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Determination of Pentachlorophenol in Hardwood Chips

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A rapid and sensitive procedure is described to determine pentachlorophenol (PCP) in hardwood chips or cardboard. The PCP is isolated via liquid–liquid partitioning steps. Pentachlorophenyl acetate is formed by acetylation using pyridine and acetic anhydride, and the derivatized material is partitioned into hexane for electron-capture GC analysis. Hardwood chips, spiked at five levels with PCP, had recoveries of 83 ± 6%. Results from 86 hardwood chip samples and 11 cardboard samples that were analyzed between 1977 and 1981 had PCP residue levels that ranged from <5 to 240 ppb and from 0.13 to 4.4 ppm, respectively.

Pentachlorophenol (PCP) is a registered fungicide used in slime control and the preservation of wood products. This pesticide is environmentally persistent and poses a potential health problem to animals exposed to PCP-treated products or to toxic PCP contaminants, such as dioxins as reported by Plimmer et al. (1973), Dickson (1980), and Lamberton et al. (1979). Hardwood chips are used as bedding for laboratory research animals while sawdust and wood shavings are used to control litter in chicken and pig pens and in holding areas for cattle. Since the presence of excessive PCP in these wood byproducts may be deleterious to animals, it is important to monitor the PCP levels in such products. Wood chips or cardboard having PCP levels above 2 ppm are not used in our laboratory to avoid biasing results from animal studies. Thus, a simple and accurate method was needed to analyze wood products for PCP at levels below 2 ppm. Establishment of the 2.0-ppm limit of PCP was to prevent biological response and restrict exposure of experimental animals to potentially deleterious chemicals that may be present in ancillary animal supplies as discussed by Greenman et al. (1980). In addition, supplies could be obtained that satisfied the arbitrary PCP limitation.

Extensive reviews of the environmental hazards and analytical procedures to determine residues of PCP in various substrates have been published by Bevenue and Beckman (1967) and by Ahlborg and Thunberg (1980). Since the introduction of PCP in the 1930s, methods developed to conduct PCP analysis include colorimetric, UV, and IR absorption plus paper, thin-layer, gas, and liquid chromatography. Many of these procedures are tedious and lack the necessary selectivity and/or sensitivity required for residue analysis. The HPLC method reported by Daniels and Swan (1979) does provide a more rapid procedure but lacks the sensitivity we require. Most of the methods reported have been for the analysis of wastewater, animal tissues, urine, air, and soil. Ting and Quick (1980) reported a TLC method to determine PCP in sawdust and wood shavings that is capable of detecting about 2 ppm of PCP. A method reported by Rudling (1970) to analyze for PCP in animal tissue or water consisted of extracting the acidified sample with *n*-hexane followed by acetylation. The method presented herein also acetylates the PCP for subsequent electron-capture GC analysis. The analytical procedure to determine PCP in hardwood chips or cardboard is sensitive and can be completed in less than 1 day. In addition, the method has good precision as demonstrated by the standard deviation obtained for the spiked recoveries, and it is free from interference by other residues that may be present in the samples.

EXPERIMENTAL SECTION

Reagents. The PCP standards (No. 3462, Eastman Kodak Co., Rochester, NY, and Reference Standard, EPA, Perrine, FL) were used as received because they contained no extraneous peaks when analyzed by flame ionization detection (FID) and electron-capture (EC) gas chromatography (GC). In addition, confirmation and purity analyses were obtained on the two standards by mass spectrometry.

The acetylating reagent was prepared by mixing 2 mL of pyridine (No. 27530, Pierce Chemical Co., Rockford, IL) with 0.8 mL of acetic anhydride (No. A-10, Fisher Scientific Co., Fair Lawn, NJ). Fresh reagent was prepared weekly and kept under refrigeration.

All solvents were Nanograde (Mallinckrodt, Inc., St. Louis, MO), and all reagents were CP grade.

Materials. *Hardwood chip* samples were taken from batches of hardwood chips purchased commercially for animal bedding. The chips were manufactured only from maple, birch, beech, or mixtures thereof with particle size specifications requiring that over 95% pass through a no. 8 mesh screen and less than 6% pass through a no. 20 mesh screen; moisture content did not exceed 8%. Shipments of hardwood chips were received in 40-lb bags, and 250-g aliquots of material were taken randomly from nine different bags. The individual collections were composited in a "V" blender to produce a homogeneous sample from each shipment for PCP analysis. In a similar fashion, commercially supplied cardboard samples were taken from a batch of gray-mist chipboard sheets (about 30 × 30 cm and 0.07 cm thick) that are used to form feeder boxes. Six

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random sheets of the cardboard from each batch were taken and cut up into 0.5-cm squares, and the squares were mixed to produce a homogeneous sample for PCP analysis.

Extraction and Recovery Experiments. Tests to determine recovery and precision of the method were conducted: 10-g portions of hardwood chips were weighed into glass culture tubes (38 × 200 mm) with Teflon-lined screw caps. The samples were then spiked with 0, 10, 50, 100, 500, and 1000 ppb of PCP by adding an appropriate amount of chemical in 2 mL of hexane. The open tubes were allowed to stand for 4 h, and then samples were extracted, cleaned up, derivatized, and analyzed for EC-GC.

