophotometry. The spectrophotometric analysis of PCP was adapted from the chemistry developed on detection of phenols (Emerson, 1943; Ettinger et al., 1951; Bencze, 1963). The acidic hydroxyl group of PCP enables its coupling with aminoantipyrine to occur under acidic pH (Figure 1). This reaction is relatively specific, since lower substituted chlorophenols and nonchlorinated phenols only react with 4-aminoantipyrine under alkaline pH conditions (Bencze, 1963). The reaction conditions were optimized by investigating the kinetics of product formation and stability as a function of time, pH, and reagent concentrations. The data are summarized in Figure 2. The reaction conditions clearly showed a pH

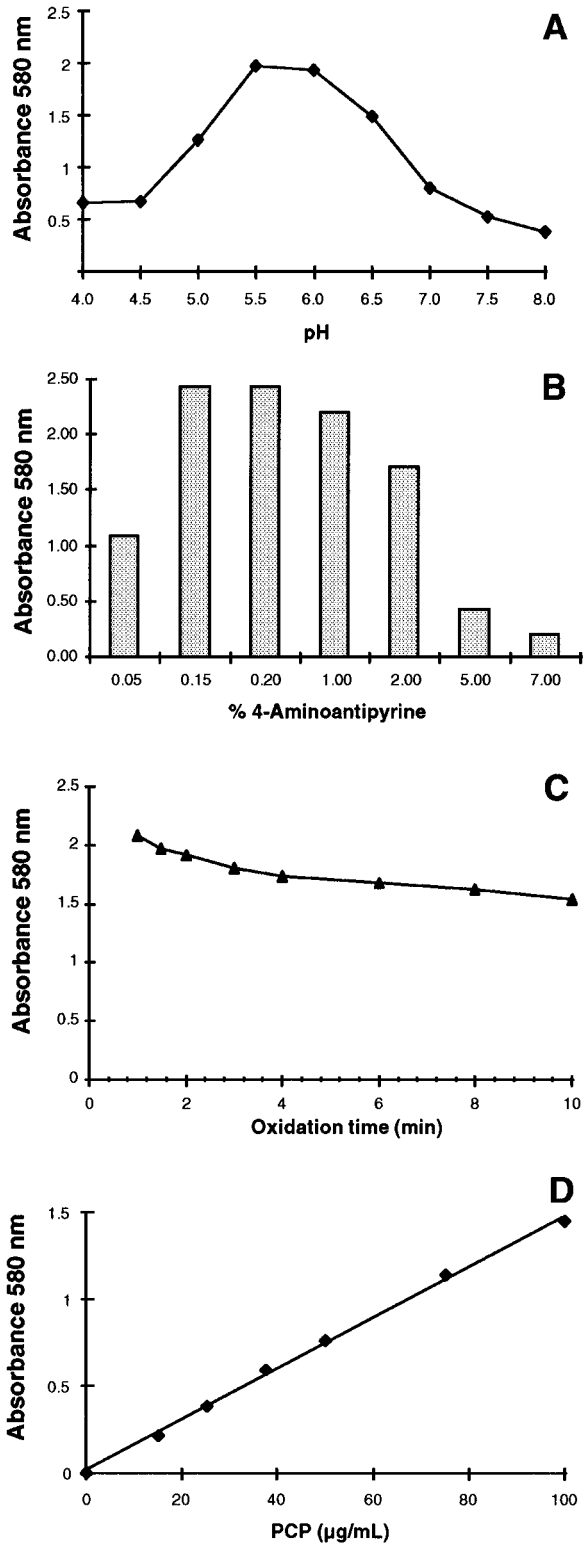


Figure 2. Kinetics of PCP reactivity with 4-aminoantipyrine as (A) a function of pH, (B) a function of 4-aminoantipyrine concentration, and (C) a function of time. The standard curve (D) for PCP oxidation by sodium persulfate (0.20%) in 20 mM NaH_2PO_4 buffer (pH 5.8) was done with 4-aminoantipyrine (0.2%) and 0.5 mL of 8.4% sodium persulfate as described in Materials and Methods. Values obtained are an average of duplicate determinations that were within 10% of each other.

optimum at pH 5.8, which is in contrast to the results of Bencze who reported an optimum at pH 7−7.5. Therefore, all ensuing reactions were conducted at pH 5.8. Kinetic studies showed that maximum dye formation occurred within 2 min of persulfate oxidation and

