Rapid Method for Detection of Parathion in Plasma by Electron Capture Gas Chromatography without Prior Cleanup

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A rapid sensitive method was developed for the detection of the organophosphate insecticide, parathion (*O,O*-diethyl *O-p*-nitrophenyl-phosphorothioate) in mouse plasma. The insecticide is extracted from acidified plasma with *n*-hexane and quantitatively measured by means of electron capture gas chromatography, with methyl parathion as an internal standard. Levels of parathion ranging from approximately 0.1 to 0.3 microgram per milliliter of plasma were measured. Average recovery of C^{14}-parathion added to plasma was 93.4%. This method may be of value in confirming suspected cases of exposure to this insecticide.

Many of the organophosphate compounds used as insecticides are thiophosphate derivatives. Since these agents are highly toxic, they are generally found in biological tissues in low concentrations after accidental exposure. Insecticides in the nanogram range are commonly measured by electron capture (Petitjean and Lantz, 1963), thermionic emission (Ford and Beroza, 1967) or flame photometric (Brody and Chaney, 1966) gas chromatography.

Jain *et al.* (1965) extracted and identified 23 pesticides from rat blood using the electron capture detector. The method used by these workers did not involve extensive cleanup procedures as did those of earlier investigators. They extracted the pesticides with ether:acetone (1:1) which was then evaporated to dryness. The residue was taken into hexane which was then analyzed gas chromatographically.

This report describes a simple gas chromatographic method for estimating nanogram quantities of the thiophosphate insecticide parathion (*O,O*-diethyl *O-p*-nitrophenyl phosphorothioate) in mouse plasma by the use of a single solvent without prior cleanup.

METHOD

Apparatus. A Hewlett-Packard model 5750 B gas chromatograph fitted with a ^{63}Ni electron capture detector was used to analyze plasma extracts for parathion. The glass column employed was 4 feet by 0.25-inch I.D. and was packed with 3.8% UCC-W982 on 80 to 100-mesh Diatoport S. Argon to methane (95 to 5) was used as the carrier gas and the flow rate was adjusted to 85 ml. per minute. Column temperature was 195° C, detector temperature 240° C, and injection port temperature 235° C.

Procedure. Mature male mice of the Swiss-Webster strain weighing 20 to 25 grams were injected intravenously with parathion at a dose of 4 mg. per kg. of body weight. The parathion was dissolved in 5% aqueous Tween 20 solution which was administered in a volume of 1% of the animal weight. The animals were bled by cervical incision at various time intervals after parathion administration and blood was collected in heparinized tubes. The red cells were sedimented by centrifugation in a Sorvall RC2-B refrigerated centrifuge at 3500 r.p.m. for 10 minutes. One milliliter of plasma was placed in a clear glass vial (1-dram capacity) fitted with a polyethylene stopper, and acidified by adding 0.02 ml. of concentrated HCl. This mixture was extracted by shaking for 20 minutes with 1.5 ml. of *n*-hexane containing methyl parathion (0.5 ng./2 μl.) as the internal standard. The emulsion formed was broken by centrifugation at 6000 r.p.m. for 10 minutes. A 2-μl. sample of the hexane phase was injected into the gas chromatograph.

Recovery. Parathion labeled with ^{14}C in the 2,6 ring position was counted in a Packard model 3375 Tri-Carb Scintillation Counter. Per cent recovery of parathion was determined by adding known quantities of ^{14}C parathion to the plasma which was then extracted as described above. The amount of plasma used in these recovery experiments did not affect the counting efficiency (Benson, 1966) which was found to be approximately 78%. Confirmation that the radioactivity present was due to parathion, and not paraoxon and/or *p*-nitrophenol, was accomplished by means of thin-layer chromatography.

Thin-Layer Chromatography. Fifty-microliter portions of plasma extracts were spotted 5 μl. at a time on a 250-micron Silica Gel G plate. To locate the radioactive material, the plates were respotted with carrier parathion and some of its metabolites in sufficient quantities to be detected by ultraviolet light. The concentrations of these carriers were as follows: parathion 100 μg./5 μl.; paraoxon 100 μg./5 μl.; *p*-nitrophenol 4 μg./5 μl. The plates were developed with benzene to ethylacetate (85 to 15), and after identification the radioactive material was scraped into 10 ml. of the fluor. The fluor used was that proposed by Benson (1966) and was chosen for its ability to solubilize aqueous solutions. It consists of 333.3 ml. of Triton X-100, 666.7 ml. of toluene, 5.5 g. of 2,5-diphenyloxazole and 100 mg. of 1,4-bis-[2,(5-phenyloxazolyl)] benzene.

