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# Determination of Polyhalogenated Phenolic Compounds in Drinking Water, Human Blood Serum and Adipose Tissue

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The blood serum of 58 white female residents of Dade County, Florida has been analyzed for the presence of eight halogenated phenols: 2,4-dichlorophenol (2,4-DCP); 2,3,5-trichlorophenol (2,3,5-TCP); 2,4,5-trichlorophenol (2,4,5-TCP); 2,4,6-trichlorophenol (2,4,6-TCP); 2,5-dichloro-4-bromophenol (2,5-DC-4-BP); 2,3,4,5-tetrachlorophenol (2,3,4,5-TTCP); 2,3,4,6-tetrachlorophenol (2,3,4,6-TTCP) and pentachlorophenol (PCP). Each resident had been following her respective drinking water usage pattern for at least the past 5 years. Thirty-three of the individuals were using chlorinated municipal drinking water; the other 25 were ingesting mostly non-chlorinated water from privately owned wells.

The drinking water of 12 of the subjects who were using chlorinated water and 10 of the subjects who were using non-chlorinated water was analyzed for the same group of compounds.

Additionally, ten samples of human adipose tissue, taken at autopsy, were analyzed for halogenated phenol content.

All three analytical methods employed were based in part on previously published procedures, but each was individually modified to accommodate the use of smaller sample sizes, modified work-up steps or different derivatizing reagents.

## EXPERIMENTAL

Apparatus. A gas chromatograph (GC) equipped with a $^{3}H$ electron capture detector (ECD) and a 1.8 m x 4 mm ID glass column packed with 4% SE-30 + 6% QF-1 on 80-100 mesh Supelcoport® was used. A nitrogen carrier gas flow-rate of 30 mL/min and inlet column and detector temperatures of 215, 165, and 210°C, respectively, were used.

Solvents and Reagents. All solvents were Nanograde® quality. The deionized water was extracted twice with benzene. N-ethyl-N'-nitro-N-nitrosoguanidine was used to prepare the ethylating reagent according to STANLEY (1966). The silica gel (Woelm, activity grade I) was prepared according to SHAFIK et al. (1973). Authentic reference compounds of 95+% purity were furnished by Mr. J. F. Thompson, HERL, USEPA, Research Triangle Park, North Carolina.

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Water and Biologic Samples. All samples were collected with and stored in clean containers removed from any source of halogenated phenol.

Drinking water samples were collected from kitchen water faucets on the same days blood samples were drawn. Each 1-l sample, contained in a glass bottle with a ground-glass stopper, was stored at $4^0$ until analyzed.

Blood samples were collected using disposable syringes, placed in Teflon®-lined screw-capped test tubes, and allowed to clot. After centrifugation, the serum was drawn off using a hexane washed disposable pipet and stored in a similar test tube at $4^0$ until analyzed.

Adipose tissue samples were obtained from a county medical examiner's laboratory. The autopsy samples were, in each instance, removed from near the anterior abdominal wall, placed in liquid scintillation vials, sealed with Teflon®-lined screw caps and frozen until analyzed.

Procedure for Drinking Water. The pH of 100 mL of water contained in a separatory funnel is adjusted to 1.5 by adding 1 mL of conc. HCl. After the addition of 10 mL of $CH_2Cl_2$, the mixture is shaken vigorously and allowed to separate. Meanwhile, a small pad of glass wool is placed at the bottom of a 2.2-cm x 30-cm Pyrex® glass filtering column, and $Na_2SO_4$ is added to a depth of 2 cm. The tip of the column is positioned over a 25-mL concentrator tube, and the lower (organic) layer is drained through the $Na_2SO_4$. The aqueous layer is re-extracted with 10 mL of $CH_2Cl_2$. The second organic layer is also passed through the $Na_2SO_4$ and into the same concentrator tube. The filtering column is rinsed with 5 mL of $CH_2Cl_2$ which is similarly collected in the tube. Ten drops of "keeper" solution (1% USP paraffin oil in hexane), which suppresses sample loss during the concentrating steps, is added to the tube and the sample is concentrated to 0.2 mL by using a nitrogen-stream evaporator. Two mL of hexane are then added, and the sample is re-concentrated to 0.5 mL. Another 2 mL of hexane are added, and sufficient freshly prepared diazoethane solution is added to give a persistent yellow coloration to the mixture. After allowing the solution to remain at room temperature for 20 min, nitrogen is bubbled through the solution and it is concentrated to 0.5 mL. Finally the sample is passed through a silica-gel column according to the method of SHAFIK et al. (1973). The first fraction is concentrated to 1 mL and analyzed by ECD-GC.

