Storage Stability of Chlorinated Phenols in Urine

The storage stability of chlorinated phenols in urine has been investigated. A decrease of greater than 40% was noted in urine fortified with known amounts of chlorophenols and analyzed over a 36-day period with repeated freezing and thawing. A sample storage technique is presented which allows for the stability of chlorophenols in urine for up to 40 days with little or no decomposition noted.

Because chlorinated phenols are metabolites of various pesticides (Menzie, 1969, 1974, 1978), the determination of chlorinated phenols in human urine can possibly be used as an index of exposure by agriculture workers, pesticide applicators, etc. These compounds also arise from other sources including the degradation or metabolism of various industrial chemicals. As a specific example, analysis of urine for 3,4-dichlorophenol could be used as an index of exposure to the herbicide diuron.

No reference to the stability of chlorinated phenols in urine has been found in the literature, although stability studies of other potentially degradable compounds (parathion; anilines; 2,4-D) have been made (Bristol, 1976; Comer et al., 1976; Ito et al., 1979; Lores et al., 1980).

It has been observed in this laboratory, during development of analytical methods for the determination of chlorinated phenols in urine (Edgerton and Moseman, 1979; Edgerton et al., 1979, 1980), that a drastic decrease in the levels of chlorinated phenols occurred in the analysis of urine samples over an extended time period for confirmation of analytical results.

For this reason, a study was initiated to determine the storage stability of chlorinated phenols in urine and to establish proper storage techniques to ensure the attainment of quantitative analytical results when samples are to be analyzed over an extended period of time.

MATERIALS AND METHODS

Apparatus. A Tracor Model 222 gas chromatograph equipped with a nickel-63 electron-capture detector was operated in the pulsed linearized mode. Borosilicate glass columns (1.8 m × 4 mm i.d. or 0.6 m × 4 mm i.d.) were packed with double support bonded diethylene glycol succinate (Edgerton and Moseman, 1980). The columns were operated at 180–210 °C with a 5% methane in argon carrier gas flow rate of 60–80 mL/min. The detector, inlet, and transfer line were maintained at 300, 225, and 220 °C, respectively.

Reagents and Materials. The following glassware was used: Chromaflex column, plain, Teflon stopcock, 250 × 10.5 mm i.d. (Kontes Catalog No. K-420280, Kontes Glass Co., Vineland, NJ 08360); Kuderna-Danish concentrator assembly (K-570000); 25-mL graduated tubes, size 2525 (K-570050); 15 × 125 mm screw-cap culture tube.

Chlorinated phenol reference standards were obtained from Aldrich Chemical Co. (Milwaukee, WI). Solutions of phenols in acetone were used for fortification.

All solvents were pesticide quality or the equivalent. Reagent materials (3 N HCl, 0.1 N NaOH, $NaHSO_3$, and deionized water) were extracted with hexane and toluene prior to use.

The macroreticular resin, XAD-4, was obtained from Rohm and Haas (Philadelphia, PA). The fines were removed by slurrying in methanol and decanting. The remaining beads were purified as described by Edgerton et al. (1980).

Analysis of Urine. Chlorinated phenols in urine were analyzed by the direct GC determination method of Edgerton et al. (1980). Briefly, 4 mL of first void, fresh urine was placed in a 15 × 125 mm screw-cap culture tube, acidified with concentrated HCl, and hydrolyzed for 1 h in a boiling water bath. The hydrolyzed urine was transferred to a XAD-4 column. After several aqueous washes, the chlorinated phenols were eluted with 10% 2-propanol in hexane. The column effluent was concentrated and analyzed by electron-capture gas chromatography.

Table I. Percent Recovery of Chlorinated Phenols in Urine—Entire Sample Frozen

	% recovery		
day of sampling[a]	2,4,6-trichlorophenol	2,3,5,6-tetrachlorophenol	pentachlorophenol
0	84, 83	88, 84	89, 89
5	81, 82	83, 87	81, 86
16	78	65	71
26	68	68	62
36	54, 60	51, 63	47, 58

[a] Duplicate analysis.