One hundred milliliters of 10% methanol in benzene was added, and the sample was mechanically extracted for 4 h on a wrist-action shaker at a rate of 200 excursions per minute. Samples that were allowed to soak overnight in the solvent after the 4 h extracting period and extracted for an additional 30 min did not show any improvement in extraction efficiency. The extract was filtered through a 5-g plug of anhydrous sodium sulfate (Mallinckrodt, Inc., No. 8024) and made to 100 mL with additional extracting solvent. A 25-mL aliquot of the filtrate was transferred to a 100-mL round-bottom flask containing two boiling beads and concentrated to 3–5 mL by using a 35 °C water bath and water pump vacuum. The concentrate was quantitatively transferred to a 20-mL culture tube and evaporated just to dryness by using a gentle stream of N_2 and a 35 °C water bath.

The residue was dissolved in 5 mL of distilled water and 1 mL of 6 M H_2SO_4 and extracted from the aqueous solution with shaking for 1 min with 5 mL of 2-propanol–hexane (3:5 v/v). The organic and aqueous layers were separated with the aid of a centrifuge, and the organic extract was quantitatively transferred to an 8-mL culture tube with a Teflon-lined screw cap. The PCP was extracted from the organic material by vigorously shaking for 1 min with two 2-mL portions of 0.1 M $Na_2B_4O_7$ solution. The layers were separated with the aid of a centrifuge, and the two aqueous extracts were combined for subsequent derivatization.

Derivatization of PCP for EC-GC Analysis. Acetate derivatives of PCP were prepared by adding 100 μL of fresh acetylating reagent (2 mL of pyridine with 0.8 mL of acetic anhydride) plus 2.5 mL of hexane to the tube containing the aqueous extract and mixing the contents with moderate shaking for 1 min. An appropriate amount of the hexane phase was analyzed by EC-GC for PCP. Standards were prepared by adding appropriate amounts of PCP in 2.5 mL of hexane to 4 mL of $Na_2B_4O_7$ and derivatized.

Gas Chromatography. The gas chromatograph used to assay for PCP was a Hewlett-Packard Model 5710A instrument equipped with a ^{63}Ni linear electron-capture detector (operated at 300 °C), a 6-ft glass column (4-mm i.d.), and a Hewlett-Packard Model 3380A recording integrator. The glass column, containing 3% Dexsil-300 on Chromosorb W HP (100–120 mesh) conditioned at 275 °C overnight prior to use, was operated isothermally at 225 °C. The carrier gas was 5% methane in argon at 40 psig and a flow of 40 mL/min. The injection port temperature was 250 °C. Under these conditions, the retention time for pentachlorophenyl acetate was 2.9 min. Tentative confirmation of the presence of PCP was made with the EC-GC described above equipped with a 6-ft glass column (4-mm i.d.) packed with 3% OV-1 plus 3.9% OV-17 on Gas-Chrom Q (100–120 mesh). The column was conditioned at 250 °C overnight prior to use and operated at 180 °C. The carrier gas was 95% argon–5% methane at 40 psig and a flow rate of 60 mL/min. Under these conditions, the retention time for pentachlorophenyl acetate was 4.6 min. Further confirmation of PCP was made by using an MT-220 GC equipped with a Hall electrolytic conductivity detector (Tracor, Inc., Austin, TX) operated in the reduction mode. The GC had a 6-ft glass column (4-mm i.d.) containing 5% QF-1 on Chromosorb W HP (80–100 mesh) that was previously conditioned at 250 °C overnight prior to use and was operated isothermally at 200 °C with a helium carrier flow rate of 55 mL/min at 40 psig. The pyrolysis furnace temperature was 900 °C; the conductivity cell was operated with an ethanol flow rate of 0.7 mL/min and a hydrogen reactant gas flow rate of 5 mL/min. Under these conditions, the retention time of acetylated pentachlorophenol was 7.0 min. The column effluent was vented to the atmosphere during the first 2 min after each injection to prevent loss of detector sensitivity.

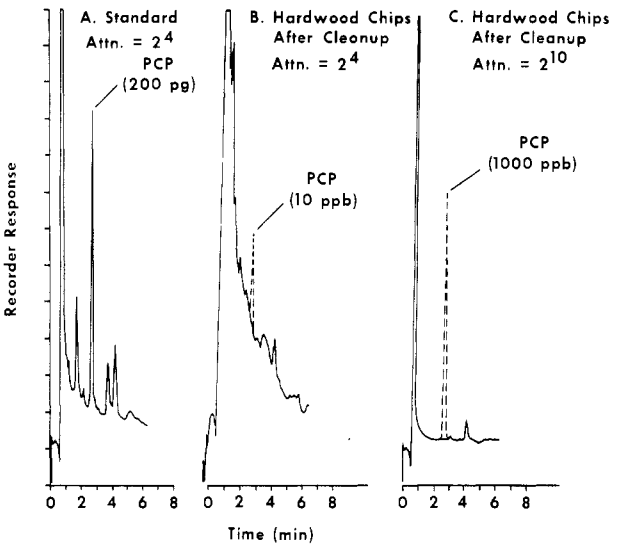

Figure 1. EC-GC chromatograms showing 4-μL injections of PCP after derivatization to the PCP-acetate.