Recovery studies are performed by adding known amounts of reference standards to 100 mL of deionized water devoid of the halogenated phenols for which the analyses are being made, then running the fortified sample through the entire procedure. If the water control sample contains any halogenated phenol, the amount is subtracted from the appropriate value obtained during the recovery studies.

Procedure for Blood Serum. To 1 mL of human serum contained in a round-bottomed tube is added one drop of conc. HCl to adjust the pH to 1. Next are added 2 mL of 2% aqueous $Na_2SO_4$ and 6 mL of $CH_2Cl_2$. The mixture is rotated in a Roto-rack® at 50 rpm for 2 h, then centrifuged about 5 min at 2000 rpm. Three mL of the organic layer are transferred to a clean 15-mL centrifuge tube and 10 drops of "keeper" solution are added. The mixture is concentrated to 0.5 mL, about 0.7 mL of iso-octane is added, and the sample is re-concentrated to 0.5 mL. The 0.5 mL of freshly prepared diazoethane solution is added, and after the mixture has been allowed to stand at room temperature for 20 min, any excess reagent is removed by a stream of dry nitrogen. The analytical sample is obtained according to the silica-gel procedure of SHAFIK et al. (1973) for the determination of phenolic compounds in urine. The first fraction is collected, concentrated to 5 mL and analyzed by ECD-GC.

Recovery studies are performed by adding known amounts of reference standards to 1 mL of blood serum, then running the fortified sample through the entire procedure. If the serum control sample contains any halogenated phenol, the amount is subtracted from the appropriate value obtained during the recovery studies.

Procedure for Adipose Tissue. One mL of hexane is added to 200 mg of tissue contained in a #22 Dual® tissue grinder and a finely divided slurry is formed by extensive use of the plunger. Then the slurry is shaken for about a minute using a Vortex® mixer. After the addition of 0.5 mL of 10% aqueous NaOH, the mixture is shaken for about a minute, then centrifuged to separate the layers. The top (hexane) layer is aspirated and discarded. The aqueous layer is re-extracted twice with 1.5 mL of hexane; in both instances the hexane is discarded. Then 0.5 mL of conc. HCl is added and the aqueous solution shaken. When the sample has cooled to room temperature, 1.5 mL of diethyl ether are added and the mixture is shaken for about 0.5 min on a Vortex® mixer. After the layers have separated, the top (ether) layer is transferred to a clean 13-mL centrifuge tube using a disposable pipet. The extraction with ether is repeated twice, each extract being transferred to the same 13-mL tube. In a ventilated hood, 2 mL of freshly prepared diazoethane reagent are added, and the reaction mixture is allowed to stand at room temperature for 20 min. Excess reagent is removed by bubbling dry nitrogen through the solution. From this point on, the method of SHAFIK et al. (1973) is followed. The first fraction is collected, concentrated to 5 mL and analyzed by ECD-GC.

Recovery studies are performed by adding known amounts of reference standards to 200 mg of tissue, then running the fortified sample through the entire procedure. If the fat control sample contains a trace of any halogenated phenol, the amount is subtracted from the appropriate value obtained during the recovery studies.

## RESULTS AND DISCUSSION

Each sample was analyzed for the eight previously mentioned halogenated phenols. Although only pentachlorophenol was detected in the water and biologic samples analyzed, this entire group of halogenated phenols is of special interest to residents of southern Florida because of the high usage rate of two pesticides: lindane and bromophos {O-(4-bromo-2,5-dichlorophenyl) O,O-dimethylphosphorothioate}. Although not yet reported for humans, laboratory rats treated perorally with γ-HCH excrete 2,3,5-TCP, 2,4,5-TCP, 2,4,6-TCP, 2,3,4,5-TTCP and 2,3,4,6-TTCP (FREAL & CHADWICK 1973). ROWLANDS (1966) has reported that 2,5-DC-4-BP is a metabolite of bromophos.

