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INSECTICIDE RESIDUES

Colorimetric Analytical Method for Bidrin Residues in Alfalfa, Celery, Lemon Peel, Lettuce, Orange Peel, Potatoes, String Beans, and Tomatoes

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A procedure is presented for the determination of microgram quantities of Bidrin in crop samples. Cleanup is accomplished with an acid reflux followed by distillation of interfering materials. The Bidrin is then hydrolyzed with alkali and the resulting dimethylamine distilled and collected. The amine is determined colorimetrically as dimethyl dithiocarbamate following the addition of cupric ion and carbon disulfide. Residues as low as 0.20 p.p.m. can be accurately determined from samples as large as 125 grams. An average of 12 samples per day can be analyzed by this procedure.

THE candidate insecticide 3-(dimethoxyphosphinyloxy) - *N,N*-dimethyl-*cis*-crotonamide, commonly called Bidrin, has been shown by various workers to be effective against certain insect pests of several crops. However, before registration of a pesticide at the state level and by the Federal Government is possible, an acceptable method of residue analysis must be submitted along with data showing persistence of the compound on and in the crops involved in the petition presented. The method should have good degrees of specificity and reliability, and also sufficient sensitivity to determine adequately the persistence characteristics of the compound and any *in situ* metabolites of pharmacological significance. The present method meets the requirements for specificity for the parent compound, reliability, and sensitivity to 0.2 p.p.m. or less in the presence of the benzene-extractable substances from alfalfa, celery, lemon peel, lettuce, orange peel, potato peel, potato pulp, string beans, and tomatoes.

Cleanup consists of refluxing the benzene extractables with acid and steam distillation of interfering materials; the nondistilled Bidrin is hydrolyzed by alkali to yield dimethylamine, which is in turn steam-distilled and determined colorimetrically by the highly specific Stanley, Baum, and Gove (*3*) procedure. Dimethylamine reacts with carbon disulfide and ammonia to form the benzene-soluble, amber-colored cupric dimethyldithiocarbamate in the presence of cupric ion. This reaction is specific for dialkylamines and therefore other pesticides with a monoalkylamine group give no interference. Other dialkylamine-containing pesticides interfere only if such compounds are hydrolyzed to steam-volatile amines by the described conditions.

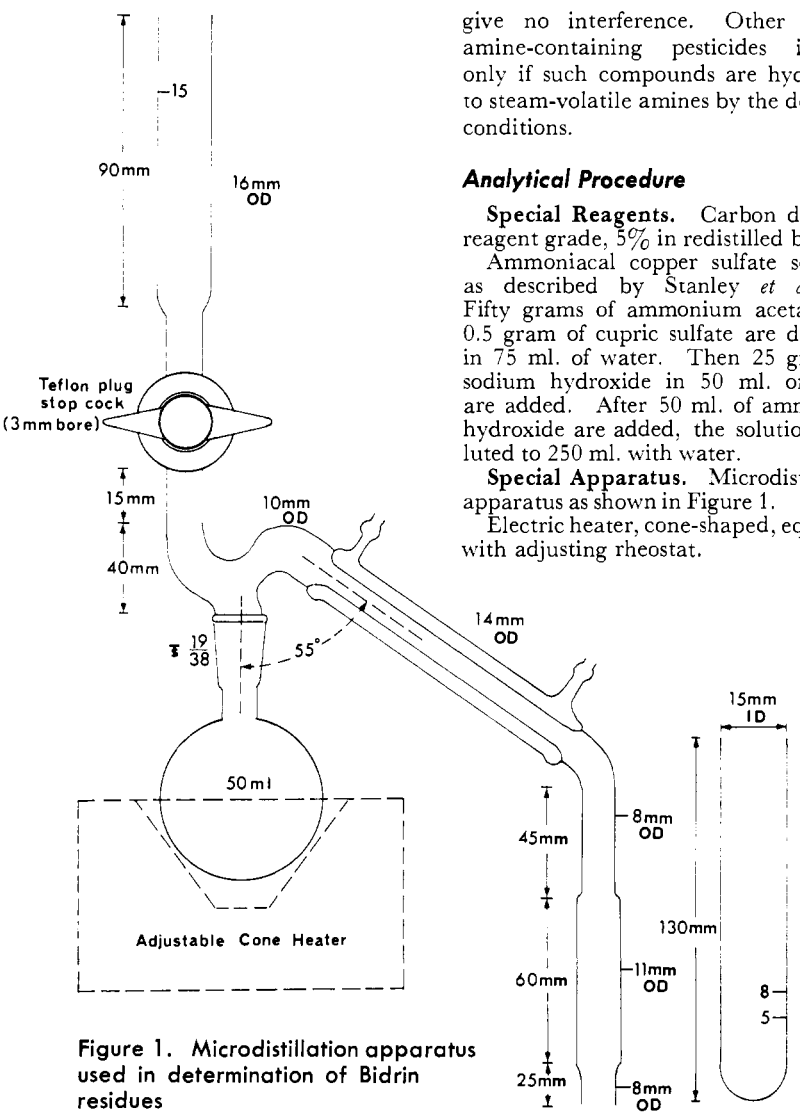


Figure 1. Microdistillation apparatus used in determination of Bidrin residues

Analytical Procedure

Special Reagents. Carbon disulfide, reagent grade, 5% in redistilled benzene.

