

Procedures for Extracting Residues of Phosphorus Insecticides and Metabolites from Field-Treated Crops

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Nine procedures for removal of six phosphorus insecticides and their metabolites from field-treated crops were compared. Recoveries were best when Soxhlet extraction with 10% methanol in chloroform was continued until appreciable amounts of residues were no longer removed. Variables considered

include extraction procedure, solvent, temperature, duration of extraction, combinations of procedures, type of compound, formulation applied, nature of crop, amounts of coextractives, and extent of weathering.

Recent reports indicate that some widely used procedures for extracting pesticides from foods and crops are inadequate (Archer and Crosby, 1967; Bertuzzi *et al.*, 1967; Burke and Porter, 1967; Mumma *et al.*, 1966; Root, 1967; Wheeler and Frear, 1966; Wheeler *et al.*, 1967). These studies deal almost entirely with residues of chlorinated hydrocarbon pesticides.

The widespread attempt to replace the persistent chlorinated hydrocarbon pesticides with nonpersistent ones led us to investigate the efficiency of various methods of extracting residues of phosphorus insecticides. The problem is not simple, because these compounds are known to change or metabolize to others after application. The P=S (P → S) group in compounds can change to P=O (P → O, O-analogs), and sulfides can be oxidized to sulfides and sulfones; these residues may be as toxic as or more toxic than the parent compound. The chemist is therefore faced with the problem of extracting and analyzing a series of compounds that can have a range of polarities, different stabilities, and—with gas chromatographic analysis—widely differing retention times. Any cleanup that is required may cause loss of some of the metabolites and perhaps rule out the possibility of analysis.

The efficiency of several procedures in extracting six insecticides and nine of their metabolites from a number of crops was determined. The samples were usually field-treated and weathered specimens, since it has been demonstrated by others—e.g., Bertuzzi *et al.*, 1967—and ourselves that extraction procedures giving complete recovery of pesticides from fortified samples often did not give adequate recovery from field samples.

The following variables were considered in the study: extraction solvent and method, time of extraction, crop extracted, temperature of extraction, combination of extraction procedures, type of compound extracted, effect of weathering, and formulation of insecticide.

Although the pesticide and its metabolites were separated by liquid chromatography, no cleanup was needed or applied, since the phosphorus determinations were made by gas chromatography with the flame photometric detector which is specific enough in response to register the phosphorus compounds without responding to the extraneous material in the crop extract. Losses through cleanup were thus avoided.

Although the primary aim of this investigation was a means of attaining complete extraction of phosphorus pesticide residues, another goal was a quantitative picture of the concentrations of parent insecticide and metabolites that occur on crops during the weathering process following the application of an insecticide. Other objectives were a minimum of coextracted material, rapid extraction, and ease and simplicity of extraction.

MATERIALS AND METHODS

Apparatus. An F & M Scientific Corp. (Avondale, Pa.) Model 700 gas chromatograph equipped with the flame photometric detector of Brody and Chaney (1966) and a 526-m μ interference filter (detects phosphorus) was used. This detector is available from the Micro Tek Instruments Corp., Baton Rouge, La.

Reagents and Solvents. Hexane was refluxed over potassium hydroxide and distilled before use. Other solvents were C.P. grade redistilled liquids, except for methanol which was used as received.

Sodium sulfate was the anhydrous C.P. chemical. Adsorbents are described in the references to the methods.

Pesticides and Metabolites. Azodrin, Bidrin, Ciba C-9491, Dursban, fenthion, Imidan, and metabolites of these compounds that were used as standards were ana-

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lytical samples supplied by their manufacturers. Their formulas are given in Figure 1. The technical grade insecticide was applied to the crops in accordance with normal agricultural practice. Metabolites were not present in the technical products.

Crops. Except for one set of samples that was treated and ensiled for 30 days in jars, the crops were all treated in the field with the parent pesticide and then allowed to weather as indicated before analysis.

Preparation of Samples for Extraction Procedures. The samples were finely chopped (about 20 mesh) in a Hobart cutter, mixed well, and held in a freezer until extracted by one of the following procedures.

Blends. Fifty grams of crop, 150 ml. of solvent, and 50 grams of sodium sulfate were blended at high speed for 5 minutes in a Waring Blendor. The product was filtered on Whatman No. 1 filter paper and the filtrate stored over sodium sulfate at 0° C. until analysis. If samples were to be extracted subsequently under reflux, 20 grams of plant sample was blended as stated with 100 ml. of solvent and without sodium sulfate; the slurry was then transferred with 50 ml. of fresh solvent to the flask for refluxing.