RESULTS AND DISCUSSION

Results of the recovery study are shown in Table I. Quantities of parathion ranging from 0.125 to 0.500 μg. were added to 1 ml. of plasma. These amounts were selected on the basis of preliminary studies in which plasma levels were determined in mice injected with ^{14}C-parathion. The average per cent recovery from plasma was 93.4%.

Table II shows the signal ratios for various concentrations of parathion in hexane relative to the internal standard, methyl parathion. As the concentration of parathion increases from 0.125 to 0.750 ng./2 μl. the signal ratios increase from 0.225 to 1.293, respectively. There is a straight line relationship between the parathion concentration and the signal ratio values. Table III shows the plasma levels and the quantities of parathion detected in hexane extracts of plasma

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Table I. Recovery of ^{14}C Parathion Added to Plasma

Added		Recovered,	Recovered,
μg./ml.	C.P.M./ml.	C.P.M./ml.	%
0.125	1160 ± 20	1080 ± 18[a]	93.1
0.250	2284 ± 26	2112 ± 24	92.5
0.500	4501 ± 80	4255 ± 66	94.5
		Average recovery	93.4

[a] Each value ± standard deviation is the mean of three determinations.

Table II. Signal Ratios of Varying Concentrations of Parathion to Internal Standard (Methyl Parathion)[a]

Parathion, ng./2μl. hexane	Signal Ratio[b]
0.125	0.225
0.250	0.438
0.375	0.673
0.500	0.888
0.625	1.084
0.750	1.293

[a] 0.5 ng./2 μl. hexane.
[b] Peak height parathion/peak height methyl parathion.

Table III. Plasma Levels of Parathion at Various Time Intervals after Intravenous Administration (4 mg./kg.)

Time after Parathion, Min.	Amount of Parathion Detected,[a] ng./2μl.	Plasma Level Parathion,[b] μg./ml.
15	0.319 ± 0.075	0.257 ± 0.061
30	0.241 ± 0.065	0.194 ± 0.053
60	0.173 ± 0.051	0.139 ± 0.041
90	0.123 ± 0.039	0.098 ± 0.031

[a] Each value is the mean ± S.D. of three determinations in each of which the pooled plasma of three animals was used.
[b] Plasma levels were calculated from the amount of parathion in 2-μl. hexane extracts after correction for recovery.

15, 30, 60, and 90 minutes after the intravenous administration of parathion. These levels are approximately one-tenth those reported by Jain *et al.* (1965) in rat whole blood. These workers administered parathion (5 mg./kg.) orally in olive oil and measured its level in the blood 24 minutes later.

Figure 1 illustrates a typical chromatogram obtained by this procedure. The retention times for methyl parathion and parathion were approximately 1.3 and 2.2 minutes, respectively. The retention times of paraoxon and *p*-nitrophenol are sufficiently different from the above compounds to allow detection if present. No paraoxon or *p*-nitrophenol was detected in mouse plasma following the intravenous administration of parathion.

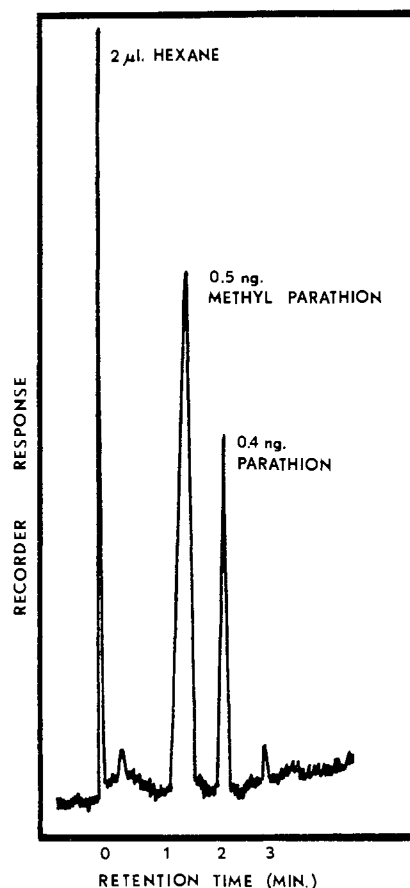

Figure 1. Chromatogram of hexane extract of plasma from mice given parathion intravenously showing 0.4 ng. parathion and 0.5 ng. methyl parathion (internal standard)

The applicability of the method described in this report to the detection and measurement of other thiophosphate insecticides should be investigated. The results of such studies may be of value in the diagnosis and management of cases of poisoning by these insecticides.

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