Rapid Gas Chromatographic Method for Analysis of *O,O*-Diethyl *O*-3,5-6-Trichloro-2-Pyridyl Phosphorothioate (Dursban) in Turkey and Chicken Tissues

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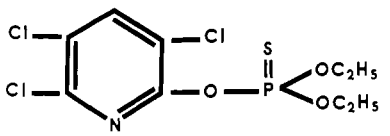
A rapid gas chromatographic method for determining *O,O*-diethyl *O*-3,5-6-trichloro-2-pyridyl phosphorothioate (Dursban) insecticide in turkey and chicken tissues is described. The residues are extracted with petroleum ether and aliquots analyzed without prior cleanup by gas chromatography using a nonpolar column and high temperature electron-capture detector. Residues as low as 0.05 p.p.m. can be detected. Recoveries of Dursban from tissues ranged from 72 to 99%. Samples of body tissues from birds dusted or fed various levels of Dursban were analyzed. Residues were found only in the skin and fat tissues.

Dursban insecticide (Dow Chemical Co.), *O,O*-diethyl *O*-3,5-6-trichloro-2-pyridyl phosphorothioate has been reported by Price (1967) to be effective for control of the chigger *Neoschongastia americana* (Hirst).



The lesions caused by the chigger feeding on the skin of turkeys has become a serious economic problem. The birds are downgraded when marketed because of the skin lesions resulting in economic loss to turkey producers. A rapid microquantitative method for the determination of Dursban in the tissues of poultry is necessary to determine detectable residues which might occur in birds exposed directly or accidentally to a Dursban application. As of January 1969, Dursban is not registered for use on poultry for chigger control.

Procedures for determining Dursban by gas chromatography have been developed (Claborn *et al.*, 1968; Gutenmann *et al.*, 1968; Rice and Dishburger, 1968). However, these methods involve elaborate and time-consuming extractions and cleanups. This paper describes a rapid, accurate method for analyzing a number of turkey and chicken tissues for Dursban.

EXPERIMENTAL

Birds were obtained from a commercial hatchery at one day of age and cared for at this laboratory. Birds on the feeding-trial were weighed at 12 weeks of age and dosed at 25, 50, and 100 p.p.m. Each bird was reweighed weekly and dosage recalculated. The phosphorothioate compound was incorporated in the feed and total consumption recorded. A 25% wettable powder (WP) formulation of the phosphorothioate compound was employed for both oral and dermal studies. The dosage was calculated on the basis of 100% of the active compound and reported in p.p.m. Treatment continued for a 4-week period or until death resulted.

Birds were dusted at 12 to 20 weeks of age with 15, 20, and 25 mg./kg. with a 25% WP. The compound was applied to the skin and feathers with the aid of a plastic shaker. Tissue samples were obtained at 9 and 28 days post-treatment from poultry dusting and feeding tests (Schlinke *et al.* 1969), (Schlinke, 1969) which were part of a preliminary toxicological study of this insecticide.

Preparation of Standard Curve and Fortified Samples. A series of Dursban standard solutions in petroleum ether from 0.2 to 0.025 ng./μl. were prepared. Two-microliter aliquots of each standard were injected into the gas chromatograph, and from these data a standard curve was plotted. Retention time for Dursban is approximately 3.5 minutes for constant volumes. Peak height is directly proportional to concentration.

To prepare the fortified samples, standards containing 12.5 and 25 ng. of Dursban in petroleum ether were added directly to 250-mg. tissue samples. Solvent was allowed to air dry. Samples were then extracted and chromatographed.

Extraction. A modified procedure of Radomski and Fiserova-Bergerova (1965) was used for the extraction. Muscle, skin, heart, gizzard, brain, fat, and liver samples were obtained. All samples, with the exception of skin, were washed in cold distilled H_2O to remove traces of blood. Entire organs were then sliced and ground in a Latapie tissue grinder. Each sample was mixed thoroughly and a 250-mg. aliquot weighed into a 7-ml. glass tissue grinder. Three milliliters of petroleum ether was added and samples were ground until the tissue was completely dispersed. The extraction solvent was transferred to a 15-ml. centrifuge tube. The tissue was re-extracted with a small volume of petroleum ether. Extracts were combined and volume was corrected to 5 ml. with petroleum ether. The extracts were dried with

Table I. Recovery of Dursban from Body Tissues of Poultry

Tissue (chicken and turkey)	Added		Recovered[a]	
	Ng.[b]	P.p.m.	Ng.[b]	%
Muscle	12.5	0.05	11.75	94
	25.0	0.10	22.50	90
Skin	12.5	0.05	11.75	94
	25.0	0.10	22.50	90
Heart	12.5	0.05	11.75	94
	25.0	0.10	23.50	94
Gizzard	12.5	0.05	7.75	82
	25.0	0.10	22.00	88
Brain	12.5	0.05	11.75	94
	25.0	0.10	22.50	90
Liver	12.5	0.05	9.00	72
	25.0	0.10	18.00	72
Fat	12.5	0.05	12.00	96
	25.0	0.10	24.75	99

[a] Mean of three determinations.
[b] Per 250 mg. of tissue sample.