Extraction under Reflux. Twenty grams of plant sample was placed in a flask with a ground-glass joint, washed in with 150 ml. of solvent, and refluxed for the specified time. The product was filtered on a Büchner funnel with Whatman No. 1 paper, the flask and filter were washed with small portions of fresh solvent, and the filtrate was passed through a plug of sodium sulfate. The total dried filtrate was equivalent to 20 grams of plant.

When studies on time of extraction were conducted, separate portions of sample were used.

Soxhlet Extraction. A plug of glass wool was placed on the bottom of the extraction tube of a Soxhlet apparatus

—e.g., Fisher Scientific Co., No. 9-556-B—to prevent plant material from siphoning over during extraction, and 20 grams of the plant material was added. Extractions were made with 150 ml. of the indicated solvent for the specified time. Extractions were at the rate of five to six exchanges of solvent per hour and were made under nitrogen to prevent possible air oxidation of the compounds. The extract was then passed through a plug of sodium sulfate.

When studies on time of extraction were conducted, flasks with fresh solvent were substituted at the appropriate time.

Cold Soxhlet Extraction. This extraction was identical to that of the regular Soxhlet extraction, except that the sample being extracted and the solvent in contact with the sample were cooled to prevent the temperature of the sample from exceeding ambient.

Preparation of Extracts for Gas Chromatography and Analysis. The procedures for preparing extracts for gas chromatography depended on the insecticide (and its metabolites) to be analyzed and have been described by Bowman and Beroza (Azodrin and Bidrin, 1967a; Ciba C-9491, 1968a; Dursban, 1967b; fenthion, 1968b; Imidan, 1966). The only departures from previous methodology were the extraction procedures applied to the raw crop itself. So that the comparisons would be as valid as possible, the procedural steps for each pesticide except the extraction were always carried out in exactly the same way on each sample.

The extracts of raw material were evaporated just to dryness with water pump vacuum and a water bath and reconstituted in the appropriate solvent, and the proper column separation was applied. The separated fractions were concentrated and made up to volume, and an aliquot was injected for gas chromatographic analysis.

The sensitivities of the gas chromatographic methods were at least 0.01 p.p.m.

RESULTS AND DISCUSSION

Inasmuch as several portions of the same sample were extracted by different procedures, every effort was made to be certain that each portion was truly representative of the whole sample. Since a certain amount of variation may be expected in spite of these precautions, differences in extraction efficiency less than 5% are probably not significant.

Every plant sample was checked by Soxhlet extraction with chloroform plus 10% methanol and by blending with a solvent that was known to yield near-quantitative recoveries of pesticide when crops were fortified with the pesticide. All residue results are expressed on a wet basis (except as noted); however, dry matter content is given for many samples. Insecticides and metabolites were absent from untreated samples.

Table I shows the efficiencies of extracting, by the various procedures, Coastal Bermuda grass treated in the field with 2 pounds per acre (2.24 kg. per hectare) of fenthion as an emulsifiable concentrate and weathered for 7 days. Fenthion may form five metabolites (Figure 1), and field exposure resulted in the formation of all five. Maximum recoveries were obtained by Soxhlet extraction with chloro-

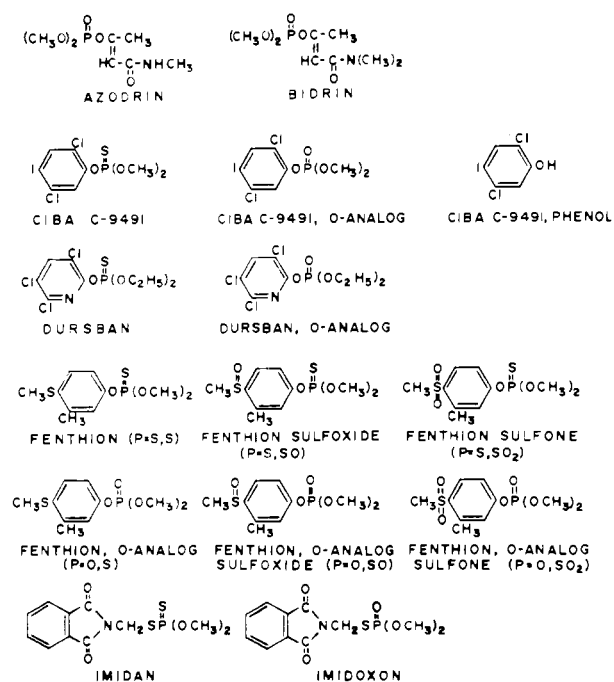


Figure 1. Formulas of insecticides and metabolites

Designations of fenthion and its metabolites in parentheses

Table I. Residues (P.P.M.) of Fenthion and Five of Its Metabolites in Coastal Bermuda Grass and Corn^a Determined by Nine Extraction Methods

