

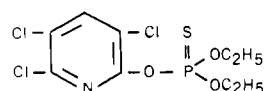
# Determination of *O,O*-Diethyl *O*-3,5,6-Trichloro-2-Pyridyl Phosphorothioate in Water and Silt by Gas Chromatography

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A gas chromatographic method for determining residues of *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (Dursban insecticide) in water and silt is described. Samples were extracted with methylene chloride and cleanup was accomplished with a silicic acid column. Residues as low as

0.0001 p.p.m. in water and 0.005 p.p.m. in silt were determined by gas chromatography using nonpolar stationary phase columns and electron-capture detection. Recoveries of Dursban insecticide from water and silt averaged 92 and 83%, respectively.

**D**ursban insecticide (Dow Chemical Co.), *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate, whose broad spectrum of activity has been reported by Gray (1965) and Kenaga *et al.* (1965), has shown great promise for the control of mosquitoes (Gahan *et al.*, 1966; Lewallen and Peters, 1966; Ludwig and McNeill, 1966; Mulla *et al.*, 1966). A method was needed to determine residues of this material in the natural waters and adjacent mud (silt) of marsh areas following spray treatments for mosquito control.



The method should separate the insecticide from these complex, natural substances, retain its identity, and accurately determine subnanogram amounts. In addition, it should be sufficiently simple and rapid to handle large numbers of samples in a brief period. A gas chromatographic method was developed to meet these requirements. Following extraction from water or silt, the Dursban residue is separated from co-extracted substances and analyzed by electron-capture gas chromatography.

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## EXPERIMENTAL

**Extraction and Liquid Chromatography.** WATER. Extract a 50-ml. sample repetitively with 100 and 50 ml. of redistilled methylene chloride in a 500-ml. separatory funnel. Shake the separatory funnel vigorously for 2 to 3 minutes each time and allow the layers to separate. Drain the methylene chloride layer following each extraction through a 1-inch column of anhydrous sodium sulfate powder into a 300-ml. Erlenmeyer flask. When all of the extract has passed through, wash the column with a 20-ml. portion of methylene chloride. Evaporate the combined dried extracts to 15 to 20 ml. on a low-heat hot plate and then to dryness under reduced pressure. Dissolve the residue in 5 ml. of redistilled *n*-hexane preparatory to transferring to a chromatographic column. Prepare a chromatographic column by adding 1.5 cm. of anhydrous sodium sulfate, followed by 5 cm. (8.5 grams) of well-packed silicic acid, and then another 1.5-cm. layer of sodium sulfate. (Each lot of silicic acid must be calibrated, and it might be necessary to change the volumes of eluting solvents given here.) Wet the column with 7.5% methylene chloride in *n*-hexane and then transfer the residue to the column, using 20 ml. more of *n*-hexane to make the transfer. Wash the column with 200 ml. more of the 7.5% methylene chloride in *n*-hexane. Change the receiver and elute the Dursban with 175 ml. of the *n*-hexane-methylene chloride solvent mixture. Concentrate the fraction to

about 10 to 20 ml. by distillation through a Snyder column. Cool the flask and evaporate to dryness, using reduced pressure with intermittent heat. Dissolve the residue in 5 ml. of *n*-hexane and inject 10  $\mu$ l. into the gas chromatograph; then dilute if necessary. Determine quantitatively the Dursban insecticide concentration of the injected aliquot by comparison of peak height with the peak height of a standard solution. Calculate the residue as parts per million based on the volume of the water sample.

**SILT.** Air-dry the samples and when the silt is dry to the touch, reduce to a fine powder using a mortar and pestle. Mix thoroughly and remove 10 grams for analysis. Blend the 10-gram sample for 5 minutes with 175 ml. of methylene chloride and about 2 grams of Celite. Filter through a folded filter into a 500-ml. Erlenmeyer flask and wash the blender and filter with another 175 ml. of methylene chloride. Concentrate the solution to a volume of about 25 ml. by distillation through a Snyder column and then to dryness at reduced pressure with intermittent low heating. Dissolve the residue and transfer to a 500-ml. separatory funnel, using 100 ml. of *n*-hexane to make the transfer. Add 50 ml. of redistilled acetonitrile to the funnel and shake for 2 to 3 minutes. Allow the layers to separate and draw off the acetonitrile layer into a second separatory funnel containing 100 ml. of *n*-hexane. Shake the second funnel for 2 to 3 minutes and after the layers have separated, draw off the acetonitrile layer into a 300-ml. Erlenmeyer flask. Repeat the process three times, using 50 ml. of acetonitrile each time. Concentrate the collection of four acetonitrile extracts to a volume of 10 to 15 ml. by distillation through a Snyder column. Remove the remaining acetonitrile by addition and evaporation of three 25-ml. portions of *n*-hexane, retaining a volume of 10 to 15 ml. Take the residue to dryness at reduced pressure with intermittent heat. Dissolve the residue in 5 ml. of *n*-hexane and proceed with the column cleanup and determination as described for water.

**Gas Chromatography.** The following conditions were employed:

**F & M MODEL 810.** Column, 4 feet  $\times$   $\frac{1}{4}$  inch o.d. borosilicate glass; packing, 5% w./w. SE-30 on 80- to 90-mesh Anakrom ABS; carrier gas, 5% methane in argon, 60 ml. per minute; detector, H<sup>3</sup> 200 mc., with pulse width of 0.75  $\mu$ sec. and pulse interval of 150  $\mu$ sec.; temperatures, column, 200° and 215° C., injection port, 245° C., detector cell, 205° C.; electrometer range setting, 10 or 10<sup>2</sup>, attenuation 8 or 16; recorder chart speed, 0.25 inch per minute.

