Comparison of Analytical Results Obtained by Gas and by High-pressure Liquid Chromatography of Parathion and Paraoxon in Extracts Prepared from Orchard Soil Dust and Dislodgable Residues on Orange Leaves

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Parathion is a major pesticide in use in California citriculture, principally as a scalicide. In use for this purpose since the early 1950's, parathion has been involved in most of the reported worker (picker) illnesses in citrus groves (BAILEY et al. 1972, SPEAR et al. 1975). Much effort is currently directed towards elucidating the underlying causes of these episodes. Research emphasis has been focused on the parathion and paraoxon levels of the foliar dislodgable residues (GUNTHER et al. 1973, ADAMS et al. 1976) and of the loose surface-dust of orchard soil (GUNTHER et al. 1975, SPENCER et al. 1975). Toxicant quantitation is generally by gas chromatography (GC) with its attendant use of elevated temperatures. Due to the importance of the research effort, it is imperative that both qualitative and quantitative results are reliable. Alteration of compounds during GC analysis, for example, the conversion of DDT to TDE (OTT and GUNTHER 1965), of Kelthane to dichlorobenzophenone (GUNTHER et al. 1962, IVES 1973), and of aldicarb to 2-methyl-2-(methylthio)-propionitrile (KNAAK et al. 1966) is not uncommon. Although a reference standard of parathion or paraoxon is injected routinely during analyses, its behavior upon injection is only assumed to be identical to that of the analyte in the sample. The reliability of the GC values for paraoxon contains an element of uncertainty as it is a difficult compound to analyze by GC. Special column preparation (e.g., IVES and GIUFFRIDA 1970) is frequently required. During a sequence of analyses, a change in the relative responses of parathion and paraoxon occurs which tends to approach a constant value after a number of injections; this number can be quite considerable. To determine if the use of elevated temperatures for parathion and paraoxon could be affecting especially the quantitative results, possibly lower temperature procedures could be applied. One approach would be cholinesterase (ChE) inhibition measurements; another would be spectrophotometry, for instance, combined with high-pressure (HP) liquid chromatography (LC) to provide cleanup and qualitative information through the retention values.

MIRER et al. (1975) used ChE inhibition and found the results for paraoxon in excellent agreement with the results obtained by GC; however, a similar investigation (STAIFF et al.

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1975) on extractives of leaf samples collected as a part of a reentry study in an apple orchard did not show such coherence in quantitative results. HPLC combined with spectrophotometry has an advantage over the anti-ChE method since quantitative results are obtained for both parathion and paraoxon.

For the reasons enumerated above both HPLC (at ambient temperature) and GC were used to analyze extracts prepared from actual field samples. The parathion and paraoxon residues in the orchard soil-dust and in the dislodgable residues on orange tree leaves were determined.

MATERIALS AND METHODS

Instruments. The Waters Associates liquid chromatograph consisted of a septum injection system, two Model 6000 reciprocating pumps, and a Model 660 solvent flow programmer. Columns used were (1) a 0.5 m X 2.2 mm i.d. stainless steel tube dry-packed with Bondapak® C_{18} ODS on 37-50 µm diameter Corasil® silica, and (2) a 0.5 m X 2.2 mm i.d. stainless steel column prepacked by Waters Associates with Carbowax 400 on 37-50 µm diameter Corasil. The detector was a tunable wavelength Perkin-Elmer Model LC-55 combined UV/VIS spectrophotometer equipped with a micro flow-cell. The output was connected to a recorder operating in the 0-100 mv range; chart speed was 0.5 in./min. Full-scale recorder deflection represented 0.2 absorbance unit (A.U.). The GC was a Varian Aerograph Model 1700 equipped with an alkali flame detector designed for phosphorous compound detection and a 1.5 m X 3.2 mm i.d. glass column packed with 5% DC-200 on 80/100 mesh Gas-Chrom Q to which Carbowax 20 M had been applied as a vapor (IVES and GIUFFRIDA 1970). Inlet, column, and detector temperatures were 220, 215, and 200°C, respectively.