Table II. Actual Levels of Biologically Incorporated Chlorinated Phenols in Urine—Entire Sample Frozen

	levels, ng/mL			
day of sampling[a]	2,5-dichlorophenol	2,4,5-trichlorophenol	2,3,4,6-tetrachlorophenol	pentachlorophenol
0	76	6	27	29
5	72	6	22	24
13	23	4	1	18
34	11	<1	<1	11

[a] Duplicate analysis.

RESULTS AND DISCUSSION

Two first void, fresh (no more than 4 h old) human urine samples were fortified at 0.10 ppm with 2,4,6-trichlorophenol, 2,3,5,6-tetrachlorophenol, and pentachlorophenol. A sample aliquot was analyzed after fortification (day 0) and the remaining samples were places in a freezer at −4 °C. The entire samples were removed from the freezer on day 5, thawed, an aliquot was analyzed, and the remainder was refrozen. This same sampling procedure was followed on days 16, 26, and 36 for one sample and days 5 and 36 on the second fortified sample. The results of these analyses are shown in Table I.

From the results of Table I, it is apparent that thawing and refreezing of urine samples results in the loss of chlorinated phenols. From day 0 to day 5, no apparent loss takes place. Thawing of the sample on day 5, refreezing, and analyzing at a later date result in some decomposition.

Table II shows the results of a similiar loss study of urine containing residue levels of biologically incorporated chlorophenols. The sampling procedure followed the procedure discussed for sample 1, but only one sample was analyzed. Drastic decreases in the residue levels of all four chlorophenols are noted on days 13 and 34.

Table III. Percent Recovery of Chlorinated Phenols in Urine—Subdivided Samples

day of sampling[a]	% recovery		
	2,4,6-trichlorophenol	2,3,5,6-tetrachlorophenol	pentachlorophenol
0	85	89	91
6	83	89	90
17	79	86	87
26	81	87	89
36	83	83	85

[a] Duplicate analysis.

Table IV. Actual Levels of Biologically Incorporated Chlorinated Phenols in Urine—Subdivided Samples

day of sampling[a]	level, ng/mL			
	2,5-dichlorophenol	2,4,5-trichlorophenol	2,3,4,6-tetrachlorophenol	pentachlorophenol
0	51	3	18	31
5	54	3	20	27
13	47	4	16	26
34	50	2	18	30

[a] Duplicate analysis.

Table III lists results from first void, fresh human urine fortified at the same levels as sample 1, but which were subdivided into 5-mL sample aliquots and frozen. Individual aliquots were removed from the freezer, thawed, and analyzed on the designated days. The remaining subsamples were left in the freezer. This sample handling technique was applied to urine containing biologically incorporated chlorophenols, and the results are shown in Table IV.

These experiments demonstrate the importance of proper sampling and storage of urine samples when analyzing for chlorophenols over an extended period of time. Our laboratory now subdivides all urine samples into 5-mL aliquots when first receiving the sample so that repetitive analysis can be performed at later times. Little loss is note when the urine samples are subdivided in this manner before freezing and subsequently analyzed over a 40-day period.

CONCLUSION

A storage technique for the assurance of quantifiable analytical results for chlorinated phenols in urine has been presented. Conventional thawing, refreezing, and rethawing of an entire urine sample exhibited as much as a 40% decrease in the levels of chlorinated phenols in fortified and biologically incurred samples. Urine samples that were subdivided into individual sample bottles and kept frozen until analysis exhibited little or no decomposition over a 40-day period.

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2-Chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide, a New Herbicide

2-Chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide (DPX 4189), a novel chemical compound, controls a wide spectrum of weeds in greenhouse studies. It is especially effective as a selective herbicide at extremely low rates in both preemergence and postemergence treatments of broadleaf and certain grass weeds commonly found in a number of cereal crops. Data demonstrating this activity and selectivity are presented.

Novel *N*-(1,3,5-triazinylaminocarbonyl)benzenesulfonamides were recently reported to be highly herbidical by Levitt (1978) and Finnerty et al. (1979). In greenhouse studies, one of these compounds, 2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide (DPX 4189), provided control of a large number of weed species. It is particularly useful at low rates of application in both preemergence and postemer-

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