Substrate	Solvent and Extraction Method	Extraction Time	Compound ^b					
			P=S, S	P=S, SO	P=S, SO ₂	P=O, S	P=O, SO	P=O, SO ₂
Coastal Bermuda grass	Benzene blend	5 min.	0.06	7.52	2.62	0.01	1.65	0.62
	Chloroform blend	5 min.	0.06	6.96	2.43	0.01	1.57	0.55
	Chloroform-10% methanol blend	5 min.	0.18	8.78	3.04	0.01	2.88	0.78
	Benzene Soxhlet	8 hr.	0.23	12.5	4.08	0.02	2.91	0.84
	Chloroform Soxhlet	8 hr.	0.25	12.0	4.19	0.02	2.82	0.80
	Chloroform-10% methanol Soxhlet	0-2 hr.	0.28	13.2	4.34	0.02	3.10	0.97
	Chloroform-10% methanol "cold" Soxhlet	2-8 hr.	0.01	0.10	0.04	0.00	0.00	0.00
	Chloroform-10% methanol reflux Soxhlet	0-2 hr.	0.23	11.0	3.95	0.02	3.00	0.77
	Chloroform-10% methanol reflux Soxhlet	2-8 hr.	0.05	0.87	0.32	0.00	0.00	0.00
	Chloroform-10% methanol reflux Soxhlet	1/2 hr.	0.18	12.2	3.77	0.01	2.90	0.91
	Chloroform-10% methanol reflux Soxhlet	1 hr.	0.19	12.0	3.75	0.01	2.85	0.91
	Chloroform-10% methanol reflux Soxhlet	2 hr.	0.22	12.2	4.21	0.02	3.00	0.94
	Blended with chloroform-10% methanol, then refluxed Soxhlet	1/2 hr.	0.18	11.7	3.64	0.01	2.85	0.91
Blended with chloroform-10% methanol, then refluxed Soxhlet	1 hr.	0.18	11.9	3.82	0.01	2.90	0.90	
Blended with chloroform-10% methanol, then refluxed Soxhlet	2 hr.	0.20	12.0	3.96	0.02	2.88	0.92	
Corn	Benzene blend	5 min.	0.022	1.47	0.42	0.00	0.62	0.17
	Chloroform blend	5 min.	0.019	1.35	0.39	0.00	0.57	0.18
	Benzene Soxhlet	8 hr.	0.067	2.97	0.97	0.00	0.96	0.35
	Chloroform Soxhlet	8 hr.	0.063	3.05	0.93	0.00	0.95	0.36
	Chloroform-10% methanol Soxhlet	8 hr.	0.070	3.59	1.03	0.00	1.05	0.38
	Chloroform-10% methanol Soxhlet	8 hr.	0.070	3.59	1.03	0.00	1.05	0.38

^a Treated with 2 pounds per acre of fenthion as an emulsifiable concentrate and weathered 7 days in the field (Tifton, Ga.). Dry matter contents of grass and corn were 38.4 and 31.2%, respectively. ^b See Figure 1 for compound designation.

form plus 10% methanol (by volume). The extraction was complete in 2 hours; an additional 6 hours of extraction yielded negligible amounts of the compounds, and the extraction was not quite complete in 1 hour. Where the plant material was kept at ambient temperature (cold) during Soxhlet extraction, complete extraction of some of the metabolites was delayed. The solvent is therefore more efficient hot than "cold." The 10% methanol in the chloroform definitely helped speed the extraction and made it more complete, since the 8-hour Soxhlet extraction with chloroform and no methanol tended to give results slightly below maximum. Chloroform containing 30% methanol was also tried as the solvent, but it removed much more plant material; this material interfered in the liquid column chromatography subsequently used to separate fenthion and its metabolites, and recoveries were no better with 30% than with 10% methanol in chloroform. [Wheeler *et al.* (1967) had indicated that 25 to 50% of the total phospholipids in fresh alfalfa and wheat were removed by Soxhlet extraction with 1-to-1 chloroform-methanol. The phospholipids could be a source of interference, since they could cause a response by the flame photometric detector, and the use of the lesser proportion of methanol in chloroform may avoid difficulty on this account.] An 8-hour Soxhlet extraction with benzene gave recoveries that were close to maximum but again tended to be consistently on the low side.

