Stability of Parathion Metabolites in Urine Samples Collected from Poisoned Individuals

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Recently our laboratory was asked to determine the cholinesterase enzyme activity of a blood sample from a child poisoned by Parathion. The child was to be in the hospital several days for observation following treatment, so it was decided to obtain blood and urine samples from the child once a day for six days. We would use these samples to correlate cholinesterase values with the concentration of o,o-Diethylphosphorothionate (DETP), o,o-Diethylphosphate (DEP) and para-nitrophenol (PNP) in urine and to study the stability of these metabolites in refrigerated urine samples.

Initial blood and urine samples were obtained before the child was treated with pralidoxime chloride. Cholinesterase values were .95 μM/min/ml for RBC and .21 μM/min/ml for plasma. DETP and PNP concentrations in the urine were 3.88 ppm DETP, .47 ppm DEP, and 3.76 ppm PNP. Subsequent samples were drawn after pralidoxime chloride treatment. Samples 99 and 100 were analyzed for DETP and DEP one week, two weeks, and then six weeks after collecting the samples. Samples 101 through 104 were analyzed after one week for PMP and then after five weeks for DETP and DEP. Since the blood enzyme values returned to normal following treatment (a normal occurrence), it was decided not to continue drawing blood samples. Results of analysis are given in Table 1.

TABLE 1

Sample Age	Sample No.	DETP ppm	DEP ppm	Parathion[a] ppm	PNP ppm	Parathion[b] ppm
Initial Analysis	99	3.88	0.47	7.53	3.76	7.87
	100	0.72	0.30	1.81	0.89	1.87
	101	0.37	0.35	1.29	1.38	2.88
	102	0.19	0.27	0.84	1.32	2.75
	103	0.19	0.15	0.61	0.55	1.15
	104	0.11	0.07	0.32	0.26	0.54

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Table 1 - continued

Sample Age	Sample No.	DETP ppm	DEP ppm	Parathion[a] ppm	PNP ppm	Parathion[b] ppm
Samples One Week Old	99	3.48	0.34	6.59	----[c]	----
	100	0.70	0.25	1.67	----	----
	101	----	----	----	1.08	2.27
	102	----	----	----	0.96	2.02
	103	----	----	----	0.44	0.93
	104	----	----	----	0.27	0.56
Samples Two Weeks Old	99	4.04	0.30	7.49	4.94	10.35
	100	0.51	0.22	1.29	0.89	1.86
	101	----	----	----	----	----
	102	----	----	----	----	----
	103	----	----	----	----	----
	104	----	----	----	----	----
Samples Five Weeks Old	99	----	----	----	----	----
	100	----	----	----	----	----
	101	0.15	0.31	0.83	----	----
	102	0.27	0.23	0.89	----	----
	103	0.15	0.12	0.48	----	----
	104	0.00	0.00	0.00	----	----
Samples Six Weeks Old	99	0.15	0.34	0.89	----	----
	100	0.14	0.23	0.67	----	----
	101	----	----	----	----	----
	102	----	----	----	----	----
	103	----	----	----	----	----
	104	----	----	----	----	----

[a]Sum of DETP and DEP as Parathion.
[b]PNP as Parathion.
[c]The amount of sample was limited, so it was necessary to alternate organophosphate (O.P.) metabolite and PNP analyses.

Urine samples were obtained each day for five days following treatment of the patient. A portion of each sample was analyzed immediately for DETP and PNP. The remainder of the sample was put into a freezer for analysis at a later date. Analysis for DETP and DEP was done by the method of SHAFIK et al (1973). Analysis for PNP was done by the method by CRANMER and PEOPLES (1970). Figure 1 shows the concentration of DETP and DEP in the fresh samples. Figure 2 shows the concentration of PNP. DETP and DEP expressed as Parathion agreed favorably with PNP values expressed as Parathion (Table 1).

