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# Determination of Atmospheric Contamination by Pentachlorophenol Using Pine Needles Located Near Treated Utility Pole Storage Sites

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Pentachlorophenol (PCP) is a synthetic chemical which has been extensively used for wood preservation and protection, with other minor applications as a herbicide. Over the past half century, PCP was used in many formulations both commercially and domestically. Laboratory animal studies have suggested that PCP is a potential carcinogen although there is a lack of human data to confirm this hypothesis. The widespread use and persistence of this chemical has resulted in its existence throughout the environment as well as the food chain. Health and Welfare Canada has estimated that approximately 78% of non-occupationally related exposure to PCP is via the food chain (Health and Welfare Canada 1992). The next most significant route of human exposure to PCP is through the ambient atmosphere. PCP is rapidly eliminated, largely as the free acid, from the human body by urinary excretion. In a study of urine samples collected from 6000 individuals from the general population of the United States, 79% of the urine samples tested were found to contain detectable quantities of PCP (Murphy 1983). In subsequent studies of smaller groups of subjects from the United States and Canada, PCP has been found in virtually 100% of the urine samples which were tested (Holler 1989; Thompson 1994, 1995a; Treble 1996). If the earlier study by Murphy had been capable of achieving a lower detection limit for PCP, it is highly likely that the frequency of positive detection would have been greater than 79%.

Many volatile and semi-volatile organic contaminants undergo extensive transport through the atmosphere. In fact, persistent organic pollutants may travel many hundreds of kilometres from their original point of emission. Chlorinated organic pollutants, for example polychlorinated biphenyls (PCBs) or insecticides such as DDT and lindane, are known to be readily partitioned into lipids and lipophilic materials in general. Pine needles are covered with a waxy coating which have been found to contain atmospherically transported pollutants (Thomas 1984; Gaggi 1985). It has been suggested that organic contaminants existing in the vapour phase partition into the lipophilic waxy coating (Eriksson 1989). The

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absorption of compounds dissolved in rainwater is believed to contribute only slightly to the levels found in the needle wax. Similarly, the absorption of particulate matter is believed to account for only a small fraction of the total amount of contaminant present in the waxy layer. Translocation of organic contaminants from the soil through the root system of the plant and into its leaves has been found to be a minor source of these compounds. This type of translocation has not been illustrated for pine needles.

Eriksson and co-workers (1989) analyzed pine needle samples collected throughout western Europe for DDT and its major metabolite (DDE), PCBs, two hexachlorocyclohexanes (the alpha and gamma isomers), and PCP. The average levels of PCP were found to range from 0.09 to 1.39 ng/g of needle for all locations while the average levels for a given region were found to be relatively consistent. In a study performed by our laboratory, pine needle samples were collected from various parts of an urban centre including golf courses, municipal parks, residential areas, and industrial areas (Thompson 1995b). PCP levels in the extracted pine needle wax were found to range from 0.42 to 2.08 ng/g of needle.

Eriksson (1989) proposed that background concentrations of global pollutants such as PCP would be expected in pine needle samples. Consequently, elevated levels should be found in samples where localized emission over an extended period of time permitted the absorption of contaminants. The concentration of the contaminants would effectively represent time-integrated ambient air concentrations. If this hypothesis holds true, pine needles collected near point sources of atmospheric contamination should have significantly higher levels of these pollutants.

Pine needle samples were collected in the vicinity of two sites in northern Saskatchewan where chemically treated utility poles were stored. These needle samples were analyzed for PCP which was suspected as being present in the solution used for preservation of the wooden poles.

## MATERIALS AND METHODS

Pentachlorophenol (greater than 98% purity) was obtained as a neat solid from BDH Chemicals Canada Limited (Toronto, Canada). Isotopically labelled pentachlorophenol having greater than 99% of all carbon atoms in the aromatic ring replaced by $^{13}C$ was purchased from Cambridge Isotope Laboratories (Woburn, MA). All organic solvents (acetone, dichloromethane, diethyl ether, hexane, and toluene) used in this study were distilled in glass and suitable for pesticide residue analysis (Anachemia; Toronto, Canada). Sulphuric acid was obtained from Caledon Laboratories Limited (Georgetown, Canada). Anhydrous sodium sulphate was purchased from Fisher Scientific (Fair Lawn, NJ). Silica gel (70-230 mesh) for column chromatography was obtained from EM Science (Gibbstown, NJ). Trifluoroacetic anhydride (TFAA), which was used to form the

trifluoroacetyl derivative of PCP, was purchased from Aldrich Chemicals (Milwaukee, WI).