Extraction under reflux for 2 hours with chloroform-10% methanol gave recoveries that approached maximum values, and there was no observable advantage in blending prior to this extraction. (The crop was finely chopped.) Soxhlet extraction was preferable to extraction under reflux because no filtration was required, the operation was simpler, recoveries were slightly better, and the product appeared to be cleaner.

Efficiencies of the blending methods were low, although

the extraction with chloroform plus 10% methanol was more complete than extractions with either chloroform or benzene alone. The superiority of dual-solvent extractions over extractions with single solvents has been cited frequently—e.g., Klein *et al.* (1959). The low results obtained by blending suggest that it is inadequate for extracting phosphorus-containing pesticides.

The close structural similarity of fenthion and its five metabolites led us to search for correlations between the functional groups in the molecules and extraction efficiency. The P=O compounds appear to be more readily extracted from plant materials than the corresponding P=S compounds—for example, the three P=O compounds were completely removed in 2 hours by Soxhlet extraction with chloroform-10% methanol, but the three P=S compounds required more time, although the amounts of P=S compounds remaining unextracted after 2 hours were small, only 1 to 4%. When the plant material was maintained at ambient temperature in the Soxhlet extractor, the P=O compounds were again completely extracted in 2 hours; however, the amounts of P=S compounds that remained unextracted after 2 hours increased to 8 to 22%.

Table I also shows data on the extraction of fenthion and its five metabolites from another field crop, corn, treated in the same way. Of the five procedures tried, Soxhlet extraction with 10% methanol in chloroform again produced the best recoveries, but Soxhlet extraction with either benzene or chloroform was almost as effective. The blending extractions with benzene or chloroform were markedly inferior. These results with a second crop again assert the superiority of Soxhlet extraction with 10% methanol in chloroform over the other procedures tried. This superiority held for the five compounds present as residues.

Table II shows the residues of fenthion and five of its

metabolites found in Coastal Bermuda grass at different intervals after field treatment with fenthion at 2 pounds per acre as an emulsifiable concentrate when two methods of extraction were used. The inadequacy of extraction by blending with benzene compared with Soxhlet extraction with 10% methanol in chloroform is again evident. In each instance, when a residue was present, recovery was inferior with the blending procedure. The data on the Soxhlet extraction in Table II are plotted (semilog) in Figure 2. Rapid decline of the parent fenthion was accompanied by a buildup, then a decline of metabolites.

Corn treated with 1 pound of Dursban per acre as an emulsifiable concentrate and ensiled 48 days was extracted by three procedures; the following results were obtained:

Extraction Method	Dursban, P.P.M.
Benzene blend	2.83
Chloroform-10% methanol Soxhlet	
0-2 hr.	3.35
2-4 hr.	0.62
4-6 hr.	0.23
6-8 hr.	0.05
Chloroform-10% methanol reflux (2 hr.)	3.08

Again the highest recovery of insecticide was obtained by Soxhlet extraction with 10% methanol in chloroform, an 8-hour extraction being required for complete recovery.

The recoveries from corn treated with Azodrin and Bidrin and ensiled in jars for 30 days follow. (Insecticides were added to the chopped corn in acetone solution prior to aging.)

Extraction Method	Insecticide	Recovery, P.P.M.
Chloroform blend	Azodrin	15.4
Chloroform-10% methanol Soxhlet		
0-2 hr.	Azodrin	15.3
2-6 hr.		0.00
Chloroform blend	Bidrin	13.9
Chloroform-10% methanol Soxhlet		
0-2 hr.	Bidrin	17.2
2-6 hr.		0.00

Table II. Residues (P.P.M.) of Fenthion and Five of Its Metabolites in Coastal Bermuda Grass^a at Various Intervals Determined by Two Extraction Methods