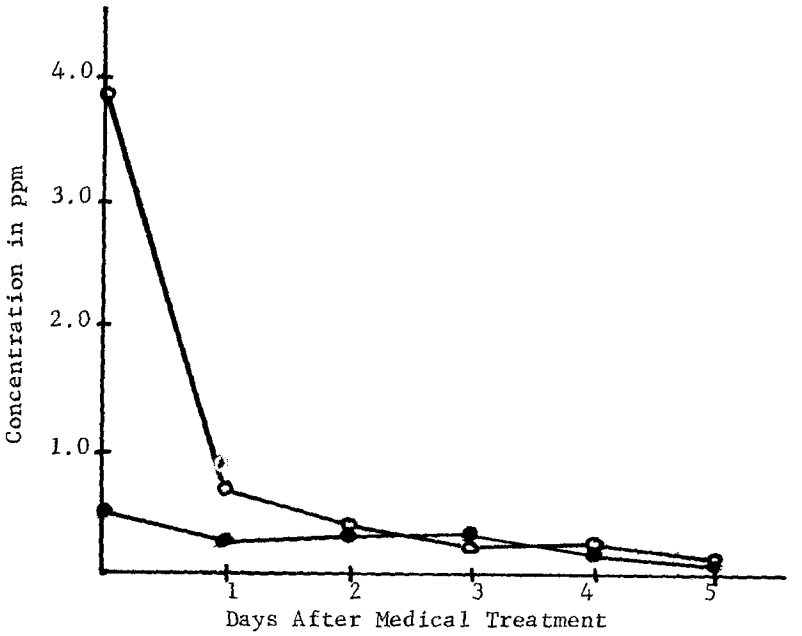

Figure 1. Concentration of DETP (o—◇) and DEP (●—●) in urine samples collected once each day for six days.

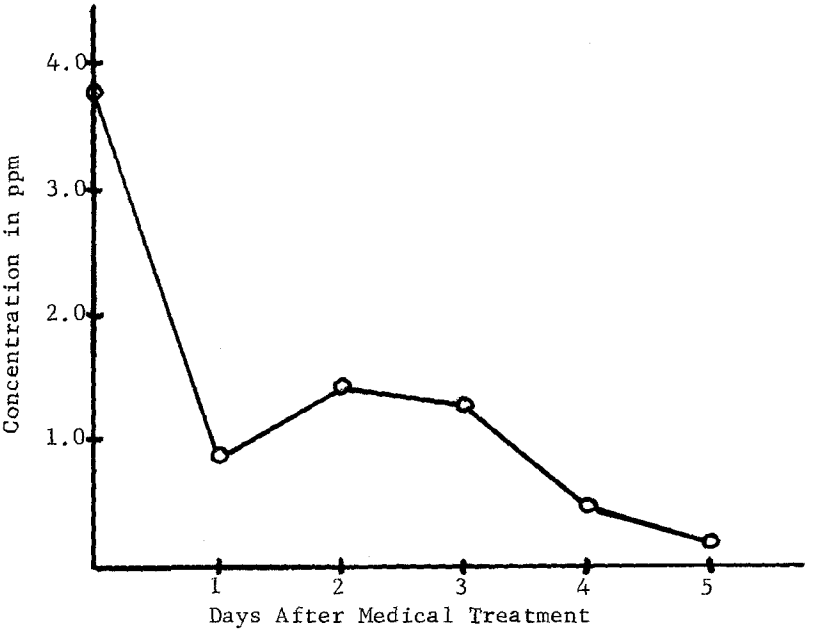

Figure 2. Concentration of PNP (o—o) in urine samples collected once each day for six days.

Figures 3, 4, and 5 are graphs of DETP and DEP concentration as a function of the age of the sample (kept in freezer). The sample was divided into 2.0 ml portions after the initial analysis so that it would not be necessary to thaw and re-freeze the sample after each analysis.