Reagents. The acetonitrile used for reverse phase chromatography was redistilled and degassed. Commercial A. R. dioxane and hexane degassed under reduced pressure were used in normal phase HPLC. The acetone used for soil sample extraction was reagent grade.

Samples. The samples were collected from an experimental orange grove on the Irvine Ranch, Tustin, California, from trees sprayed with a 25% WP parathion formulation. The surface soil dust was taken from the orchard floor by suction (SPENCER et al. 1975) 17, 33, 46, and 60 days after spraying, and extracted by shaking 15 min with an acetone-water (9+1) solvent mixture; 2 ml of solvent contained extractives from 1 g of dust. The foliar residue samples were collected as leaf discs (GUNTHER et al. 1974) 3 and 10 days after spraying. Each sample, consisting of 40 leaf discs (2.54-cm diameter), was shaken with water containing a wetting agent to remove surface dislodgable residues and then the pesticide in the aqueous extract was partitioned once into 50 ml of hexane.

LC analysis of soil dust samples. The detector wavelength setting was 272 nm, with output in absorbance units. An aliquot of the soil extract sample was drawn into a syringe with a Luer-lock, the needle was removed, and a Swinny-filter-adapter containing a Millipore® filter (FHLPO 1300) was attached. The sample was forced out through the filter into the reservoir of a 25-µl injection syringe. A 10- or 25-µl aliquot of the sample was injected into the LC equipped with the C_{18} ODS column. A linear program was used which commenced with an initial condition of 10% acetonitrile-90% water and terminated with a final condition of 100% acetonitrile over 13 min at a flow-rate of 1.0 ml/min. Prior to another injection, interfering matter was washed out of the column by pumping through acetonitrile until the UV absorption returned to baseline and the column was then reequilibrated at initial conditions. Results were compared with injected standards for quantitation by peak height.

LC analysis of foliar dust samples. The wavelength setting was 265 nm and the Carbowax 400 column was used. Due to the large difference in relative concentrations of parathion and paraoxon, quantitation for each compound was performed from different chromatograms. For parathion analysis 20 µl of the hexane extract was injected and eluted under isocratic conditions with liquid A (1.5% dioxane in hexane). For paraoxon analysis 10 ml of the hexane extract was concentrated to 1.0 ml under a gentle air stream and 200 µl of this concentrate was injected with the LC equilibrated at 80% liquid A and 20% liquid B (22.5% dioxane in hexane). A linear program was run from these initial conditions to a final condition of 100% liquid B at a flow-rate of 1.0 ml/min over 12 min.

GC analysis. An aliquot of each sample was diluted to give a suitable concentration. A volume of 2 µl was injected for the determination of parathion, with 5 µl for paraoxon. Quantitation was accomplished through the comparison of the peak height measurements obtained from the sample and from the standard.

RESULTS AND DISCUSSION

Figure 1 shows the peak shapes and separation obtained with HPLC using the described reverse phase solvent program. Figure 2 shows a typical chromatogram of a soil dust extract sample. The chromatogram shows that the acetone did not cause any analytical problems due to its UV absorbance. The reason for the use of a different wavelength for the reverse and normal phase chromatographic procedures is that a polar solvent shifts the wavelength of maximum absorbance as illustrated by GUNTHER and BLINN (1955) for parathion and paraoxon. A plot of peak height measured in A.U. vs. µg of parathion or paraoxon injected was linear up to the full-scale recorder deflection of 0.2 A.U. and is shown, in part, in Figure 3. The slope of the standard curve was 0.096 A.U./µg of parathion and 0.097 A.U./µg of paraoxon.