Days after Application	Rainfall, Inch ^b	Dry Matter Content, %	Extraction Method ^c	Compound ^d					
				P=S, S	P=S, SO	P=S, SO ₂	P=O, S	P=O, SO	P=O, SO ₂
0	...	51.8	S	47.2	75.0	1.95	0.05	1.81	0.00
			B	26.1	43.8	0.82	0.02	1.39	0.00
1	...	39.6	S	8.12	62.5	2.85	0.08	4.68	0.25
			B	3.80	36.9	1.96	0.03	3.22	0.11
2	...	35.1	S	3.77	52.6	4.90	0.04	6.46	0.58
			B	1.24	24.8	2.36	0.02	2.85	0.33
7	0.7	38.4	S	0.28	13.2	4.34	0.02	3.10	0.97
			B	0.06	7.52	2.62	0.01	1.65	0.62
14	0	40.4	S	0.049	2.23	2.15	0.00	0.69	0.25
			B	0.020	0.85	1.05	0.00	0.31	0.14
21	0.6	41.3	S	0.030	0.53	0.92	0.00	0.18	0.05
			B	0.014	0.32	0.52	0.00	0.10	0.02

^a Treated in the field with 2 pounds per acre of fenthion as an emulsifiable concentrate. ^b Between samplings. ^c S = Soxhlet extraction with chloroform-10% methanol for 2 hours. B = blended with benzene for 5 minutes. ^d See Figure 1 for compound designation.

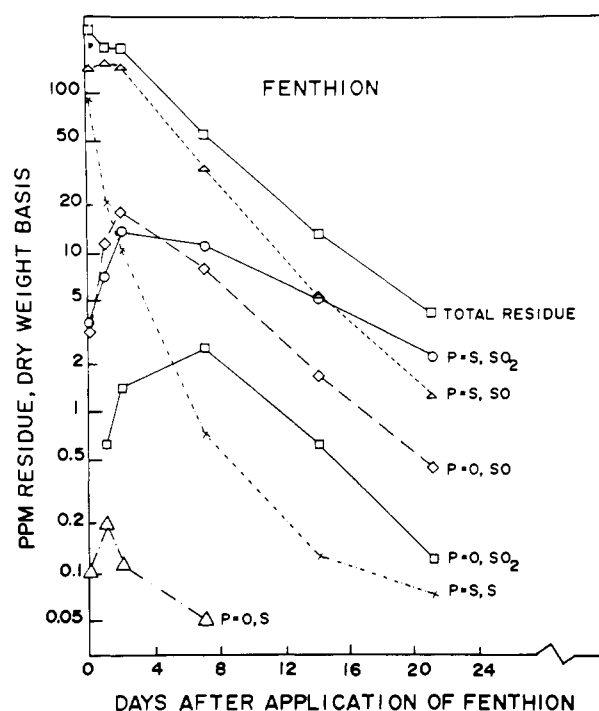


Figure 2. Residues of fenthion and five metabolites in Coastal Bermuda grass

After application of 2 pounds of fenthion per acre as an emulsifiable concentrate at Tifton, Ga., Aug. 8, 1967. See Figure 1 for designations of fenthion and its metabolites

Although maximum values were obtained by Soxhlet extraction with 10% methanol in chloroform, blending with chloroform also gave maximum recovery of Azodrin; the recovery of Bidrin by blending was low.

Pesticide residues were determined by two methods on coastal Bermuda grass weathered 0, 1, 4, and 8 days after field treatment with insecticide at 1 pound per acre. Table III shows that the residues of Bidrin and Azodrin found after blending with chloroform were within about 10% of those found after Soxhlet extraction with 10% methanol in chloroform for 2 hours, the period required for com-

Table III. Residues (P.P.M.) in Coastal Bermuda Grass at Various Times after Field Treatment with Bidrin and Azodrin^a Determined by Two Extraction Methods

Insecticide Applied	Days after Application	Dry Matter, %	Chloroform Blend		Chloroform-10% Methanol Soxhlet (2 Hr.)	
			Bidrin	Azodrin	Bidrin	Azodrin
Bidrin	0	38.8	23.2	0.00	26.8	0.00
	1	43.0	31.8	0.18	34.2	0.20
	4	44.2	21.8	0.32	24.6 ^b	0.32 ^b
	8	47.5	11.9	0.27	12.4	0.30
Azodrin	0	36.6	...	24.0	...	26.0
	1	47.0	...	29.0	...	30.8
	4	44.1	...	20.0	...	19.2 ^c
	8	45.6	...	9.52	...	10.1

^a Insecticide applied at 1 pound per acre as an emulsifiable concentrate. No rainfall on crop.

^b An additional 2 hours of Soxhlet extraction of this sample yielded 0.03 p.p.m. Bidrin and no measurable Azodrin.