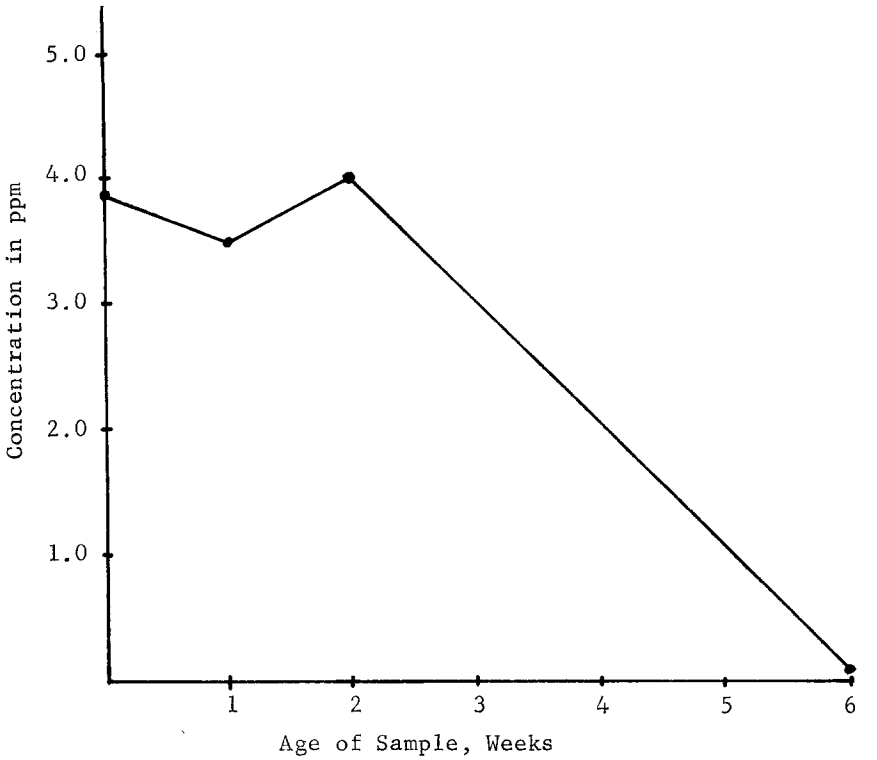

Figure 3. Concentration of DETP in urine (sample 99) as a function of the age of the sample.

At a later date, we received a blood sample from a person who had been occupationally exposed to a large amount of Parathion. Analysis of the blood sample for cholinesterase enzyme activity resulted in a RBC value of 3.06 μM/min/ml and a plasma value of .28 μM/min/ml. These values were low enough to anticipate the presence of DETP and DEP in the patient's urine. A urine specimen was requested. Unfortunately, it took four days for the sample to get to us, so it was not possible to correlate the initial O.P. metabolite concentration values with reduced cholinesterase values (one of the original aims of this study). The sample was analyzed, however, and then frozen for analysis at a later date. This data is plotted in Figure 6.

To further study the stability of DETP, an aqueous solution was made to contain 3.00 mg DETP-K per milliliter of solution. The solution was divided into halves. Each half was further divided into 2 ml portions. One half was stored at room temperature, the other half was stored in the freezer at -18°C. Room temperature samples and freezer samples were run simultaneously. Sample concentration as DETP-K is plotted as a function of age in days in Figure 7.