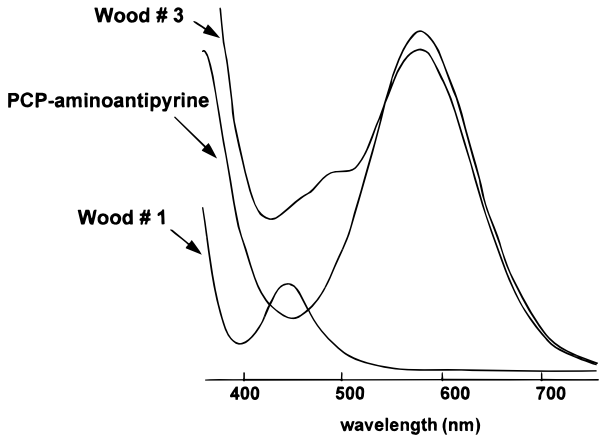


Figure 3. Visible spectrum of oxidized PCP coupled to 4-aminoantipyrine and spectra of the extracts derived from heavily (no. 3) and weakly (no. 1) contaminated wood samples.

gradually decreased over time probably due to further oxidative reactions or hydrolysis of the phenazone linkage. The PCP−antipyrine dye complex was stable up to 90 min following extraction into toluene. The range of 4-aminoantipyrine over which maximal dye formation occurred was between 0.15 and 1.0%. Based upon these conditions, a standard curve was generated where PCP was coupled to 4-aminoantipyrine (0.2%) during a 2 min oxidation with sodium persulfate in 20 mM NaH_2PO_4 (pH 5.8) followed by extraction with 2 mL of toluene. The curve was linear up to 100 μg/mL. These were the optimal conditions used to measure the PCP content in sawdust.

The ability of other phenolic wood derivatives to form coupled products with 4-aminoantipyrine following sodium persulfate oxidation was investigated. Phenol and *m*- and *p*-cresol were found to be at least 1000-fold less reactive than PCP on a per mol basis (data not shown) when reactions were conducted at pH 5.8 as described above. This difference in reactivity can be attributed to the low pH used for selective oxidation of PCP (Ettinger et al., 1951; Bencze, 1963).

The visible spectrum of oxidized PCP coupled to 4-aminoantipyrine is shown in Figure 3 along with the spectra of heavily and weakly contaminated wood samples. The chromophores of the reference compound and the heavily contaminated wood sample show maximal absorbance at 578 nm, and the spectra are similar between 500 and 700 nm, while the absorbance of the weakly contaminated sample is at the limit of detection. The PCP−antipyrine dye complex formed in the wood extract was analyzed by TLC using toluene as the developing solvent. A prominent blue band was detected ($R_f = 0.4$) which comigrated with the synthetic PCP-coupled aminoantipyrine product followed by a faint orange band ($R_f = 0.35$) (data not shown). Therefore, the visible chromophore used for quantitation of PCP is indeed attributed principally to PCP.

Quantification of PCP by Gas Chromatography−Mass Spectrometry. Derivatization of PCP by acetic anhydride in a biphasic solvent containing toluene and 0.1 M Na_2CO_3 (pH 11.4) results in complete partitioning of the pentachlorophenyl acetate into the organic and serves as a purification step as considerable interfering material is retained in the aqueous phase. The PCP acetate also displays superior chromatographic properties to underivatized PCP and results in increased sensitivity of analyte detection. The molecular ions of PCP acetate and [$^{13}C_6$]PCP acetate are observed respec-

