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Preliminary Results of a Survey of Pentachlorophenol Levels in Human Urine

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Pentachlorophenol (PCP) has been extensively manufactured and utilized worldwide for about half a century. It has been used predominantly in commercial and domestic wood preservation and protection applications. Although the use of PCP has been restricted in recent years, its persistence has resulted in its widespread existence as an environmental contaminant.

Very little information is available regarding the long-term toxic effects of pentachlorophenol upon human health. The widespread presence of PCP in the food chain has resulted in the major exposure route for humans. Previous studies have shown that PCP is present in the body fluids and tissues of humans who are not occupationally exposed to this chemical (Murphy et al. 1983; Gebefugi and Korte 1983; Williams et al. 1984; Geyer et al. 1987; Hill et al. 1989). With the increasing demand for human health indicators of the state of the environment, our laboratory has decided to investigate the presence of residual levels of pentachlorophenol in human urine.

MATERIALS AND METHODS

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 toluene, hexane 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 5 mL aliquot of each urine sample was pipetted into separate glass centrifuge tubes. 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 sulfuric acid over a period of an hour. The hydrolysis step is necessary to ensure complete recovery of native PCP present in the original urine sample (Edgerton and Moseman 1979).

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A 1 mL portion of toluene 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 aqueous and organic phases were allowed to separate. Emulsified samples were centrifuged at approximately 2500 r.p.m. for the required time necessary to allow complete separation of the phases (5 to 8 minutes).

Exactly 500 microliters of each toluene extract was transferred to clean centrifuge tubes. A fresh solution of diazomethane reagent (in hexane) was prepared daily. An aliquot of the diazomethane-hexane reagent was added to each centrifuge tube in sufficient volume so that the yellow color of the reagent solution persisted. Samples were allowed to sit for at least one hour in a fumehood in order that excess diazomethane would dissipate.

Samples were evaporated just to dryness under a gentle stream of gas with the assistance of a warm water bath. The residues were finally reconstituted with 50 microliters of toluene.

All sample extracts were analyzed using a VG TRIO 1000 GC-MS system in which the Hewlett-Packard 5890 Series II gas chromatograph was directly interfaced to a VG quadrupole mass spectrometer. Two microliters of each extract were injected into the GC-MS using an HP7673 autosampler. The split/splitless injection port was operated in the splitless mode and maintained at a temperature of 260 °C. All samples were chromatographed on a 30 meter DB-5MS fused silica capillary column having an internal diameter of 0.25 mm and a stationary phase film thickness of 0.25 microns (J & W Scientific, Folsom, CA). 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. The direct capillary interface linking the GC to the mass spectrometer was maintained at 300 °C throughout the entire run.

The mass spectrometer was operated in the electron impact ionization mode with an electron energy of 70 eV and a source temperature of 200 °C. In order to achieve the desired sensitivity, all analyses were carried out using selected ion monitoring (SIM). Three ions were monitored for native pentachlorophenol (263, 278 and 280) while two ions were monitored for the $^{13}C_6$-PCP (288 and 290). A dwell time of 50 milliseconds and a mass scan width of $\pm$ 0.25 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 ($\pm$ 0.02 minutes) in the reconstructed ion chromatograms of all three 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, 278 and 280 for the urine extract must agree within $\pm$ 15% of the relative ratios obtained for a standard solution of PCP.

The level of pentachlorophenol in each urine sample was determined using the technique of stable isotope dilution. Standard solutions containing 10, 50 and 250 picograms per microliter (pg/uL) of PCP and 250 pg/uL of $^{13}C_6$-PCP were used to establish a calibration

curve of relative response factors versus PCP concentration. The relative response factor is the ratio of the peak areas for mass 280 for native PCP and 288 for $^{13}C_6$-PCP. As $^{13}C_6$-PCP has virtually identical physical and chemical properties as PCP, it can be assumed that the recovery of $^{13}C_6$-PCP will be the same as the recovery of PCP.

RESULTS AND DISCUSSION

Figure 1 gives an example of a typical urine extract which tested positive for pentachlorophenol in accordance with the criteria listed in the experimental section. The component suspected to be PCP has peaks in all three mass chromatograms within $\pm$ 0.02 minutes of the peak due to the isotopically labelled $^{13}C_6$-PCP which was added to each sample. The relative ratios of the integrated peak areas in the mass chromatograms for m/z 263, 278 and 280 in the PCP standard solution (figure 1a) are 63:61:100. The relative ratios of the corresponding peaks in the mass chromatograms of the urine extract (figure 1b) are 70:63:100. It is evident that the aforementioned criteria are satisfied for the positive identification of pentachlorophenol in human urine.

Urine samples were selected at random from a large pool of samples submitted to our laboratory. A total of 87 urine samples were analyzed. Trace levels of pentachlorophenol were found in 100% percent of the samples which were analyzed. The results of the analyses are summarized in table 1 and figure 2.