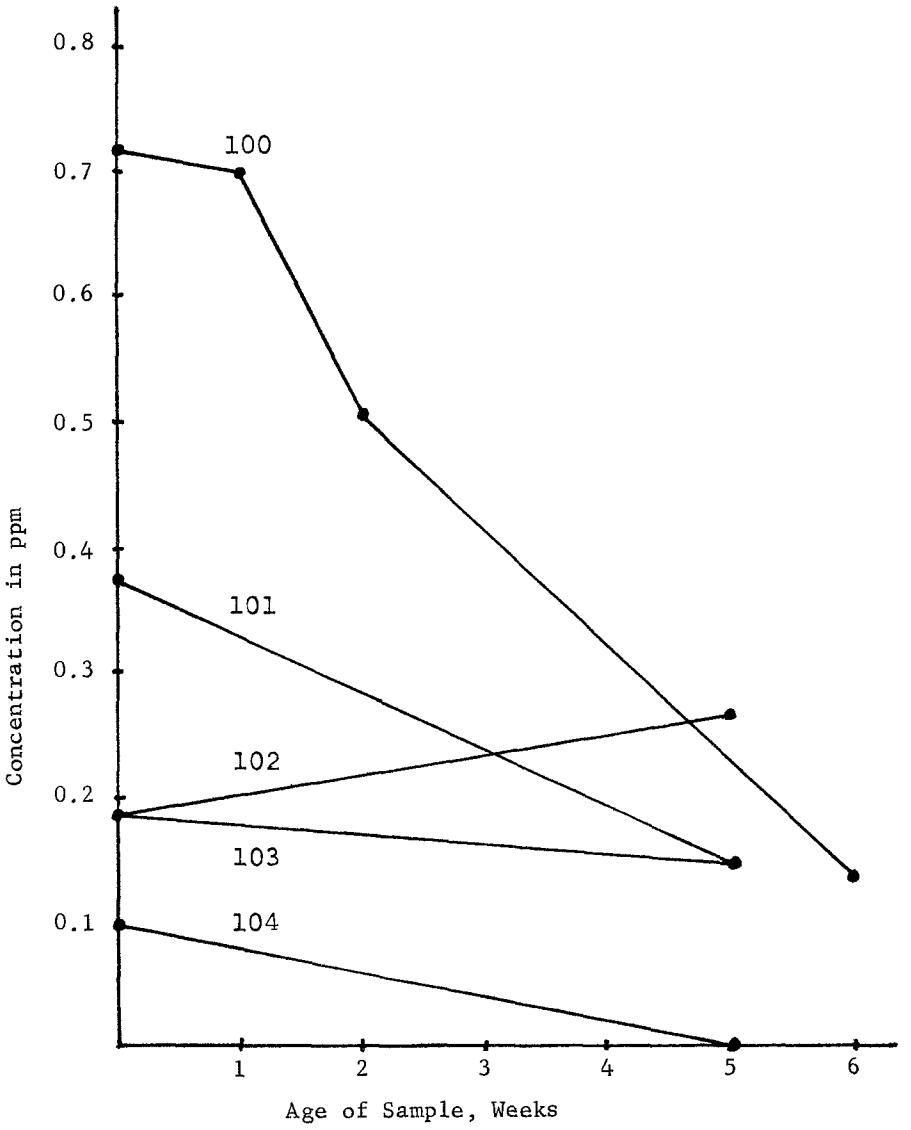

Figure 4. Concentration of DETP in urine samples (100, 101, 102, 103, and 104) as a function of the age of the sample.

DISCUSSION

Urine samples are being used to verify poisoning cases and to measure the degree of exposure of agricultural and plant formulation workers to organophosphate pesticides. To get data that accurately represent the true degree of exposure as indicated by the concentration of O. P. metabolites, it is essential to obtain and analyze samples as soon as possible after the occurrence of an exposure or a poisoning incident. As indicated by Figures 1 and 2, a urine sample received from a patient two days after poisoning contains only 18% (approximately) of the O.P. metabolite concentration that was available on the day of poisoning. The sampling protocol for some monitoring programs allows holding urine samples in a frozen condition until analyzed at a later date. This procedure will lead to inaccurate results in the case of exposure to pesticides that metabolize to DETP because DETP is apparently not stable in urine, even when the sample is kept at -18°C. Figure 3 shows that a sample containing initially 4 ppm DETP will contain approximately half of this concentration after four weeks in a freezer.

