Table IV. Reaction Products of Other Chlorinated Insecticides and Interference in Toxaphene Method

Insecticide	Product Color	Abs. Max., Mμ	% Apparent Toxaphene Recovery from Mixture[a]
Rhothane	Yellow	<420	96
DDT	Orange-red	490	102
Lindane	Colorless	None	102
Endrin, 1-hr. soln.	Lt. green	General	104
Heptachlor	Lt. green	General	107
Methoxychlor	Yellow-green	<420	113
Aldrin	Lt. purple	525, >700	129
Endrin, 72-hr. soln.	...	...	130
Dieldrin	Lt. purple	490, 680	132
Chlordan, 72-hr. soln.	...	...	138
Chlordan, 4-hr. soln.	Blue	650	175

[a] Approximately 300 γ of each insecticide combined with 300 γ of toxaphene just before analysis.

but one exception. Rice straw control extract was contaminated with toxaphene, or contained an impurity which was incompletely removed by Procedure A and reacted with diphenylamine. Recovery of 0 to 100 γ of toxaphene in the absence of plant extractives was 98 ± 10% from Procedure A after correction for the separation procedure blank.

Comparison of Spectrophotometric and Organic Chlorine Analyses. Grain extract analyses by spectrophotometric and organic chlorine (*5*) methods, corrected for the appropriate control, are in good agreement, as shown in Table III. Comparison of data obtained from the whole grain and mealed grain extracts of the same sample shows clearly that all of the toxaphene is at or near the surface. The barley contains about 2 p.p.m. of naturally occurring organic chlorine, which is extracted from the mealed samples only.

The organic chlorine method was not applied to many of the other samples because they had been treated with other chlorinated materials in addition to toxaphene or because they contained high and variable amounts of naturally occurring organic chlorine.

Interference from Solvents and Other Insecticides. Interferences in the spectrophotometric method for toxaphene occur, but can usually be detected and eliminated. A trace impurity in carbon tetrachloride and chloroform (possibly phosgene) gives an azure blue color with an absorbance maximum at 600 mμ. Redistilled methylene chloride is free of such impurities. Nitromethane gives the same type of interference and is not recommended for preliminary separations or cleanup. Sulfur, which may be present in dust formulations, gives an emerald green color with toxaphene in the diphenylamine method, and hydrogen sulfide is evolved during the fusion reaction. Sulfur is difficult to separate from toxaphene at the residue level, and greatly enhanced absorbance results if the amount present is more than 10% of the toxaphene residue. Interference in the assay procedure is eliminated by using a limited volume of methanol, in which sulfur has a very low solubility, as the extracting solvent. Pentachlorophenol, which may be sprayed just prior to harvest to reduce moisture in rice, does not interfere.

A number of other chlorinated insecticides have been tested alone and in 1 to 1 mixtures with toxaphene. All except lindane give some color, but absorbance at 640 mμ is weak for most. Aldrin, dieldrin, and chlordan have absorbance peaks at other wave lengths which overlap the 640-mμ band. Toxaphene recoveries from mixtures (300 γ of each insecticide) are shown in Table IV. A freshly prepared solution of endrin in acetone did not interfere, but upon aging the reactivity approached that of dieldrin and aldrin. Chlordan solutions in acetone showed a decrease in reactivity after aging, but interference in toxaphene determinations was still serious. In practice, no serious interference problem from other chlorinated organic insecticides in this method is anticipated, as 1 to 1 residue mixtures of toxaphene with dieldrin, aldrin, or chlordan are unlikely. They are seldom, if ever, applied together and dieldrin and aldrin are used at much lower dosage levels than toxaphene. There is no problem in distinguishing between chlordan and toxaphene, because methods specific for chlordan are known.

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INSECTICIDE RESIDUES

Toxaphene Residues on Pangolagrass

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THERE are approximately one-half million acres of improved pangolagrass in Florida, and toxaphene is one of the leading commercial insecticides recommended for the control of caterpillars on this pasture grass. One of the primary needs of all cattlemen at this time is definite information on a safe interval between pasture grass insecticide treatment and turning in beef cattle for indefinite periods of grazing.

