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Effect of Freezer Storage on Alkyl Phosphate Metabolites in Urine

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For the past two years our laboratory has been involved in the monitoring of alkyl phosphate metabolites of various organophosphorus insecticides in the urine of farm workers (KRAUS et al. 1977). Because of the large number of samples involved, the samples were stored frozen until analyzed. Recently, however, it was reported that the parathion metabolites, 0,0-diethylphosphoric acid (DEP) and 0,0-diethyl phosphorothioate K^+ (DETP, thionate isomer), are not stable in urine samples stored at -18°C (COMER et al. 1976). Thus, the purpose of this study was to investigate these findings further to determine the validity of data previously obtained by ourselves and other investigators. All urine metabolites under investigation were studied over a twenty week period.

EXPERIMENTAL PROCEDURE

Standards. The alkyl phosphate standards, 0,0-diethylphosphoric acid and 0,0-diethyl phosphorothioate K^+, were supplied by American Cyanamid Corporation. Working solutions were prepared from stock solutions (1 mg metabolite/mL acetone) by diluting aliquots with acetone to provide final solutions containing 0.01 mg metabolite/mL acetone.

Reagents. DOWEX 1-X8 anion exchange resin, 50/100 mesh, chloride form (BIO-RAD Laboratories); pesticide grade acetone; diazopentane as described by SHAFIK et al. (1973); and acetone containing 1% formic acid.

Equipment. A gas liquid chromatograph (GLC) equipped with a flame photometric detector was used to separate and quantitate the metabolites. The glass column used was 1.8 m x 4 mm i.d. that had been silane-treated and packed with 4% OV-210 on acid washed, silane-treated 80/100 mesh Gas Chrom Q (LEIBRAND and DUNHAM 1973). The GLC parameters were as follows: column oven temp., 155°C; inlet temp., 200°C; detector temp., 200°C;

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nitrogen carrier gas flow rate, 85 mL/min. A Corning, Model 12, Research pH Meter, calibrated at pH 4.01 and pH 7.00, was used for pH measurements.

Method. Each of six male volunteers collected urine samples in one-quart plastic bottles during a normal working day. A 100 mL aliquot of urine from each subject was fortified at 5 ppm 0,0-diethylphosphoric acid and 10 ppm 0,0-diethyl phosphorothioate K^+. The samples were mixed thoroughly, divided into 10 mL aliquots (one 10 mL aliquot for each analysis), and then frozen. All 10 mL aliquots were stored in glass scintillation vials, covered with Parafilm, and stored at -25°C until analyzed. The samples were allowed to thaw at room temperature for 2-3 h before analysis.

Each 10 mL aliquot was sufficient to run duplicate analyses. The fortified samples were analyzed at 0, 1, 2, 3, 4, 6, 8, 12, 16, and 20 weeks. Three 10 mL aliquots of urine from each subject were analyzed in duplicate as controls at -2 days, 19 days, and 37 days after freezing. Recovery studies were also performed in duplicate from 10 mL aliquots of control urine that had been frozen.

The metabolites were analyzed as the derivatives 0,0-diethyl 0-amyl phosphate (DEAmP) and 0,0-diethyl S-amyl phosphorothiolate (DEAmPTH, thiolate isomer), which were prepared by a slight modification (WINTERLIN et al. 1975) of the method of LORES and BRADWAY (1977). Quantitation was accomplished by comparing peak heights to external standards. The sensitivity of the analysis was 0.1 ppm for a 2 mL urine sample when a 6-µL aliquot from 6 mL of final solution was injected onto the GLC column (minimum detectable limit of 200 pg of alkyl phosphate). The pH of the urines of each subject was also monitored.

RESULTS AND DISCUSSION

The results of COMER et al. (1976) indicated considerable breakdown or disappearance of 0,0-diethyl phosphorothioate K^+ (DETP, thionate isomer) over a six week period. This was contrary to our findings in this study. Under our conditions there was no apparent disappearance of either DEP or DEPTh even after the urine samples were stored in the freezer for 20 weeks, as shown in Figures 1 and 2. Though the results of COMER et al. (1976) also indicated the disappearance