^c An additional 2 hours of Soxhlet extraction of this sample yielded 0.03 p.p.m. Azodrin.

plete extraction of the pesticides. (A plot of the data is shown in Figure 3.) Recoveries by the blending procedure using benzene were seriously below those obtained by Soxhlet extraction with 10% methanol in chloroform for Ciba C-9491, its oxygen analog, and its phenol, Dursban and its oxygen analog (Table IV), Imidan, and Imidoxon (Table V). The data on Soxhlet extraction in Tables IV and V are plotted in Figure 4 to show the amount of parent and metabolites recovered. Table V shows no great difference in residues of Imidan and its oxygen analog Imidoxon following application of Imidan either as an emulsifiable concentrate or an aqueous acetone suspension. Also, the presence of the emulsifier did not seem to affect the recovery of the compounds by the two extraction methods—i.e., results from the blending were about one half those obtained by Soxhlet extraction for all samples, regardless of the time of weathering.

Wheeler *et al.* (1967) studied the extraction of dieldrin from wheat and grass and compared the efficiency of extraction by blending with 2-to-1 hexane-isopropyl alcohol *vs.* overnight Soxhlet extraction with 1-to-1 chloroform-methanol. They found that the effectiveness of the blending procedure decreased with successive cuttings (or samplings) of the plants. A comparison of the blending and Soxhlet extraction data in Tables II through V did not show a consistent trend of this kind.

Acetonitrile was also used in extractions under reflux and in Soxhlet extractions, but recoveries were invariably lower than those obtained in the 10% methanol-chloroform Soxhlet extraction, even though the acetonitrile extracts contained more plant extractives. Also, the evaporation of the acetonitrile left a gummy residue that would not dissolve completely in the benzene or chloroform required in the procedures used for the subsequent column separations of the metabolites and the parent insecticide; the residue was even difficult to dissolve in acetonitrile. Moreover, the evaporation of acetonitrile caused losses of pesticide residues. Thus, the analysis of a control extract fortified before and after the evaporation indicated that 40 to 50% of the fenthion and metabolite residues added were "lost" during evaporation. In a repetition of this same experiment with the 10% methanol-chloroform Soxhlet extraction, no residue was lost. Possibly acetonitrile may be a satisfactory solvent for extraction if its use is accompanied by transfer of the pesticide to another

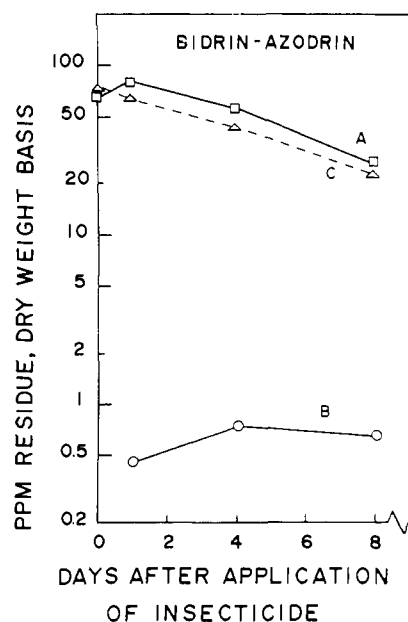


Figure 3. Residues of Bidrin (A) and Azodrin (B) in Coastal Bermuda grass

After application of 1 pound of Bidrin per acre as an emulsifiable concentrate. Residues of Azodrin (C) after application of 1 pound of Azodrin per acre as an emulsifiable concentrate

solvent (thus avoiding the acetonitrile evaporation) and subsequent cleanup; those advocating the use of acetonitrile for extraction (usually for chlorinated hydrocarbons) have always employed such a cleanup (Bertuzzi *et al.*, 1967; Burke and Porter, 1967; Johnson, 1962; Root, 1967) which included the use of column chromatography. For the organophosphorus pesticides of this study the 10% methanol-chloroform extraction was preferable because a cleanup was not required, a feature which is especially welcome because the differences in solubility and other properties of each insecticide and its metabolites would make the development of such a cleanup procedure difficult, and a different procedure would be needed for each pesticide.