This paper summarizes data involving toxaphene residues on pangolagrass exposed to the varying weather conditions of north, central, and south Florida

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A tolerance of 7.0 p.p.m. of toxaphene residues in the fat of beef cattle has been established by the Food and Drug Administration. Toxaphene residues were determined on pangolagrass sampled 2 hours, and 3, 7, and 14 days after treatment. Range grass treatments included three formulations and two dosage rates at three widely separated locations in the state. Seven days after insecticide application, all toxaphene residues on the pangolagrass were below 20.0 p.p.m., regardless of previous treatment. Two weeks after application, the highest toxaphene residues were 12.0 p.p.m. U. S. Department of Agriculture tests indicate that beef cattle may be fed 25 p.p.m. or less of toxaphene in the diet for 10 consecutive weeks without exceeding the beef fat tolerance. Therefore, a 7-day interval has been recommended to local cattlemen between toxaphene applications on pangolagrass and the time cattle are allowed to graze on the treated range.

in late summer. All three experiments were coordinated as much as possible, including a common source of three formulations of toxaphene, in order to eliminate differences other than climatic. Applications and samplings were supervised by staff entomologists who had previously decided on standardized mode of application and approximate height of grass at application time. Experiments were designed in randomized blocks and the pangolagrass was sampled and extracted in a uniform manner.

Materials and Methods

At all locations a plot size of 1/100 acre (21 $\times$ 21 feet) was used. Each treated field and check plot was replicated three times at the three locations (location A, Gainesville; location B, Bradenton; and location C, Belle Glade). Adequate border areas were maintained to avoid excess contamination between treatments. One insecticide application was made using 1 and 2 pounds of active ingredient of the following formulation: 10% dust, 40% wettable powder (W.P.), and 60% emulsifiable concentrate (E.C.). Random sampling of grass was performed in nine individual 1-sq. foot areas in each plot per cutting. The grass from these nine subsamples was cut into 1-inch lengths and mixed and a 100-gram sample was extracted with 600 ml. of *n*-hexane for 1 hour. Extractions were placed in sealed Mason jars and stored at 40° F. until analyzed.

Considerable effort was made to have all coworkers apply the toxaphene to grass of uniform height. However, because of factors beyond control, at locations A and B the toxaphene was applied to grass approximately 18 to 24 inches high, whereas at location C it was applied to grass only 10 to 15 inches high. Variations in height could have considerable effect on the calculated parts per million, particularly in the first samplings.

All samples were analyzed by the colorimetric diphenylamine procedure for toxaphene (*4*), with minor modifications. Chromatographic columns used for cleanup were 19 mm. in inside diameter and were packed to a height of 18 cm. with 60- to 100-mesh Florisil previously conditioned at 120° C. for 7 hours in a forced-draft oven. Elution curves were run on each new batch of Florisil. A typical elution curve is illustrated in Figure 1. For all batches of Florisil treated, 90 to 95% of the toxaphene added to the chromatographic column generally came through in the 80- to 130-ml. elution fraction. Therefore, depending on the Florisil batch, a 50- to 60-ml. wax forecut was discarded before the eluent was collected for analysis. On the basis of elution curve studies, approximately 95% of the toxaphene was collected from the column subsequent to the wax forecut.

Standard curves were run in the 100- to 700-γ toxaphene range. Table I indicates recoveries observed using untreated pangolagrass extract and carried through entire cleanup and subsequent analytical steps.

Results

The toxaphene residue data obtained in these studies are indicated in Table II. Only insignificant differences in relative humidity and mean daily temperatures were recorded at the three locations. However, considerable rainfall occurred during the critical first 3-day period at locations A and B, whereas practically no rain fell at location C during this period. Locations A and C reported rather consistently heavy precipitation between the 3- and 14-day sampling dates, whereas at location B less than 0.5 inch of rainfall was recorded between the 3- and 7-day and the 14-day samplings.

