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Pentachlorophenol Levels in Human Urine

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Pentachlorophenol is perhaps one of the most persistent and widespread pollutants in existence today. The ubiquitous nature of this molecule and its toxicological properties have aroused the interest of numerous researchers in diverse areas of environmental chemistry, occupational health and safety, and environmental and occupational medicine. Unfortunately, due to the unavailability of data indicative of "normal" or background levels of PCP exposure in the general population, researchers have encountered difficulty assessing long-term toxicological effects. It is desirable to be able to determine the minimum level of long-term exposure which will result in an adverse effect to human health.

There have been a limited number of studies examining PCP levels in the urine of non-occupationally exposed individuals (Murphy et al. 1983; Hill et al. 1989; Holler et al. 1989; Thompson and Treble 1994). In continuation of previous work carried out at our laboratory, we have analyzed a series of urine samples which were collected in the middle of winter as opposed to early in the fall. This work will provide further information regarding background levels of PCP and also determine whether or not there is potentially seasonal variation.

MATERIALS AND METHODS

Due to the potentially infectious nature of the specimens being analyzed, it is essential that this type of analysis only be performed by specially trained laboratory staff in a properly equipped clinical laboratory.

The precursor compound used for the synthesis of diazomethane is suspected to be both a potent carcinogen and mutagen. Diazomethane itself is toxic and may potentially explode. Therefore appropriate measures must be taken in exercising the necessary precautions during

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the derivatization step.

Neat pentachlorophenol, greater than 98% purity, was obtained from BDH Chemicals Canada Limited (Toronto, Ontario). Carbon-13 isotopically labelled pentachlorophenol ($^{13}C_6$-PCP) having a purity of 99% was purchased from Cambridge Isotope Laboratories (Woburn, MA). Glass distilled petroleum ether, diethyl ether and acetone (all suitable for residue analysis) were obtained from BDH Chemicals Canada Limited. Sulfuric acid (ultra pure reagent grade) was acquired from J.T. Baker Inc. (Phillipsburg, NJ). 1-Methyl-3-nitro-1-nitrosoguanidine (MNNG) used for the synthesis of diazomethane was purchased from the Aldrich Chemical Company (St. Louis, MO).

A 10 mL aliquot of each urine sample was pipetted into a 15 mL glass centrifuge tube. All samples were fortified with 50 microliters of a solution containing 1 nanogram of $^{13}C_6$-PCP per microliter of acetone. Prior to extraction, the samples were hydrolyzed with 200 microliters of concentrated sulfuric acid. The hydrolysis step is necessary to ensure complete recovery of naturally incurred PCP (Edgerton and Moseman 1979).

A 3 mL portion of petroleum ether was pipetted into each centrifuge tube. The tubes were tightly sealed with Teflon-lined caps and gently mixed for half an hour using a rocking platform. After mixing, the resulting emulsions were eliminated by centrifuging the samples for 10 minutes at 2500 r.p.m. The organic layer was removed and dried by passing it through a disposable glass pipette packed with a 1 inch layer of acidified sodium sulphate. The dried extracts were collected in centrifuge tubes. The extraction procedure was repeated with two additional 3 mL aliquots of petroleum ether with the extracts were dried and combined.

The extract volumes were reduced to approximately 1 mL using a gentle stream of ultrahigh purity nitrogen gas. All solutions were derivatized with a freshly prepared solution of diazomethane in diethyl ether. After allowing the derivatized extracts to sit in a fumehood for half an hour (to permit excess diazomethane to dissipate), the solutions were gently blown just to dryness. The residues were reconstituted with 100 microliters of a solution of 200 picograms of 2,3,5,6-tetrachloroxylene in toluene. The sole purpose of the tetrachloroxylene is for monitoring the between run performance of the GC-MS system.

All sample extracts were analyzed using a Fisons MD800 gas chromatograph-mass spectrometer system. The Carlo Erba 8000 GC was directly interfaced to the Fisons quadrupole mass spectrometer via a heated capillary interface. The GC was equipped with a splitless

injection port and a 15 metre DB-5MS fused silica capillary column (0.25 mm i.d.) coated with a 0.25 micron thick film of stationary phase (J & W Scientific, Folsom, CA). Two microliters of each extract were injected into the GC-MS using a Fisons AS800 autosampler. The oven temperature program consisted of an initial temperature of 120 °C held for 2 minutes and then ramped to 220 °C at 8 °C/min and finally to 300 °C at 20 °C/min. The final oven temperature was held for 10 minutes. A 1 minute stabilization was employed to ensure that the system had reached proper temperature prior to the commencement of the next analytical run.