Pentachlorophenol and/or its sodium salt has several industrial and agricultural uses. It is often used as a wood perservative in locations where infestation by insects is problematic, as a household treatment for termites, as a mildew retardant and disinfectant, and as a contact herbicide in agricultural areas. Heavy usage of PCP occurs in Hawaii, and routine blood levels for its citizens have been reported by BEVENUE et al. (1968) to be in the range of 0.07 to 5.2 ppm. In their studies of workers regularly and directly exposed to PCP, plasma levels ranged from 0.99 to 9.1 ppm. The lowest values were those obtained for office workers of wood-treatment plants. BEVENUE et al. (1968) also reported that the blood cell fraction contains only about one percent of the PCP present in whole blood samples. For a recent review of the chemistry, pharmacology and environmental toxicology of pentachlorophenol, the reader is referred to RAO et al. (1978).

Water and Serum Analysis Parameters. Table 1 contains averaged recovery, sensitivity and detectability data from duplicate analyses of three 100-mL samples of benzene extracted, deionized water. The first sample served as the control. To the second was added 450 ng 2,3,4,6-TTCP. To the third sample was added 4.69 µg 2,4-DCP, 348 ng 2,4,5-TCP, 200 ng 2,5-DC-4-BP and 117 ng PCP. (This grouping of authentic reference materials was adopted because 2,3,5-TCP and 2,4,5-TCP as well as 2,5-DC-4-BP and 2,3,4,6-TTCP co-elute under the analytical parameters employed. Inasmuch as only PCP was detected, a search for different parameters or GC columns was not made.) A trace of PCP was detected in the control sample; before the recovery data were calculated, its recorder response was subtracted from the PCP response obtained from the fortified sample. No other interfering component or halogenated phenol was found at a concentration exceeding its corresponding level of detectability.

TABLE 3
Drinking Water and Blood Serum Levels of
Pentachlorophenol in Dade County, Florida

| Users of City Water | | Users of Well Water | |
|---|---|---|---|
| Water (ppt) | Serum (ppb) | Water (ppt) | Serum (ppb) |
| 128 | 15 | 110 | 67 |
| 120 | 30 | 60 | 29 |
| 60 | 10 | ND | 39 |
| ND* | 23 | ND | 26 |
| 170 | 55 | 50 | 17 |
| 340 | 17 | 40 | 71 |
| 100 | 27 | 44 | 20 |
| 93 | 26 | 50 | 28 |
| 90 | 98 | 40 | 11 |
| ND | 22 | 42 | 42 |
| 34 | 17 | | 61 |
| 37 | 17 | | 33 |
| | 29 | | 36 |
| | 31 | | 118 |
| | 23 | | 26 |
| | 15 | | 69 |
| | 20 | | 41 |
| | 18 | | 42 |
| | 68 | | 110 |
| | 25 | | 18 |
| | 25 | | 42 |
| | 51 | | 99 |
| | 15 | | 53 |
| | 25 | | 64 |
| | 19 | | 50 |
| | 12 | | |
| | 20 | | |
| | 54 | | |
| | 20 | | |
| | 27 | | |
| | 16 | | |
| | 51 | | |
| | 40 | | |

*ND = not detected

Representative Water and Serum Analyses. Table 3 contains both the drinking water and blood serum levels of PCP for the 58 participants included in this investigation. PCP levels were about twice as high in municipal drinking water as in well water. The municipal water levels ranged from <30 to 340 ppt with an average value of 98 ppt; well water levels ranged from <30 to 110 ppt with an average value of 44 ppt. Blood serum levels of PCP were not dramatically different for the two groups. Participants who drank municipal water had serum levels between 10 and 98 ppb; those who

drank mainly well water had serum levels between 11 and 120 ppb. Both ranges were significantly below those reported for citizens of Hawaii (BEVENUE et al. 1968).

Adipose Tissue Analysis Parameters. Table 4 contains averaged recovery, sensitivity and detectability data from duplicate analyses of three 200-mg samples of tissue. The first sample served as the control. To the second was added 450 ng 2,3,5-TCP, 162 ng 2,4,6-TCP, 188 ng 2,3,4,5-TTCP and 278 ng 2,3,4,6-TTCP. To the third sample was added 4.69 µg 2,4-DCP, 348 ng 2,4,5-TCP, 200 ng 2,5-DC-4-BP and 117 ng PCP. The fat control contained no PCP but did contain a component which had a retention time identical to that of 2,4,6-TCP. Before the recovery data were calculated, the recorder response of this component was subtracted from the 2,4,6-TCP response obtained from the spiked samples. No other halogenated phenol was detected in chromatograms of the control sample at a concentration exceeding its corresponding level of detectability.