Thus, in the present study, the best method of extraction in every instance was Soxhlet extraction of the finely chopped crop with 10% methanol in chloroform for whatever time was necessary to accomplish maximum removal

Table IV. Residues (P.P.M.) in Coastal Bermuda Grass at Various Times after Field Treatment with Ciba C-9491 and Dursban^a Determined by Two Extraction Methods

Insecticide Applied	Days after Application	Dry Matter, %	Benzene Blend			Chloroform-10% Methanol Soxhlet (4 Hr.)		
			C-9491	O-analog	Phenol ^b	C-9491	O-analog	Phenol ^b
Ciba C-9491	0	39.5	28.1	0.049	0.68	42.6	0.126	1.31
	1	45.9	22.3	0.116	0.77	35.9	0.254	1.35
	4	44.9	13.4	0.250	1.31	24.1 ^c	0.493 ^c	2.38 ^c
	8	47.2	9.38	0.207	1.54	17.0	0.416	2.52
Dursban			Dursban	O-analog		Dursban	O-analog	
	0	39.2	17.3	0.07		35.0	0.15	
	1	45.2	15.5	0.08		30.2	0.17	
	4	45.5	11.2	0.04		20.1 ^d	0.09 ^d	
	8	46.1	6.90	0.02		13.2	0.05	

^a Insecticide applied at 1 pound per acre as an emulsifiable concentrate. No rainfall on crop.

^b Analyzed by electron-capture gas chromatography as described by Bowman and Beroza (1968a).

^c An additional 4 hours of extraction of this sample yielded in p.p.m. 0.18 C-9491, <0.002 O-analog, 0.014 phenol.

^d An additional 4 hours of extraction of this sample yielded 0.18 p.p.m. of Dursban and none of its O-analog.

Table V. Residues (P.P.M.) in Coastal Bermuda Grass at Various Times after Field Treatment with Imidan^a in Different Formulations Determined by Two Extraction Methods

Formulation	Days after Application	Dry Matter, %	Benzene Blend		Chloroform-10% Methanol Soxhlet (4 Hr.)	
			Imidan	Imidoxon	Imidan	Imidoxon
Emulsifiable concentrate	0	36.5	23.5	0.04	49.3	0.10
	1	41.8	21.1	0.08	48.8	0.18
	4	43.1	19.2	0.40	37.2 ^b	0.82 ^b
	8	47.6	16.9	0.38	28.1	0.62
Aqueous acetone suspension	0	34.9	23.6	0.06	41.1	0.11
	1	43.8	29.5	0.09	56.6	0.19
	4	45.2	16.5	0.50	34.8 ^c	0.90 ^c
	8	45.1	9.2	0.40	18.3	0.66

^a Insecticide applied at 1 pound per acre. No rainfall on crop.

^b An additional 4 hours of extraction of this sample yielded 0.10 p.p.m. Imidan and no Imidoxon.

^c An additional 4 hours of extraction of this sample yielded 0.07 p.p.m. Imidan and no Imidoxon.