Table 1. Summary of analytical results (n = 87).

	PCP CONCENTRATION (ng/mL)
Method detection limit	0.2
Minimum quantity detected in samples analyzed	0.5
Maximum quantity detected in samples analyzed	9.1
Median concentration of all samples analyzed	1.3
Average concentration of all samples analyzed	1.6

In previous studies examining the background levels of pentachlorophenol in a population not known to have direct exposure to pentachlorophenol via occupation, PCP levels were found to range in the low nanograms per milliliter of urine (Murphy et al. 1983; Hill et al. 1989). In one study carried out as part of the National Health and Nutrition Examination Survey (NHANES), 79% of the 6000 samples analyzed were found to contain detectable levels of PCP. The method detection limit for their study was 2 ng/mL. In a second study, 100% of the 197 samples analyzed had detectable quantities of PCP based on a method detection limit of 1 ng/mL (Hill et al. 1989). It should be noted that all 197 samples had PCP concentrations greater than the 2 ng/mL detection limit reported in the previous NHANES study (Holler et al. 1989). Preliminary results of our study revealed pentachlorophenol levels ranging from 0.5 to 9.1 ng/mL, with the bulk of the urine samples analyzed containing less than 2 ng/mL. With a detection limit of 0.2 ng/mL, all 87 samples were found to contain detectable levels of PCP. If our method detection limit was 1 or 2 ng/mL, the percent of urine samples which would have tested positive for PCP would have been 67% and 26%, respectively. Clearly the improvement in detection limit results in additional information regarding background residual levels of PCP in human urine.