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Table II. Residues of Dursban Found in Body Tissues of Chickens and Turkeys

Fowl	Treatment	Days	Residues (P.P.M.)[a] Skin	Fat
CHICKENS	IN FEED	ON TREATMENT		
1	Control	...	[b]	[b]
2	Control	...	[b]	[b]
3	25 p.p.m.	28	[b]	0.181
4	25 p.p.m.	28	0.123	0.181
5	50 p.p.m.	28	0.123	0.544
6	50 p.p.m.	28	0.123	0.471
7	100 p.p.m.	28	0.384	0.870
8	100 p.p.m.	28	0.384	0.653
TURKEYS	IN FEED	ON TREATMENT		
10	Control	...	[b]	[b]
11	50 p.p.m.	14	[b]	0.121
12	50 p.p.m.	28	[b]	0.161
13	100 p.p.m.	28	[b]	0.343
TURKEYS	DUSTED	AFTER TREATMENT		
14	15 mg./kg.	9	0.191	[b]
15	20 mg./kg.	9	1.100	0.220
16	15 mg./kg.	28	[b]	[b]
17	20 mg./kg.	28	[b]	[b]
18	25 mg./kg.	28	[b]	[b]

[a] Less than 0.05 p.p.m. found in muscle, liver, heart, brain, and gizzard.
[b] Contained less than 0.05 p.p.m.

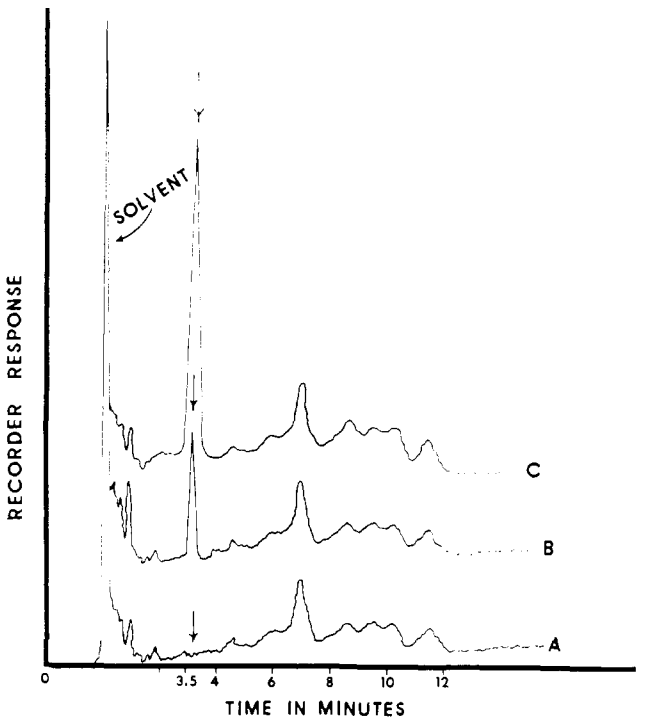

Figure 1. Typical chromatograms (fat)
A. Control
B. 100 p.p.m. in feed daily for 4 weeks
C. Fortified at 2 p.p.m.
Arrows represent retention time of *O,O*-diethyl *O*-3,5-6-trichloro-2-pyridyl phosphorothioate

100 mg. of anhydrous sodium sulfate and centrifuged for 5 minutes at 3000 RPM. Two microliters of tissue extract was injected into the gas chromatograph, and then diluted if necessary. The Dursban insecticide was determined quantitatively by comparison of peak height with the peak height of a standard solution. The concentration was calculated as parts per million (p.p.m.) based on the weight of the sample.

Gas Chromatography. A Micro-Tek, 2000-R, Model 2003 gas chromatograph equipped with a Ni^{63} electron-capture detector was used with a 1-mv. strip chart recorder. The following conditions were employed: column, 5 feet × 1/4-inch o.d. stainless steel; packing 5% SE-30 on 80- to 100-mesh Chromosorb W. DMAS; carrier gas, prepurified nitrogen at 90 ml. per minute. Temperatures, column, 215° C.; injection port, 250° C.; detector, 270° C.; electrometer setting, High 10 × 16.

RESULTS AND DISCUSSION

The results of analysis for Dursban in fortified samples of muscle, skin, fat, heart, gizzard, brain, and liver are given in Table I. Recovery of Dursban was 72 to 99% from body tissues of chickens and turkeys at 0.05 and 0.10 p.p.m. (Figure 1). Tissue samples of chickens and turkeys treated with Dursban were analyzed. Residues were found in fat and skin tissue (Table II).

This simplified method eliminates tedious and time consuming extraction and cleanup procedures normally associated with analysis of samples by electron-capture gas chromatography. A small amount of glassware is used, and the few steps involved greatly minimize the loss of the pesticide. The microsample size (1 gram or less) would allow the use of biopsy tissue. The procedure described is rapid; one person could easily run 30 samples a day. Satisfactory reproducibility and sensitivity are obtained.

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Received for review February 14, 1969. Accepted June 19, 1969.