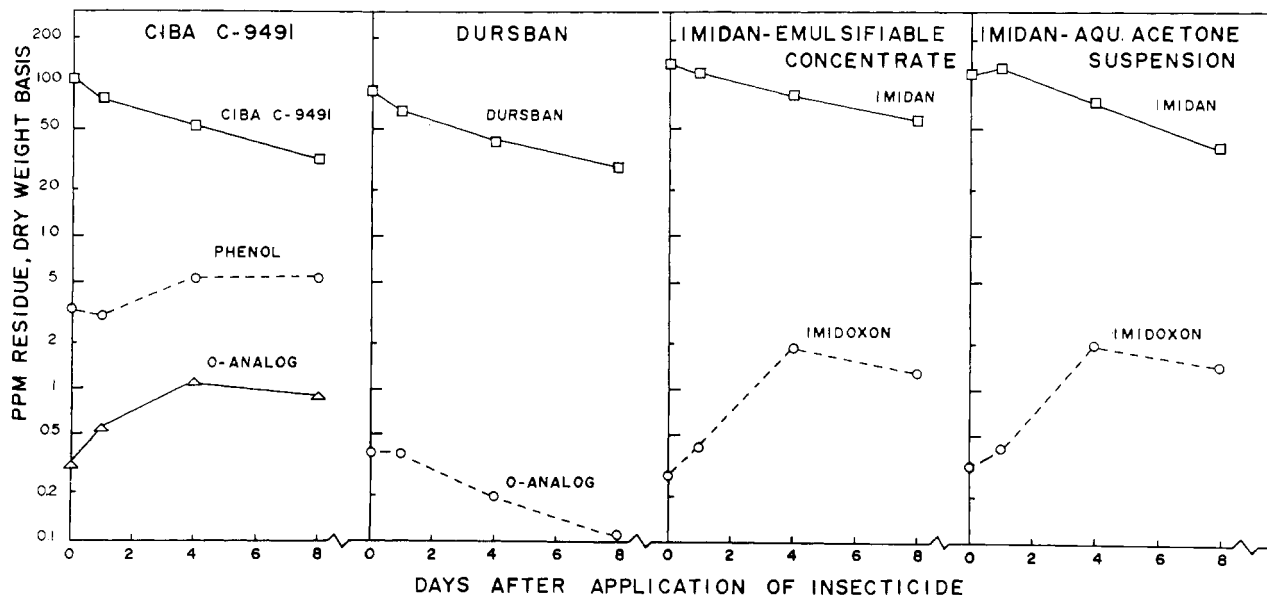


Figure 4. Residues in Coastal Bermuda grass

After application of Ciba C-9491, Dursban, and Imidan as emulsifiable concentrates and Imidan as an aqueous acetone suspension, all at level of 1 pound of insecticide per acre

Table VI. Weights of Extractives Obtained from Corn and Grass by Nine Extraction Procedures

Solvent and Extraction Method	Extraction Time	Extractive, Mg./Gram of Plant Material ^a			
		Corn		Grass	
		Wet basis	Dry basis	Wet basis	Dry basis
Benzene blend	5 min.	0.88	5.83	1.68	4.23
Chloroform blend	5 min.	0.83	5.50	2.70	6.80
Chloroform-10% methanol blend	5 min.	1.26	8.34	3.38	8.51
Benzene Soxhlet	0-2 hr.	0.62	4.11	3.76	9.47
	2-8 hr.	0.19	1.26	0.98	2.47
Chloroform Soxhlet	0-2 hr.	0.81	5.36	4.52	11.39
	2-8 hr.	0.35	2.32	1.32	3.32
Acetonitrile Soxhlet	0-2 hr.	10.99	72.78	11.69	29.45
	2-8 hr.	1.74	11.52	2.64	6.65
Chloroform-10% methanol Soxhlet	0-2 hr.	1.02	6.75	7.31	18.41
	2-8 hr.	0.84	5.56	3.90	9.82
Acetonitrile reflux	2 hr.	10.60	70.20	9.70	24.43
Chloroform-10% methanol reflux	2 hr.	1.30	8.61	5.79	14.58

^a Dry matter content: grass = 39.7%, corn = 15.1%. Raw extracts were concentrated to a small volume, placed in aluminum foil dish (W. H. Curtin Co., No. 6761, 2³/₈-inch diameter, 5/₈ inch deep), and allowed to dry in a hood. Dried residue was put in an oven at 110° C. for 5 minutes and weighed after it cooled to room temperature.

of the residues. If one excludes the use of radiolabeled pesticides to assure complete extraction, which itself is based on the assumption that no *in situ* decomposition to insoluble products occurs (Wheeler and Frear, 1966) and that the necessary radiolabeled compounds are available, this exhaustive extraction procedure appears to be the practical method of choice for removing residues of phosphorus pesticides from field samples for analysis. Blending, extraction under reflux, or other procedures or combination of procedures may save time and still give complete or adequate recoveries, but the analyst may wish to check recoveries by the extraction procedure he selects against those obtained by the exhaustive Soxhlet extraction, at least until a better procedure is found. Of course, the proposed extraction procedure may not be suitable for use with less specific methods of analysis such as electron-capture gas chromatography, since the method of extraction and degree of cleanup necessary depend on the specificity of the analytical method (electron-capture detector responds poorly or not at all to some phosphorus compounds). In this connection the highly specific response and high sensitivity of the flame photometric detector in the analysis of residues of phosphorus-containing insecticides has been recently described (Beroza and Bowman, 1968). Good quantification of pesticides in samples of very small size is possible, and contamination of the column by extracts has not been a problem because sample size is small.

Table VI gives the weights of material removed from corn and grass after extraction by nine procedures. With corn, the weight of extractives was seven times greater by the acetonitrile Soxhlet method than by the chloroform-10% methanol Soxhlet method; with grass, the acetonitrile extractive was only slightly higher. The weight of the extractives (or co-extractives) provides some idea of the amount of material injected into the gas chromatographic column. Obviously, it should be kept to a minimum. Table VI shows that the weight of extractives obtained by

the chloroform-10% methanol Soxhlet procedure was not excessive.

Figures 2, 3, and 4 show the residues that were found on field crops at specific intervals following insecticide application. Figure 2 is especially interesting because it shows the nature of the residues and how complex such a picture can be. However, it is a picture that is more complete and useful than those available in the past.

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