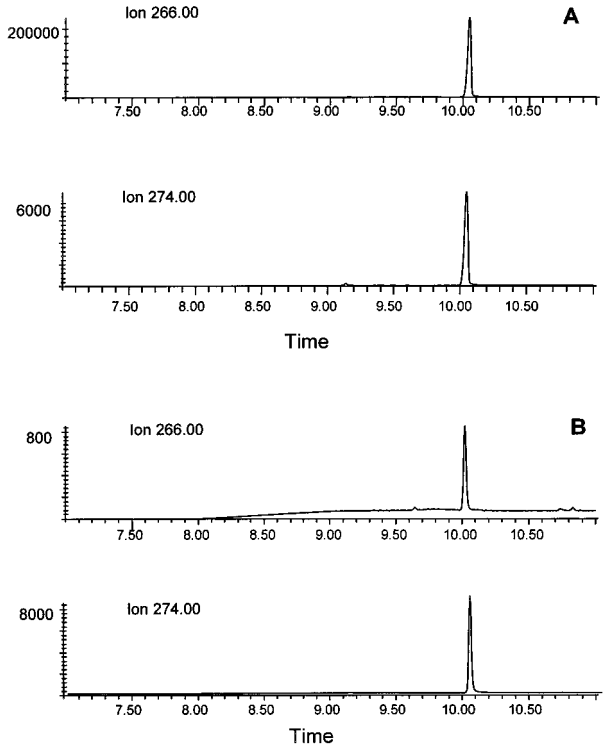
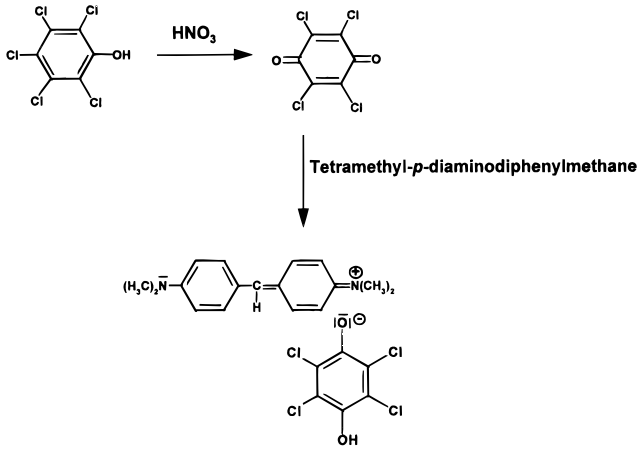


Figure 4. SIM−GC−MS analyses of PCP in two wood samples with [$^{13}C_6$]PCP internal standard added at 10 ppm for (A) the heavily contaminated wood sample and (B) the weakly contaminated wood sample. Weakly contaminated samples were quantified with internal standard added at 1 ppm.

tively at *m*/*z* 308 and 314, with loss of acetate $(M - 42)^+$ as the principal fragment and base peak. Analyses of PCP in sawdust were performed using the target ion $(M - 42)^+$ at *m*/*z* 266 for PCP; however, the target ion of [$^{13}C_6$]PCP acetate was chosen at *m*/*z* 274, not the base peak $(M - 42)^+$ at *m*/*z* 272, because of the "isotope cluster" mass ion overlap of the five chlorine atoms with the unlabeled derivative. Calibration curves were linear from 0.05 to 300 ppm (data not shown). The SIM−GC−MS analyses of PCP in two wood samples are presented in Figure 4. High levels of PCP are present in palette 3 (250 ppm), while low levels were found in palette 1 (<1 ppm).

Detection of PCP by Thin Layer Chromatography. Following nitric acid-mediated oxidation of PCP to TCBQ, the wood extract was analyzed by TLC using toluene as the developing solvent. TCBQ was visualized by reaction with tetramethyl-*p*-diaminodiphenylmethane which results in an electron transfer complex and produces the semiquinone−immonium radical complex that exhibits a rich blue chromophore (Kovar et al., 1971) (Figure 5). The TLC analyses of PCP (0, 1, 5, 25, 100, and 200 ppm) and wood extracts are presented in Figure 5. The limit of PCP detection under these conditions of analysis is approximately 1 ppm (approximately 320 ng of PCP applied to the plate). The PCP band is readily detected in palette 3, and the band intensity is comparable to the 200 ppm band of synthetically oxidized PCP. A minor band (R_f = 0.8) is present in the wood and also detected in the reference chemical TCBQ. This band is not attributed to the 1,2-tetrachlorobenzoquinone isomer which can form during the nitric acid-mediated oxidation of PCP in the presence of metal salts (Crivello, 1980). Under these oxidation condtions, this isomer did not react with tetramethyl-*p*-diaminodiphenylmethane (unpublished observations),



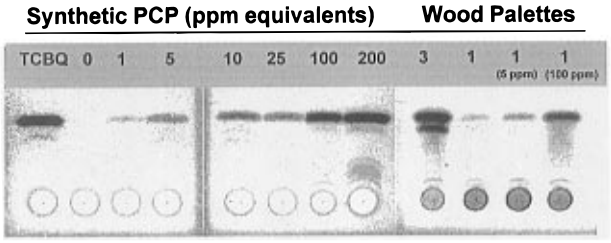


Figure 5. Chemistry of nitric acid-mediated oxidation of PCP to TCBQ followed by coupling to tetramethyl-*p*-diaminophenylmethane. TLC analyses are of TCBQ, PCP, and heavily contaminated (palette 3) and weakly contaminated (palette 1) wood palettes. The weakly contaminated wood palette was also spiked with PCP in the sawdust at 5 or 100 ppm prior to solvent extraction.