Acidified sodium sulphate was prepared by adding concentrated sulphuric acid to a beaker containing a slurry of anhydrous sodium sulphate in diethyl ether. The acid was added at a ratio of 0.1 mL per 100 grams of sodium sulphate. After thorough mixing, the beaker was covered with a watchglass and the ether was allowed to evaporate in a fumehood. The acidified sodium sulphate was stored in an oven at 110 °C until required.

Sulphuric acid modified silica gel (50% v/w) was prepared by adding 50 mL of concentrated sulphuric acid per 100 g of silica gel. The acid-silica gel mixture was combined in a 1-L amber glass bottle which was sealed with a Teflon-lined screwcap. The contents of the bottle were mixed with vigorous shaking until a free-flowing powder was obtained. This reagent was stored in the capped glass bottle and utilized as required.

Samples were collected by carefully removing small sections of branches from pine trees using pruning shears. These branches were placed in wide-mouthed glass jars which were covered with clean aluminum foil and capped tightly. Samples were stored in a freezer at -4 °C until analyzed.

Needles were carefully removed from the branches using stainless steel scissors. The clippings were collected on a piece of solvent-rinsed aluminum foil and subsequently transferred to a 100-mL amber glass screwcap bottle, the weight of which was tared to zero on an analytical balance. Approximately 10 g of pine needles were collected in each sample bottle, with all sample weights recorded to ± 0.001 g. Each sample was fortified with 100 µL of a solution containing 1 ng of $^{13}C_6$-labelled PCP per microlitre of acetone. After allowing the fortification standard to be fully absorbed onto the surface of the needles (approximately 10 min), 50 mL of dichloromethane was added to each bottle. The bottles were tightly sealed with Teflon-lined caps and placed in a mechanical shaker. The contents of the bottles were gently mixed (with a cycle time of 1 sec) for 30 min.

A liquid chromatographic column was prepared by plugging the tip of a 25-mL disposable glass pipette with acid-washed glass wool and then packing it with 23 mL of acidified silica gel topped by 2 mL of acidified sodium sulphate. The column was pre-washed with 20 mL of dichloromethane which was discarded. The sample extract was loaded onto the column and the eluate was collected in a clean 250-mL round-bottom flask. The sample bottle was rinsed with 3 x 2-mL portions of dichloromethane which were added to the column. Finally, the column was eluted with 100 mL of dichloromethane which was also collected in the 250-mL flask.

The sample extracts were evaporated to approximately 2 mL using a rotary evaporator with the assistance of a gentle vacuum and a warm water bath set to 35 °C. The extracts were quantitatively transferred to 15 mL centrifuge tubes. The extracts were gently evaporated just to dryness using a gentle stream of nitrogen gas. The residue was reconstituted in 5 mL of hexane. A 3-mL aliquot of concentrated sulphuric acid was added to each tube which was then sealed with Teflon-lined caps and shaken vigorously for 30 sec. Centrifugation at 2500 r.p.m. for 5 min was used to break up the resulting emulsions. The upper hexane layer was quantitatively removed with a disposable glass pipette and dried by passing it through a disposable Pasteur pipette plugged with glass wool and 3-cm of acidified sodium sulphate. The final acid washing step was found to be necessary to remove the remaining waxy components.

The extract volumes were reduced just to dryness using nitrogen gas. A 200-µL aliquot of TFAA was added to each tube and the contents were vortexed at high speed for 10 sec. The tubes were sealed with Teflon-lined screwcaps and placed in a hot water bath (75 °C) for 1 hr. The derivatized extracts were evaporated just to dryness using nitrogen gas and were reconstituted in 200 µL of toluene.

Analyses were performed using a GC-MS system which consisted of a Carlo Erba 8000 GC directly interfaced to a Fisons MD800 quadrupole mass spectrometer via a heated capillary interface. A 15-m DB-5MS fused silica capillary GC column with an inner diameter of 0.25 mm and a stationary phase film thickness of 0.25 microns (J & W Scientific, Folsom, CA) was used for all analyses. A Fisons AS800 autosampler was programmed to inject 2 µL of each extract or standard solution into the split/splitless injection port. The split/splitless injector was operated in the splitless mode with the purge time set to 1 min. The GC oven temperature profile consisted of an initial temperature of 120 °C, held for 1 min, increased to 220 °C at 8 °C/min and finally to 300 °C at 20 °C/min. The final temperature was held for 10 min to ensure that all components had been eluted.