TABLE 4
Percent Recovery, Detector Sensitivity and Limits of Detectability of Halogenated Phenols in Adipose Tissue

| Compound | Recovery (%) | Detector Sensitivity (pg) | Limit of Detectability (ppm) |
|---|---|---|---|
| 2,4-DCP | 101 | 469 | 4.69 |
| 2,3,5-TCP | 91 | 23 | 0.24 |
| 2,4,5-TCP | 97 | 32 | 0.32 |
| 2,4,6-TCP | 85 | 9 | 0.09 |
| 2,5-DC-4-BP | 98 | 15 | 0.15 |
| 2,3,4,5-TTCP | 90 | 18 | 0.19 |
| 2,3,4,6-TTCP | 84 | 9 | 0.09 |
| PCP | 91 | 13 | 0.14 |

Representative Adipose Tissue Analyses. Table 5 contains the adipose tissue levels of PCP obtained for the 10 necropsy samples included in this investigation. Values ranged from 10 to 80 ppb with an average value of only 23 ppb. These data correlate well with the findings of SHAFIK (1973) who reported PCP levels of 5 to 52 ppb with an average value of 25 ppb. Apparently PCP does not bioconcentrate in human adipose tissue to the same extent that DDT and DDE do. As a point of comparison, DAVIES & EDMUNDSON (1972) reported for the period 1965-1967 a range of 50-86 ppm for total DDT-derived material in necropsy adipose tissue obtained from white females of Dade County, Florida.

TABLE 1
Percent Recovery, Detector Sensitivity and Limits of Detectability of Halogenated Phenols in Water

| Compound | Recovery (%) | Detector Sensitivity (pg) | Limit of Detectability (ppb) |
|---|---|---|---|
| 2,4-DCP | 52 | 400 | 8.1 |
| 2,3,5-TCP | 79 | 22 | 0.4 |
| 2,4,5-TCP | 87 | 29 | 0.6 |
| 2,4,6-TCP | 73 | 8 | 0.2 |
| 2,5-DC-4-BP | 94 | 14 | 0.3 |
| 2,3,4,5-TTCP | 98 | 18 | 0.4 |
| 2,3,4,6-TTCP | 96 | 9 | 0.9 |
| PCP | 64 | 12 | 0.3 |

TABLE 2
Percent Recovery, Detector Sensitivity and Limits of Detectability of Halogenated Phenols in Blood Serum

| Compound | Recovery (%) | Detector Sensitivity (pg) | Limit of Detectability (ppm) |
|---|---|---|---|
| 2,4-DCP | 63 | 195 | 0.78 |
| 2,3,5-TCP | 76 | 24 | 0.10 |
| 2,4,5-TCP | 77 | 16 | 0.07 |
| 2,4,6-TCP | 89 | 8 | 0.03 |
| 2,5-DC-4-BP | 83 | 8 | 0.03 |
| 2,3,4,5-TTCP | 81 | 20 | 0.08 |
| 2,3,4,6-TTCP | 102 | 10 | 0.04 |
| PCP | 99 | 7 | 0.03 |

Table 2 contains similar data from duplicate analyses of three 1-mL samples of human blood serum. The first sample served as the control. To the second was added 450 ng 2,3,5-TCP, 162 ng 2,4,6-TCP, 188 ng 2,3,4,5-TTCP and 278 ng 2,3,4,6-TTCP. To the third sample was added 4.69 µg 2,4-DCP, 348 ng 2,4,5-TCP, 200 ng 2,5-DC-4-BP and 117 ng PCP The serum control contained a trace of PCP; before the recovery data were calculated, its recorder response was subtracted from the PCP response obtained from the fortified samples. No other halogenated phenol was detected in chromatograms of the control sample at a concentration exceeding its corresponding level of detectability.

TABLE 5
Necropsy Adipose Tissue Levels of
Pentachlorophenol in Dade County

| Sample | ppm | Sample | ppm |
|---|---|---|---|
| F-1 | 0.01 | F-6 | 0.02 |
| F-2 | 0.08 | F-7 | 0.01 |
| F-3 | 0.01 | F-8 | 0.02 |
| F-4 | 0.02 | F-9 | 0.01 |
| F-5 | 0.01 | F-10 | 0.04 |


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