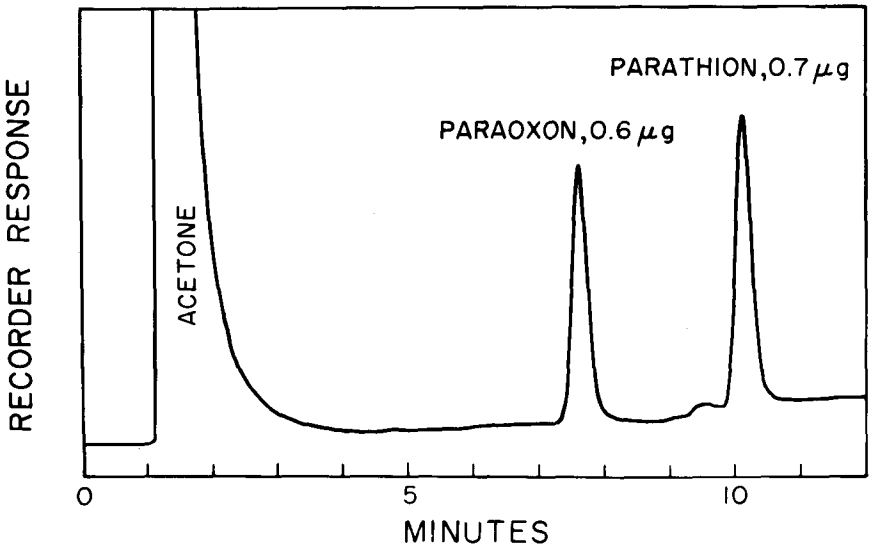

Figure 1. Liquid chromatogram of a standard mixture of parathion and paraoxon.

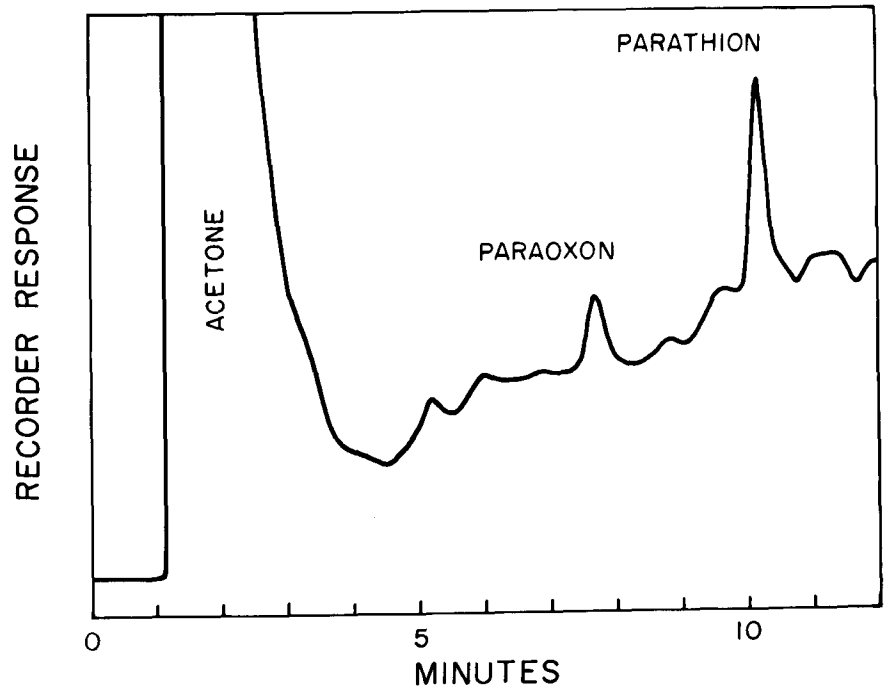

Figure 2. Liquid chromatogram of soil dust extract containing parathion and paraoxon.