The MS was operated in the electron impact ionization mode with the electron energy set at 70 eV. Selected ion monitoring (SIM) was used in order to achieve the desired sensitivity. Five ions were selected for the SIM analysis; four corresponding to the naturally incurred PCP and one for the isotopically labelled surrogate standard. The ions which were selected are summarized in Table 1. In each mass-monitoring cycle, a dwell time of 80 msec was used for each ion with a mass scan width of ± 0.1 a.m.u.

Four criteria were required to positively identify PCP in a given pine needle extract:

1. A peak must appear at the same retention time as the $^{13}C_6$-PCP (± 0.02 min) in the reconstructed ion chromatograms (RICs) of all four ions monitored for naturally incurred PCP.

2. The RIC peaks of all four ions monitored for naturally incurred PCP must have a signal-to-noise ratio of greater than 3 to 1.
3. The relative ratio of the peak areas for m/z 334, 360, 362 and 364 for the pine needle extract must agree within ± 15% of the relative ratios obtained for a standard solution of PCP analyzed under identical conditions (see Table 1).
4. Reagent blanks processed with the samples must be demonstrated to be free of PCP.

Table 1. Ions monitored for GC-MS analysis of PCP.

| analyte | ion | m/z value* |
|---|---|---|
| PCP | $C_6{}^{35}Cl_4{}^{37}ClOCF_3{}^+$ | 334 (100) |
| | $C_6{}^{35}Cl_5O(CO)CF_3{}^+$ | 360 (61) |
| | $C_6{}^{35}Cl_4{}^{37}ClO(CO)CF_3{}^+$ | 362 (99) |
| | $C_6{}^{35}Cl_3{}^{37}Cl_2O(CO)CF_3{}^+$ | 364 (63) |
| $^{13}C_6$-PCP | $^{13}C_6{}^{35}Cl_3{}^{37}Cl_2O(CO)CF_3{}^+$ | 370 |

* Values in parentheses represent relative RIC peak areas based on average values taken from injection of 3 standards.

The technique of stable isotope dilution was used to calculate the concentration of PCP in the pine needle extracts. Since isotopically labelled $^{13}C_6$-PCP has virtually identical physical and chemical properties as PCP, it can therefore be assumed that the recovery of $^{13}C_6$-PCP will be the same as the recovery of PCP. Using known quantities of each compound, relative response factors based on the ratio of the peak areas for m/z 362 for unlabelled PCP and m/z 370 for $^{13}C_6$-PCP were calculated. By comparing response factors obtained for standard solutions and pine needle extracts, the concentration of PCP in the sample extract was accurately determined. Combining this information with the original sample weight, the concentration of PCP per gram of pine needle was calculated.

## RESULTS AND DISCUSSION

Pine needle samples were collected just north of La Ronge in the province of Saskatchewan (see map in figure 1). The town of La Ronge has a population of 2,400 people and is located on a major lake, Lac La Ronge. The town itself acts as a transportation hub for many different hunting and fishing lodges as well as

various mining operations located in the northern part of the province. Approximately 30 km north of La Ronge, the paved road is replaced by gravel roads leading to various fishing lodges and mining operations. Many of these roads and side roads are not maintained in the winter.

There were two utility pole storage sites located along the main gravel road which extended from the highway north of La Ronge. The two sites, located approximately 10 and 35 km south of Missinipe (no permanent residents; site of fishing/hunting lodges), were located on the west side of the main gravel road. Both sites were easily accessed by short gravel roads which were linked to the main road. Each site had several piles of chemically treated utility poles which were left sitting in an area that had been cleared of foliage and trees. Presumably these poles were intended for use in supporting electrical power transmission lines to various sites. Both clearings were surrounded by dense brush and trees including jack pine.