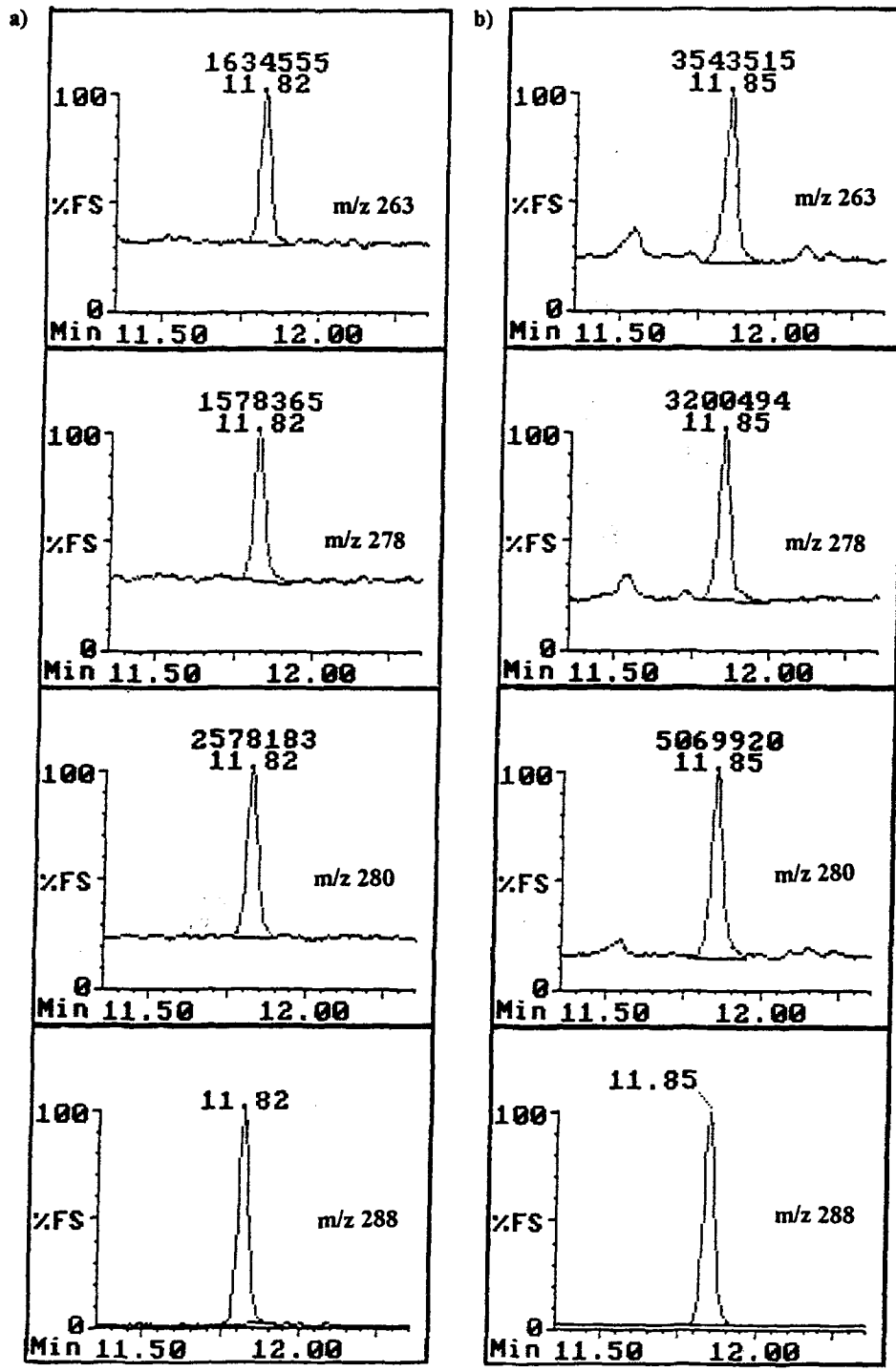

Figure 1. Mass chromatograms for: a) pentachlorophenol standard solution and b) urine extract. See text for GC-MS operating parameters.

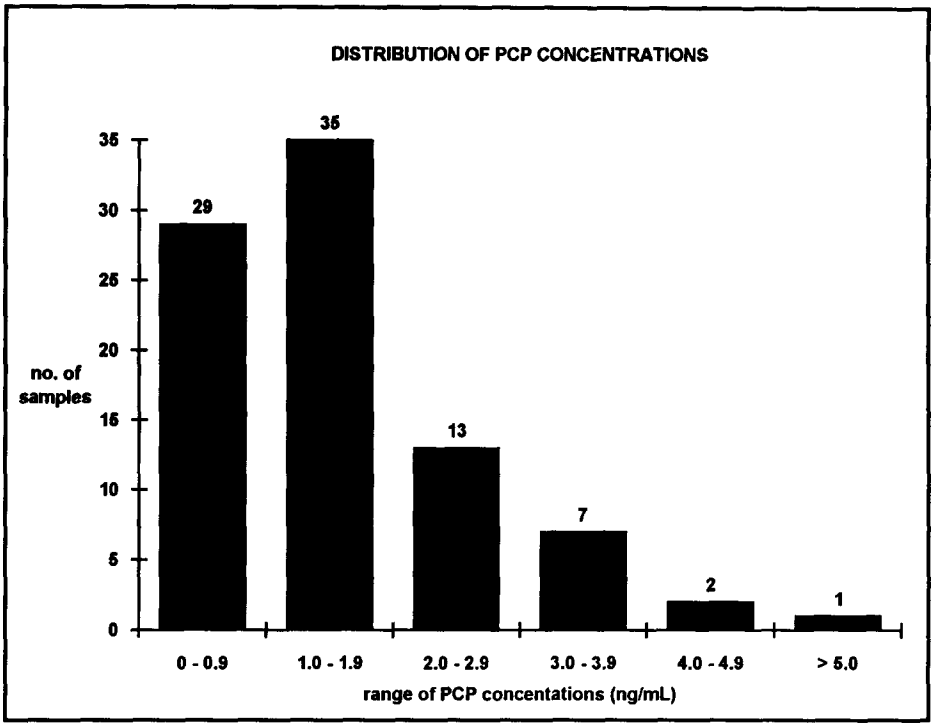

Figure 2. Distribution of pentachlorophenol levels in human urine (n = 87).

Since the samples were selected completely at random, a cross-section of the province's population was represented. A summary of the characteristics (i.e. age and sex) of the subjects is given in table 2. Approximately 50% of the samples analyzed were collected from individuals who reside in rural areas of the province. It is recognized that compilation of much more data would be necessary to lend any statistical validity in determining differences for PCP levels found in urban and rural residents. Our future studies will include an attempt to include a larger sample population.

While there is not enough data available at this time to establish any links between PCP concentration and age, sex or region of residence, the data does give a reasonable estimation of the background levels across the province. Approximately 74% of the donors tested had background urine levels between 0.5 and 2.0 ng/mL. Both the average and the median urine PCP concentrations of the 87 samples analyzed were less than 2 ng/mL.

The studies previously performed in the United States found background levels of PCP in human urine which were slightly higher than those found in this study. The differences in the levels found may be the result of the differences in the amounts of PCP-containing products used in the United States versus Saskatchewan. It would be interesting to compare the background PCP levels in human urine between a region which is heavily dependent upon the lumber industry (such as British Columbia or the northwestern United States) and one in which little or no lumber industry exists (such as Saskatchewan or midwestern states).

Table 2. Summary of subject ages and sex (n = 87).

Number of males	50
Number of females	37
Minimum age	4 years
Maximum age	86 years
Average age	50 years
Median age	58 years

The high rate of incidence of detectable quantitities of PCP in human urine as reported in this and previous studies confirms the widespread existence of PCP throughout the environment. Further investigation of human exposure to PCP in Saskatchewan is planned, however our laboratory is currently evaluating a solid phase extraction procedure for the isolation of PCP from urine.

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