The rather high initial residues at location C can probably be partially explained by the fact that the grass was considerably shorter than at the other two locations. Despite the initial high values, the final toxaphene residues were the lowest at this location. Contrary to the data from the other two locations, the Belle Glade initial toxaphene residues, resulting from the 10% dust treatment, were significantly higher than from the wettable powder or emulsifiable concentrate treatments. However, at the 3-day sampling, the location C samples had the highest residues following the wettable powder treatment. The 7- and 14-day samplings at Belle Glade resulted in the highest toxaphene residues following the emulsifiable concentrate treatments. At Bradenton and Gainesville the emulsifiable concentrate treatment resulted consistently in the highest toxaphene residues, followed closely by the wettable powder applica-

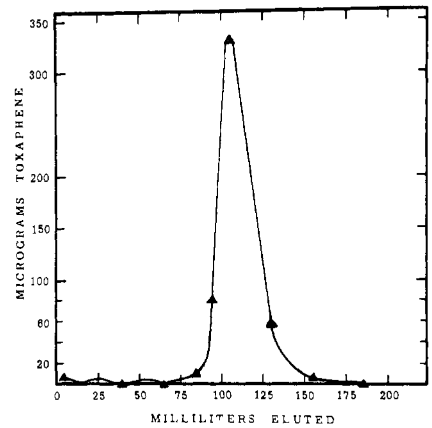
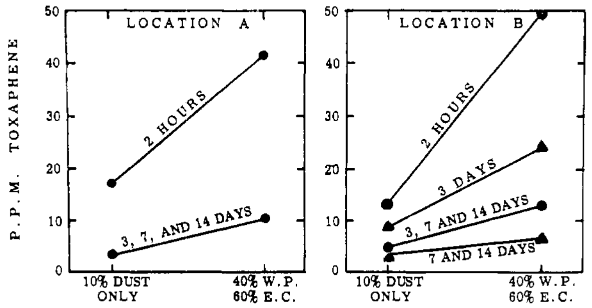


Figure 1. Toxaphene residue in hexane extract of pangolagrass

496 γ of toxaphene added to 100 ml. of extract 18-cm. column packed to 15-cm. height

Table I. Toxaphene Recovery from 100-Ml. Samples of Pangolagrass Hexane Extract

Toxaphene Added, γ	*Recovery, %*
100	100
200	102
300	95
400	97
500	98
600	97
700	103
Av.	98.9



TOXAPHENE RESIDUES ON PANGOLAGRASS

Figure 2. Sampling periods after application of toxaphene

Location A. Significant interaction. Sampling × formulation ($S_3 \times F_2$)

S_3. 2-hour sampling vs. 3-, 7-, and 14-day samplings combined

F_2. 10% dust formulation vs. 40 % W.P. and 60 % E.C.

Location B. Significant interactions. Sampling × formulation ($S_2 \times F_2$), ($S_3 \times F_2$)

S_2. 3-day vs. 7- and 14-day samplings

S_3. 2-hour vs. 3-, 7-, and 14-day samplings

F_2. 10 % dust vs. 40 % W.P. and 60 % E.C.

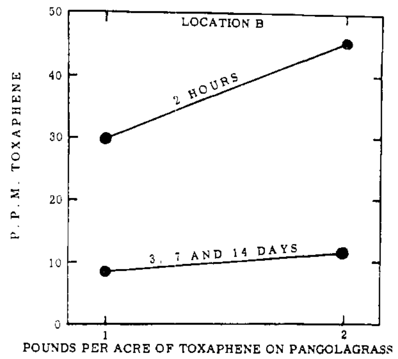


Figure 3. Sampling periods after application

Significant interactions. Sampling × level ($S_3 \times L$)

S_3. 2-hour vs. 3-, 7-, and 14-day samplings combined

L. Pounds per acre of technical insecticide applied

tion. The authors have no explanation for the apparent reversal of residue data for the 1- and 2-pound 10% dust treatment at location A.

Statistical Interpretations

Statistical analysis was performed on toxaphene residue data from identically designed field experiments at three widely separated areas of the state. Table III is an example of the analysis of variance of the Bradenton data, resulting in significant first-order interactions. The pooled error mean square (46 degrees of freedom) was used to test the significance of $S \times F \times L$ interaction. As this second-order interaction was significant at the 5% level, its mean square value was used to test the component first-order interactions for possible significance. The significant first-order interactions were used to test for possible significance of their component main effects. In all three experiments the main effect, S_3(2 hours *vs.* 3, 7, and 14 days), was highly significant. This is to be expected, as during the first few days after the last application of an insecticide, the toxicant usually disappears most rapidly. The only other main effect that showed consistent significance at all locations was F_2, a comparison of the 10% dust treatment *vs.* the 40% wettable powder and 60% emulsifiable concentrate treatments combined.