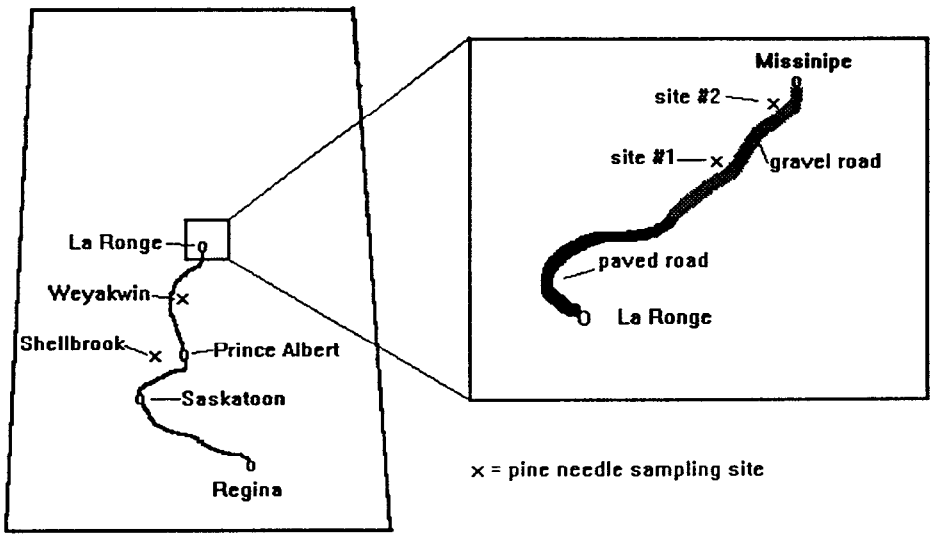


Figure 1. Map of Saskatchewan with sampling sites designated by “x”.

Pine needle samples were collected from each site. The results of the determination of PCP in these needle samples are summarized in Table 2. Pine needle samples were also collected from two sites located south of the pole storage sites. One was collected near the town of Weyakwin (population 140), approximately 100 km south of La Ronge. The other sample was collected at Shellbrook (population 1,300) approximately 50 km west of Prince Albert, which itself is about 240 km south of La Ronge. These two sites should provide an indication of the background concentration of PCP in pine needle samples.

Table 2. Determination of PCP in pine needle samples.

| description of site | PCP concentration (ng/g of pine needle) |
|---|---|
| pole storage site #1: 30 m north of poles | 570 |
| pole storage site #1: 30 m west of poles | 29 |
| pole storage site #1: 40 m west of poles | 60 |
| pole storage site #2: 30 m north of poles | 320 |
| pole storage site #2: 30 m south of poles | 77 |
| near Weyakwin (100 km south of La Ronge) | < 0.5 |
| near Shellbrook (50 km west of Prince Albert) | < 0.5 |

PCP concentrations in pine needle samples collected from trees immediately surrounding the pole storage sites were found to range from 29 to 570 ng/g of needle. By contrast, both of the pine needle samples collected at locations well removed from the pole storage sites were below 0.5 ng/g. This is consistent with our previous study where the levels of PCP in pine needle samples collected at random sites (i.e., not close to any known source of PCP emission) were found to range from 0.44 to 2.1 ng/g (Thompson 1995b). Clearly there were much higher levels of PCP present in the ambient atmosphere surrounding the pole storage sites. This was expected since a noticeable chemical odour was present in the air during the interval of sampling.

Further work is warranted to investigate the use of pine needles to monitor the rate at which the level of contamination declines with distance from the point source. This could be accomplished by sampling pine needles at concentric locations surrounding the pole storage site. In other words, collect samples at known intervals (for example, 25 m, 50 m, 75 m, etc.) directly north, south, east, and west of the storage site. In this manner the extent of contamination of the surrounding environment could be determined.

Worthy of mention are two modifications which were made in our sample preparation procedure. Firstly, instead of performing multiple acid washes of the initial sample extract, an acidified silica gel cleanup step was incorporated. It was found that this step was less labour intensive and not as hazardous in nature. The initial acid wash had sometimes resulted in a vigorous reaction with the components in the organic extract. The second modification to the method involved the use of TFAA as a derivatizing reagent instead of diazomethane. The precursor compound used for the synthesis of diazomethane is highly toxic. Also,

there are various hazards involved in the production and use of diazomethane. An additional benefit of utilizing TFAA was the fact that the resulting derivative yields ions with larger m/z values in the MS analysis. This helps to reduce the potential for interference by co-extracted compounds.

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