Table I. Recoveries of PCP from Hardwood Chips Spiked at Five Levels

spiked level, ppb	no. of anaylses	amount added, μg	amount observed	
			μg	% ± SD
10	40	0.1	0.085	85 ± 5
50	24	0.5	0.411	82 ± 6
100	48	1.0	0.84	84 ± 6
500	24	5.0	4.0	80 ± 5
1000	48	10.0	8.3	83 ± 4

RESULTS AND DISCUSSION

Panels B and C in Figure 1 show typical chromatograms obtained from analysis of hardwood chips after acetylation to the PCP-acetate (PCP-Ac) with the peaks superimposed at 10 and 1000 ppb, respectively. Panel A shows the background from the derivatizing agents used in acetylation for a 50-ppb (200-pg injection) PCP-Ac standard. The background of the spiked samples ranged from about 0.5 to 5.0 ppb and averaged slightly over 2 ppb. This established minimum detectable limit (MDL) of the procedure at 5 ppb (twice background).

Table I shows recoveries obtained from 184 analyses conducted on hardwood chip samples spiked at five different levels. The average recovery percentage for PCP spiked at the five different levels listed in Table I was 83 ± 6%. Recoveries of PCP from cardboard samples did not

significantly differ from values for hardwood chips. Data in Table I show the efficiency of the extraction procedure to be concentration independent and to have excellent precision. Quantitation of PCP in unknown samples was conducted by comparison to a standard that was near the sample value and the recovery factor applied to obtain sample concentration. This analytical procedure has been successfully used to analyze 86 samples of hardwood chips between 1977 and 1981. Observed concentrations of PCP residues in the hardwood chips ranged from less than MDL to 240 ppb with an average level of 44 ppb. All of the hardwood chip samples analyzed met the 2-ppm experimental specifications for PCP.

The procedure was also used to analyze for PCP in cardboard, a gray-mist chipboard, used to make animal feeder boxes for rodent feeders (Fullerton et al., 1981). Levels in 11 cardboard samples ranged from 0.13 to 4.4 ppm with an average level of 1.8 ppm. Three of the cardboard samples analyzed were rejected because they exceeded the 2-ppm limitation established at our laboratory.

The method presented provides sensitivity in the analysis of wood products not previously available. In addition, the method has good precision as evidence by the reproducibility of the results reported in Table I, which were obtained from replicate analyses.

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Analysis of Neutral Sugar Hydrolysates of Forage Cell Walls by High-Pressure Liquid Chromatography

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The separation and analysis of monosaccharides were accomplished by high-pressure liquid chromatography using an amine bonded phase column and acetonitrile–water solvent system. Excellent separation was achieved for rhamnose, xylose, arabinose, and glucose, while adequate separation of glucose and galactose was obtained. Monosaccharide samples were obtained by hydrolyzing the cell walls of forage grasses with 2 N trifluoroacetic acid. The hydrolysates were prepared for HPLC analysis with a Waters Associates C_{18} Sep-PAK. The precision of the analysis (10 injections of the same sample preparation) as measured by standard deviation was ±0.38, ±0.24, and ±0.35% for xylose, arabinose, and glucose, respectively. Analysis of variance indicated no difference in recovery of sugars from triplicate preparations of the same sample. Analysis of variance did show significant differences in the composition of three different samples of the same species. Thus, it is possible to accurately analyze for differences in the composition of the component sugars hydrolyzed from forage cell walls and know that the method is sensitive enough to reflect differences in composition that could be related to nutritional qualities of the feed.

One of the differences between temperate and tropical grasses is the amount of readily hydrolyzable polysaccharides in the plant cell wall. Readily hydrolyzable polysaccharides are defined as that portion of the forage cell wall hydrolyzed by dilute acid. Barton et al. (1976) showed that tropical grasses contain 30–35% more "hemicellulose" than temperate grasses (i.e., tropical 28–35%, temperate 22–26%). Further, they showed that this difference in the amount of hemicellulose could account for some of the difference in digestibility of temperate and tropical grasses.

Albersheim et al. (1967) used a gas–liquid chromatographic method to analyze the cell walls of "pinto bean" hypocotyls. This method is an improvement over earlier methods in that 2.0 N trifluoroacetic acid (TFA), which is easily removed by evaporation, replaced 1.0 N sulfuric acid as the hydrolytic agent; however, several steps are still required. In addition, Collings and Yokoyama (1979) used a modification of the alditol acetate technique of Albersheim et al. (1967) to determine cellulose and hemicellulosic sugars of delignified forages and feeds by 1-h hy-

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