Table 2. Comparative Analysis of PCP in Wood Palettes by Colorimetry, GC−MS, and TLC[a]

	PCP ± SD (ppm)		
sample	colorimetry	GC−MS	TLC
palette 1	1.20 ± 0.50	0.40 ± 0.00	+/−
palette 2	<1	1.03 ± 0.62	+/−
palette 2	4.53 ± 2.91	1.40 ± 0.40	+/−
palette 3	275 ± 35	300 ± 20	+++
palette 4	1.74 ± 0.5	1.30 ± 0.20	+/−
palette 5	254 ± 20	281 ± 27	+++
palette 6	212 ± 24	309 ± 69	+++

[a] Analyses were done in triplicate: +++, heavy contamination, strong band; +/−, weak contamination, faint, detectable band approaching the limit of TLC detection ca. 1 ppm. For the spectrophotometric method, [^{14}C]PCP was added to the sawdust at a level of 1 ppm prior to solvent extraction and quantitation was determined by accounting for recovery of radioactivity and subtracting the amount of [^{14}C]PCP recovered from the final extract.

and the identity of this product remains unknown. Palette 1 also contains faint, detectable levels of PCP which approach 1 ppm. This estimate is in agreement with the quantitative data generated by SIM−GC−MS. When wood palette 1 is spiked with 5 ppm of PCP, there is a noticeable increase in the PCP band detected by TLC. It is also possible to qualitatively distinguish PCP spiked in wood palette 1 at levels ranging from 5 to 300 ppm with the unaided eye (unpublished observations).

Comparative Data Analysis of PCP by GC−MS, Colorimetry, and TLC. The results presented in Table 2 show that the estimates of PCP obtained by the colorimetric and TLC methods are in good agreement with the quantitative data obtained by SIM−GC−MS. Thus, all three screening methods provide reliable estimates of PCP in wood palettes. The values obtained

by the TLC method are semiquantitative; the employment of a visible scanner would make the method quantitative. However, this adaptation does not appear necessary. PCP contamination in wood can be readily discerned at the 0−10, 25−100, and 100−300 ppm levels by the unaided eye.

The results from the TLC and colorimetric assays indicate that they are relatively specific toward PCP detection and cross reaction with other phenols that occur naturally in wood does not take place and result in artifact formation. The selectivity of the colorimetric and TLC assays is partly attributed to the acidic HO-phenolic group of PCP which enables the oxidation reactions to be performed under acidic pH and thus minimizes interfering reactions with other phenolics which require alkaline pH conditions. In addition, lower substituted chlorophenol derivatives, such as TCP and TeCP, are volatile, and when spiked in sawdust at 10−50 ppm, they are removed during the evaporation of the methanol:acetic acid solvent from the sawdust as demonstrated by SIM−GC−MS analysis (unpublished observations). Thus, these detection methods appear to be relatively specific for PCP.

The SIM−GC−MS method provides unequivocal identification and quantitation of PCP. The extraction method described in this article is far more rapid than other methods previously reported for GC−MS and GC−ECD (Fullerton et al., 1982; Lee et al., 1989; Sequeira and Taylor, 1991; Cui and Ruddick, 1994; Cooper et al., 1994). However, the GC−MS method requires sophisticated analytical equipment and a skilled chemist. The spectrophotometric and TLC screening methods are easy to perform and can be conducted by personnel with minimal technical training. Therefore, such methods could be incorporated directly on-site in factories/production centers to identify PCP-contaminated wood-based containers and avoid their usage in transportation and storage of foods.

ABBREVIATIONS USED

PCP, pentachlorophenol; TCP, 2,4,6-trichlorophenol; TeCP, 2,3,4,6-tetrachlorophenol; TCBQ, tetrachloro-1,4-benzoquinone, GC−MS, gas chromatography−mass spectrometry; SIM−GC−MS, select ion monitoring-gas chromatography−mass spectrometry; GC−ECD, gas chromatography−electron capture detection; TLC, thin layer chromatography.

ACKNOWLEDGMENT

The contributions of Mr. Antonio Miguel G. N. Freitas to the spectrophotometric method development are greatly appreciated. The critical comments of Dr. Laurent Fay and Dr. Sebastien Raoul, Nestlé Research Center, are greatly appreciated.

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Received for review August 7, 1996. Revised manuscript received December 19, 1996. Accepted January 7, 1997.[⊗]

JF9605963

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1997.