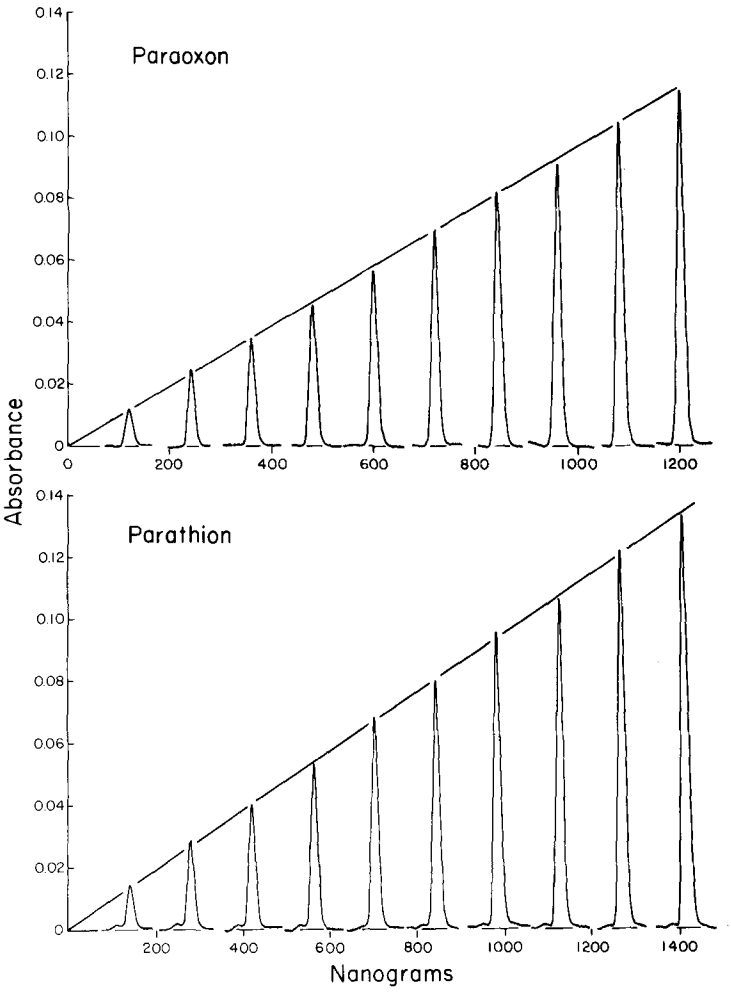

Figure 3. Liquid chromatographic standard curves for parathion and paraoxon obtained using reverse phase conditions and the UV detector set at 272 nm.

TABLE I

Residues of parathion and paraoxon found in extracts of orchard soil dust as determined by GC and HPLC.

Parathion, µg/g		Difference,	Paraoxon, µg/g		Difference,
GC	HPLC	%	GC	HPLC	%
27.8	29.8	+ 7.2	11.7	10.3	- 12.0
173	161	- 6.9	31.8	26.7	- 16.0
218	220	+ 0.9	40.2	38.5	- 4.2
37.8	38.8	+ 2.6	12.6	13.1	+ 4.0
27.8	30.9	+ 11.4	9.7	10.0	+ 3.1
161	171	+ 6.2	29.0	29.2	+ 0.7
21.6	19.5	- 9.7	16.2	16.4	+ 1.2
87.0	96.0	+ 10.3	31.0	27.7	- 10.6
23.0	23.1	+ 0.4	14.4	13.4	- 6.9
122	130	+ 6.6	43.2	41.7	- 3.5
114	117	+ 2.6	42.6	40.7	- 4.5
54.0	52.8	- 2.2	22.7	22.7	0
73.5	64.4	- 12.4	33.1	30.3	- 8.5
29.1	25.0	- 14.1	22.2	18.1	- 18.5
93.3	81.9	- 12.2	42.2	45.1	+ 6.9
65.2	60.0	- 8.0	37.2	35.3	- 5.1
12.2	11.2	- 8.2	16.9	14.6	- 13.6
56.7	58.6	- 5.5	32.1	28.4	- 11.5
24.7	25.0	+ 1.2	23.2	22.9	- 1.3
64.0	60.0	- 6.3	36.6	34.5	- 5.7
101	107	+ 5.9	52.6	47.6	- 9.5
10.9	12.2	+ 11.9	12.7	12.5	- 1.6

TABLE II

Residues of parathion and paraoxon found in extracts of dislodgable residues from leaf surfaces as determined by GC and HPLC.