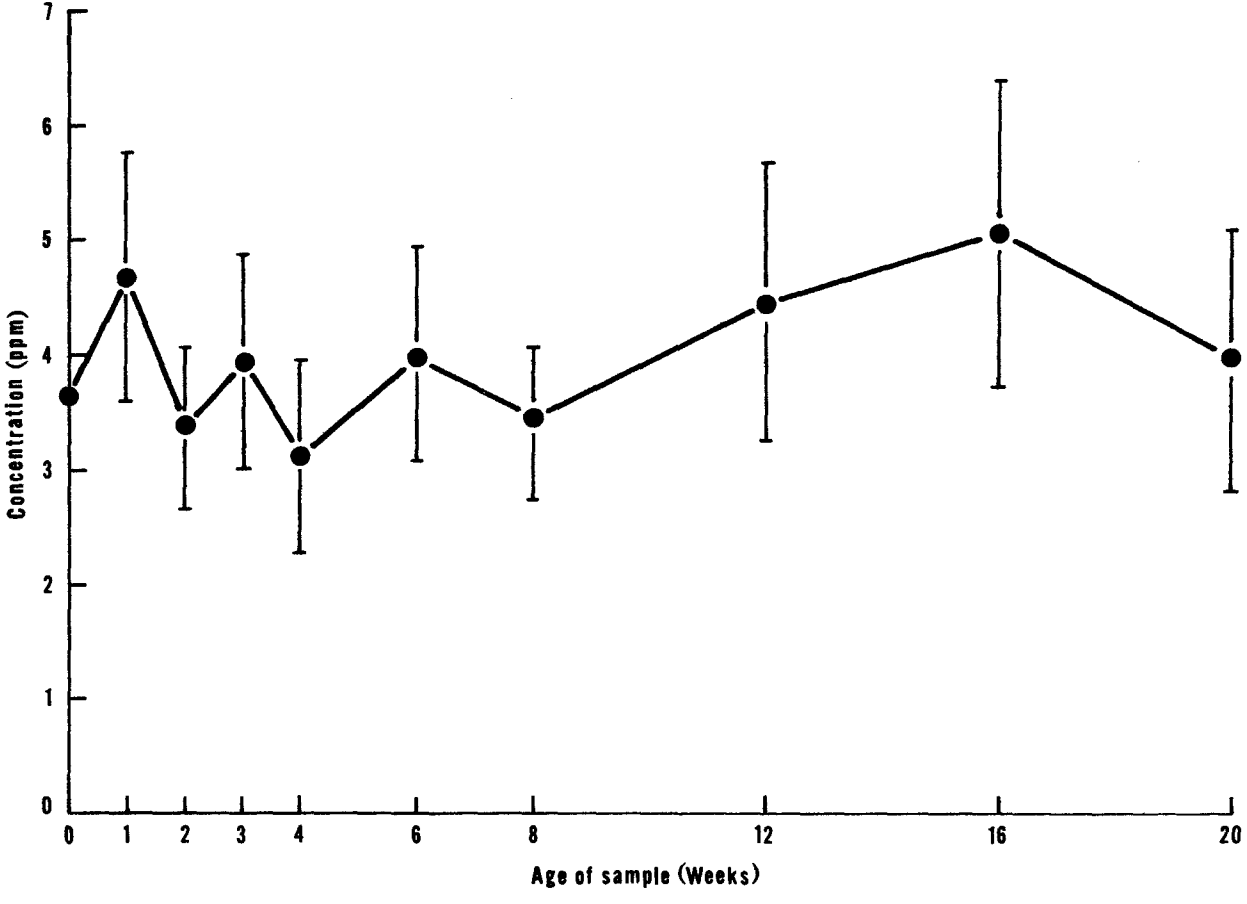

Figure 1. Concentration of DEP as a function of the age of the sample. Group mean values are plotted. Standard deviation is indicated.