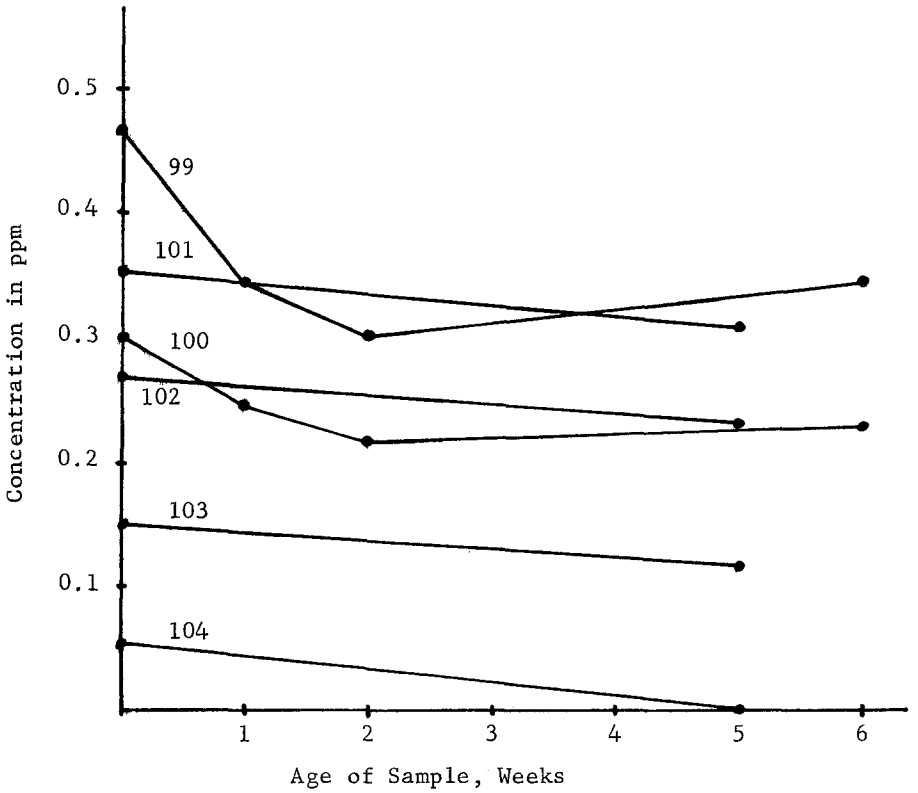

Figure 5. Concentration of DEP in urine samples as a function of the age of the samples.

An aqueous solution of the potassium salt of DETP was made to evaluate the possibility of using a master, or stock, solution as a quality control recovery standard. However, the rate at which DETP decomposes (or for other reasons becomes not available for measurement), as illustrated in Figure 7, indicates that it is not feasible to work from a stock standard solution. It is interesting that the rate at which the concentration of DETP decreased was nearly the same for the solution kept at room temperature as it was for the sample kept at -18°C. in the freezer.

Three pertinent facts were brought out by this study: (1) urine samples must be obtained from a patient within 24 hours of poisoning if the sample is to reflect the concentration of O.P. metabolites at the time of poisoning, (2) the sample should be analyzed as soon as possible, certainly not later than one week after obtaining the sample from the patient, and (3) an aqueous solution of the potassium salt of DETP cannot be stored for use as a stock standard solution.

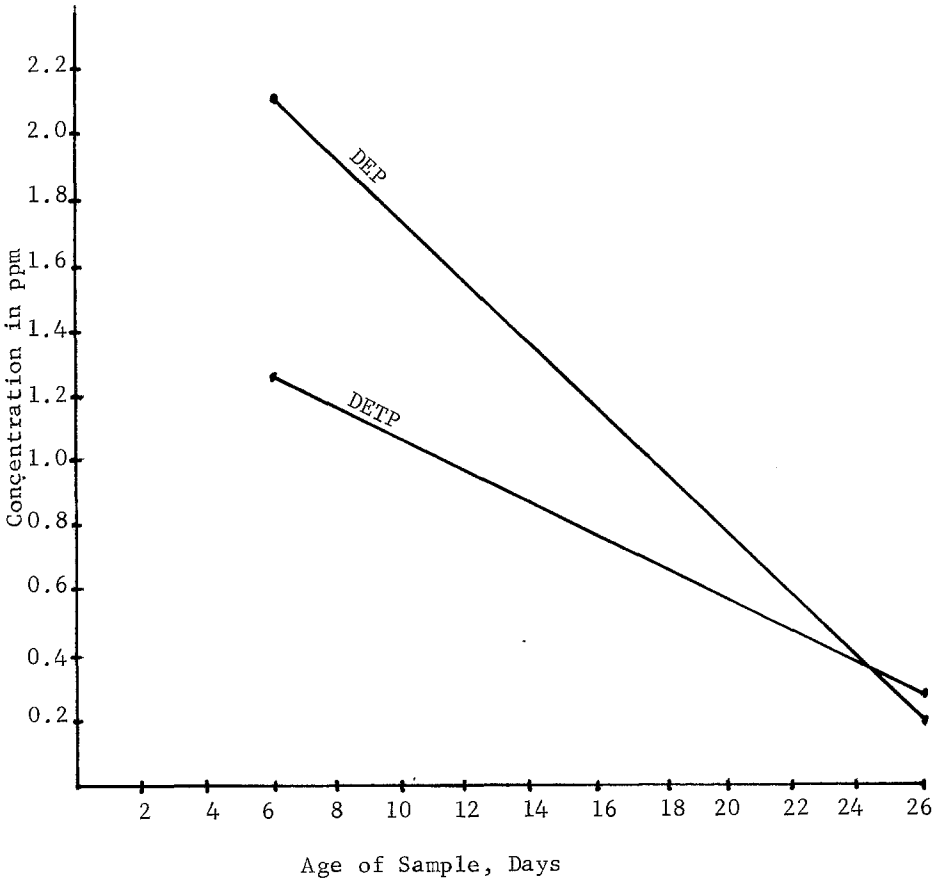

Figure 6. Concentration of DETP and DEP in a urine sample as a function of the age of the sample.