All mass spectral data were obtained with the instrument operated in the electron impact ionization mode. The electron energy was set to 70 eV for all analyses and the source temperature was maintained at 200 °C. The quadrupole mass spectrometer was operated in the selected ion monitoring (SIM) mode where four ions were monitored for the naturally incurred pentachlorophenol (263, 265, 278 and 280) and two ions were chosen for the isotopically labelled $^{13}C_6$-PCP (288 and 290). Masses 242 and 244 were selected for the measurement of the internal standard, tetrachloroxylene. A dwell time of 80 milliseconds and a mass scan width of ± 0.1 a.m.u. was used for each ion monitored.

In order to confirm the presence of pentachlorophenol in a given urine extract, the following criteria must be satisfied:

1. A peak must appear at the same retention time as the $^{13}C_6$-PCP (± 0.02 minutes) in the reconstructed ion chromatograms of all four ions monitored for native PCP. The peaks must have a signal-to-noise ratio of greater than 5 to 1.

2. The relative ratio of the peak areas for m/z 263, 265, 278 and 280 for the urine extract must agree within ± 15% of the relative ratios obtained for a standard solution of PCP analyzed under identical conditions.

3. Reagent blanks processed with the samples must be free of PCP contamination.

The level of pentachlorophenol in each urine sample was determined using the technique of stable isotope dilution. $^{13}C_6$-PCP has virtually identical physical and chemical properties as PCP and therefore it can be assumed that the recovery of $^{13}C_6$-PCP will be the same as the recovery of PCP. This permits the most accurate determination of the naturally incurred PCP. Relative response factors based on the ratio of the peak areas for mass 280 for native PCP and 288 for $^{13}C_6$-PCP were used for all subsequent calculations.

RESULTS AND DISCUSSION

A total of 38 randomly sampled urine specimens were analyzed for PCP. As in our previous study (Thompson and Treble 1994), 100 percent of the samples analyzed were found to contain quantifiable concentrations of PCP. A comparison of the results obtained in this study and the previous one is contained within table 1. The difference in the distribution of urinary PCP levels for the two studies is illustrated in figure 1.

Table 1. Summary of analytical results.

	STUDY I	STUDY II
Date of collection/analysis	Sept. 1992	Jan. 1995
Number of males	50	17
Number of females	37	21
Minimum age (years)	4	6
Maximum age (years)	86	87
Average age (years)	50	58
Median age (years)	58	62
Method detection limit (ng/mL)	0.2	0.05
Minimum level of PCP in urines analyzed (ng/mL)	0.5	0.1
Maximum level of PCP in urines analyzed (ng/mL)	9.1	3.6
Median urinary PCP concentration (ng/mL)	1.3	0.5
Average urinary PCP concentration (ng/mL)	1.6	0.9

Based on these results, it would appear that there is a slight difference in the average levels of PCP found in the urines analyzed. Approximately half of the urine samples analyzed in this second study had PCP concentrations less than or equal to the lowest level detected in all of the urines analyzed in the previous study which was carried out in our laboratory. While a smaller number of samples were analyzed in this study, the range of subject ages were virtually identical and therefore a reasonable cross-section has been taken. The most likely reason for the difference in the average and median urinary PCP concentrations may be the difference in the time of year during which the samples were collected. In our previous study the samples were collected in early autumn while the samples analyzed in the current study were collected in early January.

While the food chain is generally acknowledged as being the major route of exposure to PCP, there is also potentially significant exposure via