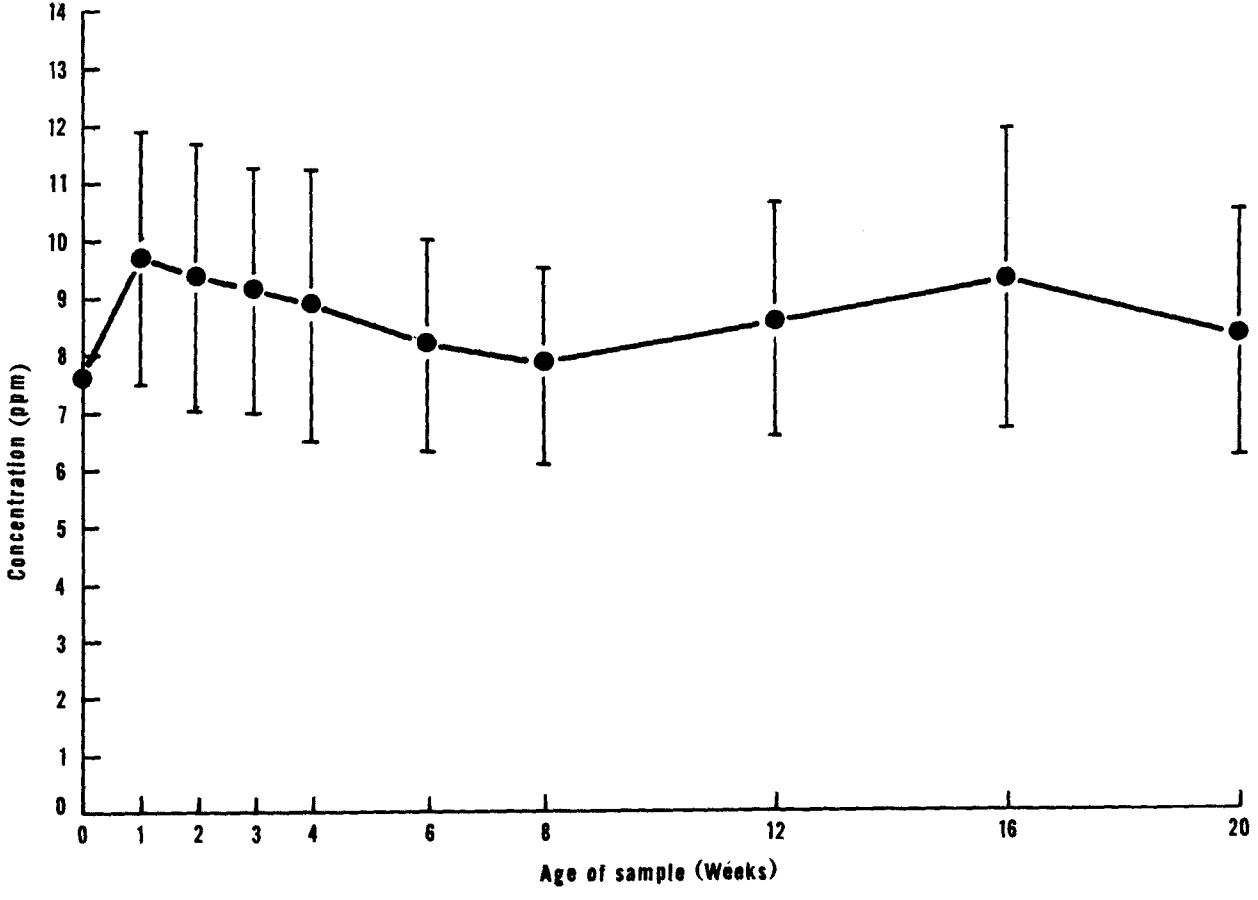

Figure 2. Concentration of DEPTh as a function of the age of the sample. Group mean values are plotted. Standard deviation is indicated.

of O,O-diethyl phosphorothioate K^+ over a range of approximately 4 ppm, we have found fluctuations of up to 4 ppm in a sample analyzed one week apart.

In their investigation LORES and BRADWAY (1977) found a significant amount of variability in their recoveries. Our results were also quite variable, as shown in Table 1.

TABLE 1

Percent Recovery of Alkyl Phosphate Metabolites in Urine

WEEK	DEP	DEPTh
0	43	46
1	38	61
2	47	35
3	44	37
4	46	33
6	53	94
8	49	77
12	39	73
16	37	68
20	38	27

Because the low recoveries did not give us as reliable quantitation as desired, the analytical method was used only as a semi-quantitative measurement.

Also, the pH of the urine of each subject was monitored as a rough indicator of microbial growth and, thus, any possible breakdown. Although a considerable variation existed between subjects, the pH did not change significantly during the storage period.

In conclusion, it is apparent from this study that dialkyl phosphate metabolites do not break down or disappear in urine samples stored frozen for up to 20 weeks. It is evident, however, that the analytical procedure used gives rise to highly variable data that could be interpreted as a loss or disappearance of the metabolites.

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