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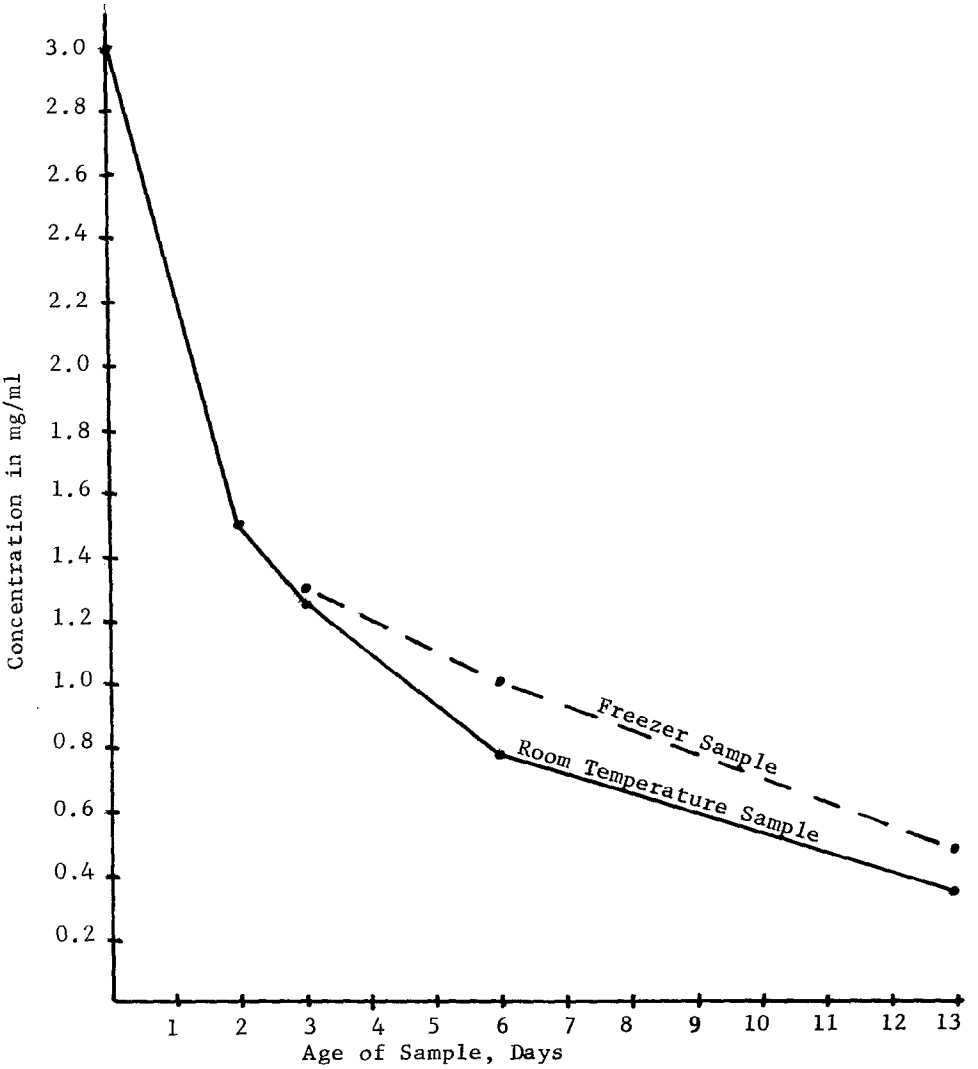

Figure 7. Concentration of an aqueous solution of the potassium salt of DETP as a function of the age of the sample. Part of the sample was kept at room temperature (●—●) and part was kept in the freezer (●--●).