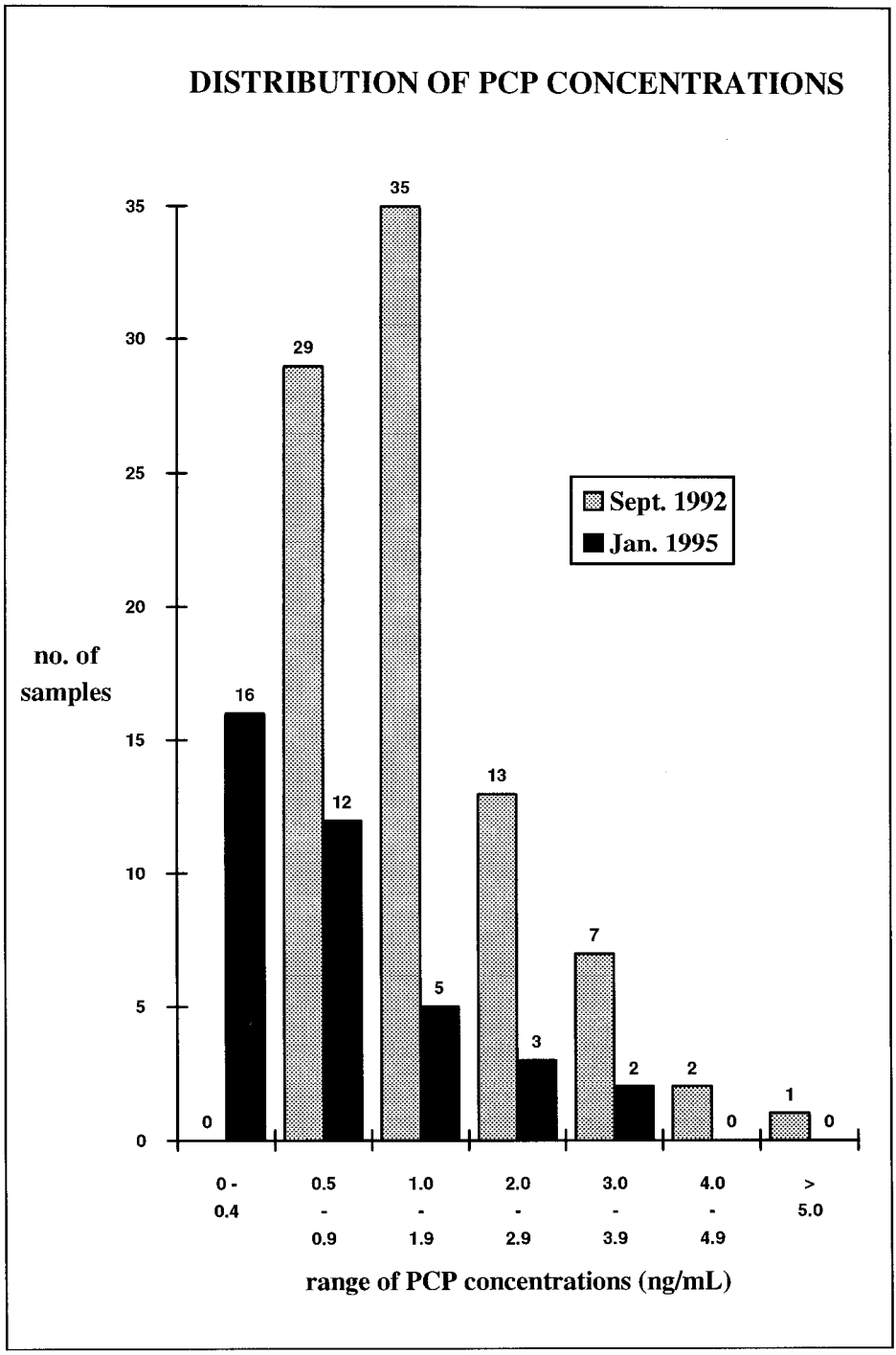

Figure 1. Distribution of PCP concentrations from two studies.

inhalation. A recent study by Waite (1995) reported the detection of PCP in ambient air samples collected in Regina, Prince Albert National Park and Yellowknife.

As in our previous study of Saskatchewan residents (Thompson and Treble 1994), the range of urinary PCP concentrations were in the low ng/mL range. These levels are on average lower than the levels typically reported in studies performed in the United States (Murphy et al. 1983; Hill et al. 1989; Holler et al. 1989). In all of the previously mentioned studies, the urinary PCP concentrations have been determined on a study of “normal” healthy individuals.

The two studies performed by our laboratory do not indicate that there is any general trend in urinary PCP levels based on either the sex or age of the subjects. However, in comparing the two sets of data, one immediately questions the reason why the second set appears to have somewhat lower PCP levels than the first. Presumably the exposure to PCP via atmospheric transport and inhalation would vary considerably from one season to another. It is quite likely that in colder climates, the rate of PCP exposure would be reduced due to the decreased level of PCP in the air.

In order to determine whether or not there is a seasonal variation in urinary PCP levels, it would be necessary to monitor a controlled group of individuals over a lengthy period of time. This would be required in order to rule out major differences in dietary intake which is possible if urine samples from different groups of people are used in each discreet sampling subset.

The fact that PCP was found in all of the ambient air samples analyzed in a recent study by Environment Canada (Waite 1995) coupled with the 100 percent detection of PCP in 125 urine samples indicates a need for further investigation of inhalation as a route of exposure to this compound. It is entirely possible that this route of exposure is more significant than currently recognized.

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