As it is somewhat difficult to explain a significant first-order interaction, graphical presentation simplifies interpretations. The less the degree of parallelism between the two lines being compared, the greater the significance of the interaction. The significant interaction ($S_3 \times F_2$) for location A is shown graphically in Figure 2. At the initial sampling (2 hours after application) the toxaphene residues due to the 10% dust treatment are significantly lower in comparison with the residues from treatment with the 40% wettable powder or 60% emulsifiable concentrate. However, the toxaphene residues on samples taken 3, 7, and 14 days after application from the 10% dust plots were much more comparable to those found on the grass following the other two formulations. In other words, the great dif-

Table II. Toxaphene Residues on Pangolagrass

(Expressed in parts per million and average of three field replications. Dosages expressed in active ingredient per 100 gallons per acre)

Test Location	R. H., %[a]	Temp., °F.[b]	Rainfall, Inches[c]	Sampling Dates[d]	10% Dust		40% W.P.		60% E.C.	
					1 lb.	2 lb.	1 lb.	2 lb.	1 lb.	2 lb.
Gainesville (Loc. A)				2 hours	19.9	13.9	22.7	52.3	35.7	55.4
	Min. 51 Max. 100	82.2	1.67	3 days	4.5	3.1	7.5	14.9	8.8	22.8
	Min. 56 Max. 100	79.6	1.23	7 days	3.4	2.4	5.4	10.9	7.5	18.2
	Min. 55 Max. 100	80.8	1.80	14 days	3.0	1.9	4.4	9.3	4.9	12.0
Bradenton (Loc. B)				2 hours	10.8	15.0	33.4	60.2	45.0	61.4
	Noon 70 Mid. 98	80.7	1.83	3 days	6.6	8.8	17.3	23.5	24.3	31.7
	Noon 81 Mid. 97	79.9	0.46	7 days	2.8	4.3	6.5	8.5	7.1	9.8
	Noon 74 Mid. 97	81.5	0.47	14 days	2.0	3.2	3.9	6.7	5.2	8.6
Belle Glade (Loc. C)				2 hours	193.1	277.2	86.0	166.2	103.3	138.0
	Min. 47 Max. 100	80.2	0.28	3 days	11.8	30.0	20.8	37.2	17.5	32.2
	Min. 50 Max. 100	80.6	1.51	7 days	3.9	8.9	4.6	11.4	10.6	14.9
	Min. 50 Max. 100	79.6	1.84	14 days	1.3	1.7	1.6	1.9	2.4	6.3

[a] Average daily relative humidity for interval between indicated and previous sampling date.
[b] Average daily temperature for interval between indicated and previous sampling date.
[c] Rainfall for period between indicated and previous sampling date.
[d] Time interval between field application and sampling date.

ference in residues between the 10% dust and the other formulations 2 hours after treatment was much less apparent on subsequent samplings following the insecticide application.

Also in Figure 2, two significant first-order interactions ($S_2 \times F_2$ and $S_3 \times F_2$) selected from location B experimental data are graphically illustrated. Both interactions also indicate that the large differences in toxaphene residues immediately after application resulting from the 10% dust and the other two formulations combined are decreased with each subsequent sampling. After 2 weeks of weathering, very little difference in residues was found among the three formulations. Graphically illustrated in Figure 3 is the significant interaction $S_3 \times L$ for location B. This interaction indicates that at the initial sampling (2 hours after application) the difference in toxaphene residues resulting from 1 pound of active ingredient per acre is significantly lower than from 2 pounds per acre. This difference became less apparent with each subsequent sampling date following the insecticide applications.