Parathion, µg/cm^2		Difference,	Paraoxon, µg/cm^2		Difference,
GC	HPLC	%	GC	HPLC	%
2.94	3.10	+ 5.4	0.045	0.030	- 33
3.77	3.91	+ 3.7	0.052	0.048	- 8
2.71	2.51	- 7.4	0.046	0.028	- 39
2.79	2.88	+ 3.2	0.034	0.027	- 21
2.92	3.04	+ 4.1	0.028	0.029	+ 4
2.94	3.06	+ 4.1	0.034	0.027	- 21
1.02	1.07	+ 4.9	0.055	0.062	+ 13
0.84	0.81	- 3.6	0.045	0.050	+ 11
0.84	0.78	- 7.1	0.032	0.026	- 19
0.43	0.40	- 7.0	0.025	0.028	+ 12
0.73	0.73	0	0.032	0.033	+ 3
0.78	0.78	0	0.029	0.025	- 14

Since the leaf dust extracts contained relatively less paraoxon than the orchard soil dust extracts, the minimum detectability in the method for the leaf dust extracts had to be lower than that for the soil dust extracts for the HPLC procedure. This was accomplished by using a larger injection volume for the leaf extracts. KIRKLAND (1974) suggested that an upper limit for the injected volume that does not lead to peak broadening is one-third of the volume that passes the detector during the elution of a peak. Though it is pointed out that the initial mobile phase must have a low eluting power, the disturbance of the elution caused by the injected solvent is not considered. However, a large volume of the solvent cannot be injected without deforming the peak shape if the solvent exceeds a certain polarity in normal phase chromatography, nor can it be too lipophilic in reverse phase chromatography. This condition limits the volume of acetone that can be injected and run through the reverse phase solvent program, without pronounced peak-broadening, to approximately 25 µl. This restriction, together with the fact that the leaf-dust samples were in hexane demanding evaporation to dryness and subsequent redissolving in a solvent compatible with reverse phase, made the choice of normal phase conditions convenient for the analysis of those samples. As the described reverse phase program offers sharper peaks with the low volume injections ($\leq$ 25 µl), the apparent gain in detectability for the leaf-dust samples derives solely from an increase in the allowable injection-volume (< 25 µl) with the normal phase conditions. The slope of the standard curve for the paraoxon for the normal phase solvent-programmed condition is only 0.043 A.U./µg injected; the slope for parathion is 0.075 A.U./µg injected under the described normal phase isocratic conditions. The results from analyzing 22 soil dust samples and 12 foliar dust samples in accordance with the GC and HPLC procedures described are collated in Tables I and II, together with the difference between the methods given in percent of the value obtained by GC. The standard deviations of the percent differences between the GC and HPLC results and the algebraic mean of the percent differences are given in Table III.

TABLE III

Statistical parameters calculated from Tables I and II for parathion and paraoxon in extracts of foliar dust and soil dust analyzed by GC and HPLC.

	Parathion		Paraoxon	
Sample	Standard deviation	Algebraic mean	Standard deviation	Algebraic mean
Soil dust	8.1	-0.8	6.6	-5.3
Foliar dust	5.0	0.0	18	-9

It is concluded that in the fairly wide concentration range of contaminating parathion and paraoxon in orchard dusts from leaves and soil investigated there is no systematic disagreement in either the qualitative or the quantitative analytical results

for parathion by GC and HPLC; although the results seem to be systematically a little higher for paraoxon when GC is used, the difference is not significant when the magnitude of the standard deviations is taken into account. Hence, a conversion of parathion to paraoxon was not observed on the GC column, and the change in relative response for parathion and paraoxon often observed during GC is likely due to adsorption on the column. The good agreement of the HPLC and GC results for paraoxon is an indirect proof of its identity and occurrence as foliar dislodgable residues on citrus leaves and in orchard soil dust. Further confirmation of the identities of parathion and paraoxon in the latter extracts was made using a chemical reaction detector and is reported by OTT (1977). Due to the rapidity of sample analysis, GC is the technique of choice for quantitation of samples containing parathion and paraoxon.

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