**JARRELL-ASH MODEL 28-700.** Column, 4 feet  $\times$   $\frac{1}{4}$  inch o.d. borosilicate glass; packing, 5% w./w. SF-96 on 80- to 100-mesh Chromosorb W; carrier gas, nitrogen, 90 ml. per minute; detector, H<sup>3</sup> 100 mc., d.c. mode; temperatures, column, 215° C., injection port, 245° C., detector cell, 205° C.; electrometer sensitivity,  $1 \times 10^{-10}$  ampere, recorder chart speed, 0.25 inch per minute.

## RESULTS AND DISCUSSION

The chief difference between the two instruments was in the column packing, as shown above. The columns were conditioned for 96 hours, 10° to 25° C. above operating temperature. Both the SE-30 and SF-96 substrates permitted satisfactory elution of the insecticide. Peak

**Table I. Gas Chromatographic Analyses of Dursban in Water and Silt**

Substance	Added		Recovered	
	P.p.m.	$\mu$ g. <sup>a</sup>	$\mu$ g. <sup>b</sup>	%
Water	0	0	0	...
	0.0001	0.005	0.005	100
	0.0002	0.010	0.010	100
	0.0004	0.020	0.015	75
	0.0006	0.030	0.030	100
	0.0008	0.040	0.028	70
	0.0010	0.050	0.050	100
	0.0020	0.100	0.105	105
		Av.	92	
Silt	0	0	0	...
	0.005	0.05	0.041	82
	0.010	0.10	0.081	81
	0.020	0.20	0.14	70
	0.040	0.40	0.35	88
	0.050	0.50	0.44	88
			Av.	83

<sup>a</sup> Per 50 grams of water and 10 grams of silt.

<sup>b</sup> Average of one to four analyses.

height and resolution were improved by increasing the column temperature from 200° to 215° C.

Using the stated conditions, the retention time of Dursban on the F & M chromatograph, operating with a column temperature of 200° C., was 5.8 minutes; at 215° C., 4.1 minutes. The retention time on the Jarrell-Ash instrument using the stated conditions was 2.7 minutes.

The results of typical analyses of Dursban insecticide in fortified and unfortified samples of water and silt are given in Table I. The concentration ranges from 0.0001 to 0.002 p.p.m. in water and 0.005 to 0.050 p.p.m. in silt were evaluated. Recovery averaged 92% in water and 83% in silt over the range studied. In the materials studied, control values were negligible at the lowest sensitivity limit. The determinations in silt were limited to 0.005 p.p.m. because substances not removed during cleanup interfered below this limit.

A series of standard solutions of Dursban insecticide in *n*-hexane, ranging in concentration from 0.001 to 0.20  $\mu$ g. per ml., was prepared and chromatographed, and response (peak height) was recorded. Based on these data, a standard solution, producing approximately the same response as a test sample, was injected into the chromatograph after each analysis to determine the concentration of the material accurately. Such a procedure corrects for any variations in the response of the electron-capture detector.

Figure 1 shows typical chromatograms of extracts from control and fortified control samples of water and silt.

The solvents and volumes used in the procedures for water and silt permit determination of Dursban insecticide in these substances over a wide range of composition. Water analyses were performed equally well with fresh, brackish, and salt water. In all cases, the silicic acid column cleanup step was necessary. All water was from natural sites. When river water samples of unusual clarity (for this work) were carried through to detection without

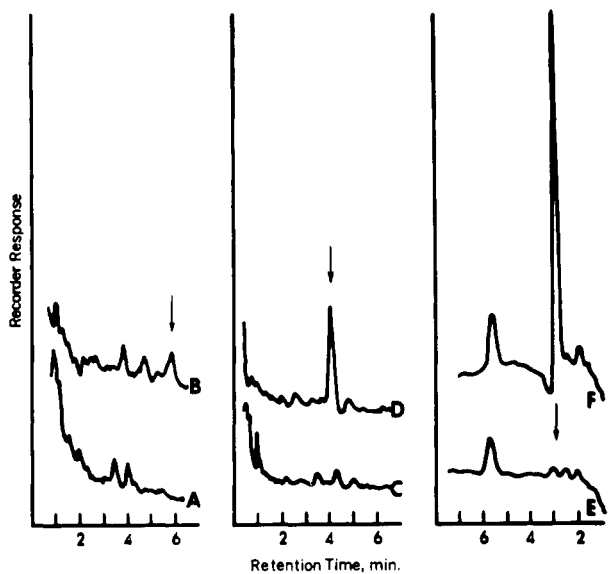


Figure 1. Typical chromatograms of Dursban insecticide. Arrows indicate retention time of *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate at column temperatures of *A-B*, 200° C.; *C-D* and *E-F*, 215° C.

- A.* Control water
- B.* Recovery at 0.0001-p.p.m. level
- C.* Control silt
- D.* Recovery at 0.01-p.p.m. level
- E.* Control silt
- F.* Recovery at 0.05-p.p.m. level

column cleanup, large peaks occurred near or at the retention time of residues, making quantitation of Dursban insecticide impossible. These peaks were removed by column cleanup, permitting accurate determination of the chemical. Several different soil types were analyzed successfully. No attempt has been made to classify them according to specific compositions. In general, those treated were sand, sandy loam, and clay. The term "silt" has been used here to describe the various sedimentary deposits common to ponds and marshes, the substrates with which this work is largely concerned.

Of the solvents tried, methylene chloride was the most effective in the extraction of Dursban insecticide from water and from silt. Since all traces of the methylene chloride were removed before final redissolving of the residue in *n*-hexane, no interference with the electron-capture detection system was experienced.

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