Table III. Analysis of Variance of Toxaphene Residues Remaining on Pangolagrass

(Location B, August 1958)

Variance Source	Degrees of Freedom	Sums of Squares	Mean Squares	F Value Pooled	F Value $S \times F \times L$	F Value $S \times F$
Blocks, B	2	85				
Samplings, S						
S_1	1	22	22			
S_2	1	2025	2025			N.S.[a]
S_3	1	10238	10238			20.1[b]
Formulations, F						
F_1	1	207	207			N.S.[a]
F_2	1	3807	3807			7.5[c]
$S \times F$	6	3059	510		12.7[b]	
$S_2 \times F_2$	1	421	421		10.5[b]	
$S_3 \times F_2$	1	2535	2535		63.2[b]	
Levels, L	1	743	743		18.5[b]	
$S \times L$	3	565	188		4.7[b]	
$S_3 \times L$	1	539	539		13.4[b]	
$F \times L$	2	165	83		N.S.[a]	
$S \times F \times L$	6	241	40	2.8[b]		
Error						
$S \times B$	6	72	12			
$F \times B$	4	78	19			
$S \times F \times B$	12	145	12			
$L \times B$	2	111	55			
$S \times L \times B$	6	41	7			
$F \times L \times B$	4	50	13			
$S \times F \times L \times B$	12	167	14			
Total	71					
Pooled error	46	663	14			

S_1 7 days *vs.* 14 days.
S_2 3 days *vs.* 7 and 14 days
S_3 2 hours *vs.* 3, 7, and 14 days

F_1 40% W.P. *vs.* 60% E.C.
F_2 10% dust *vs.* other formulations

[a] Not significant.
[b] Significance at 5% level.
[c] Significance at 1% level.

Discussion

A tolerance of 7.0 p.p.m. of toxaphene in beef cattle fat has been established by the Food and Drug Administration. In the three experiments reported here, the toxaphene residues following the highest dosage treatments were all below 20.0 p.p.m., 7 days after the grass had been sprayed or dusted. Two weeks after treatment, the highest toxaphene residues were 12 p.p.m. Allowing for the normal toxaphene breakdown pattern, toxaphene residues on the grass plots treated with the higher dosages should have been somewhat below 10 p.p.m. 3 weeks after treatment.

Normally, in Florida an insecticide is applied to pasture grass only once during a grazing period. A field may be grazed from 3 to 4 weeks to 3 to 4 months, depending on number of head grazed, weather, etc. Consequently, even following the 2 pounds of active toxaphene application per acre, grazing cattle would not be ingesting more than 10 p.p.m. in the grass after the 3-week interval subsequent to treatment. If cattle were allowed in a field treated with 2 pounds of active ingredient per acre 1 week after one toxaphene application, it could be estimated that during a subsequent 1-month grazing period the cattle would ingest in daily grass intake the following quantities of toxaphene:

Grazing Week	P.P.M.	Grazing Week	P.P.M.
1st	12–20	3rd	7–9
2nd	9–12	4th	5–7

Claborn *et al.* (*2*, *3*), after extensive U.S. Department of Agriculture tests at Kerrville, Tex., presented data on toxaphene residues in the fatty tissues of steers and heifers. This study indicated that any feed containing less than 25 p.p.m. of toxaphene residue can be fed to beef cattle daily up to 10 consecutive weeks without exceeding the beef fat tolerance at slaughtering. Of the nine chlorinated hydrocarbon insecticides tested, toxaphene tended to be the least stored in the fatty tissues of cattle or sheep. Methoxychlor was the only chlorinated hydrocarbon tested that did not cause some detectable insecticide storage in the fat according to Carter *et al.* (*1*).

The insecticide residues in meat are present only in the fat of the meat. Because the edible meat of a medium fat carcass trimmed for retail trade contains approximately 20% fat, the parts per million of residues in edible meat may be obtained by dividing the results of the fat analysis by a factor of 5.

On the basis of residues remaining on three widely separated pangolagrass experiments in Florida and U. S. Department of Agriculture information on contaminated beef, a 7-day interval is recommended after a toxaphene application before the cattle are allowed to graze on the treated range. This interval should be more than sufficient, as no toxaphene application (up to 2 pounds of active ingredient per acre of three commercial formulations) resulted in residues over 20 p.p.m., 7 days after treatment.

Acknowledgment

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