Ammoniacal copper sulfate solution, as described by Stanley *et al.* (*3*). Fifty grams of ammonium acetate and 0.5 gram of cupric sulfate are dissolved in 75 ml. of water. Then 25 grams of sodium hydroxide in 50 ml. of water are added. After 50 ml. of ammonium hydroxide are added, the solution is diluted to 250 ml. with water.

Special Apparatus. Microdistillation apparatus as shown in Figure 1.

Electric heater, cone-shaped, equipped with adjusting rheostat.

Table I. Residue Values for Bidrin on Duplicate Analyses of Triplicate Samples of Field-Sprayed Valencia Oranges

Replicate	Days after Treatment	Bidrin, P.P.M.	Dev. from Av., P.P.M.
A1	2	6.7	0.5
		6.9	0.7
A2	2	5.8	0.4
		6.2	0.0
A3	2	5.7	0.5
		5.8	0.4
		Av. 6.2	0.4
A1	7	2.3	0.3
		2.4	0.2
A2	7	2.8	0.2
		2.7	0.1
A3	7	2.8	0.2
		2.8	0.2
		Av. 2.6	0.2

Procedure. REMOVAL OF INTERFERING MATERIALS. All samples were equilibrated with benzene as previously described (*2*). A 250-ml. aliquot (0.5 gram of substrate per ml.) of stripping solution was placed in a 500-ml. Erlenmeyer flask and the benzene was evaporated on a steam bath. In the presence of plant material, no condenser was needed to prevent loss of Bidrin during this evaporation. The remaining residue was quantitatively transferred to a 50-ml. $\overline{\S}$ 19/38 round-bottomed flask using 15 ml. of methylene chloride. This solution was evaporated through a three-ball Snyder column until only solvent-moist plant extractives remained, to which 5 ml. of 95% ethyl alcohol and 4 ml. of 12*N* sulfuric acid were added. This mixture was then refluxed vigorously on a steam bath for 90 minutes under a three-ball Snyder column. Fifteen milliliters of water were then added to the flask through the Snyder column, the flask was attached to the microdistillation head (Figure 1), and 8 ml. were distilled and discarded.

HYDROLYSIS AND COLOR DEVELOPMENT. While still connected to the distillation apparatus, the flask was cooled with ice water. The collection tube containing 5 ml. of 0.02*N* hydrochloric acid solution was positioned so that the delivery tube of the condenser was just below the surface of the liquid. Fifteen milliliters of 10*N* sodium hydroxide solution were then added to the flask through the stopcock side arm, and a Carborundum boiling chip was introduced into the side arm and washed into the flask with 3 to 4 ml. of water. Heat was applied until 3 ml. of distillate were collected.

The contents of the collection tube were shaken with two 3-ml. portions of the carbon disulfide–benzene reagent. These organic washings (top layer) were carefully removed and discarded. Five milliliters of the carbon disulfide–benzene reagent and 2 ml. of the ammoniacal copper sulfate solution were added to the collection tube, which was then capped and shaken vigorously for 3 minutes. The upper layer, which contained the amber-colored cupric dimethyl dithiocarbamate, was filtered into a 1-cm. cell and read on a Beckman Model B spectrophotometer at 434 mμ; the instrument was set with benzene.

Typical standard curves prepared from analytical grade Bidrin supplied by the manufacturer read 28 μg. per 0.100 ± 0.005 absorbance unit. Best results were obtained by reading the solutions immediately after color development.

RESULTS OF FIELD AND FORTIFIED SAMPLES. On July 7, 1964, mature Valencia orange trees were sprayed in a commercial manner with 1 pint (7.5 pounds per gallon) of Bidrin per 100 gallons of finished spray at a rate of 2500 gallons per acre (90 trees per acre). Triplicate 32-fruit samples were collected on July 7 (pretreatment), 9, and 14. The peel was removed and chopped, and 500-gram subsamples were equilibrated by tumbling, as described by

Table II. Recovery of Bidrin Added to Stripping Solutions of Various Crops

Crop or Crop Part	Sample Weight, G.	Added μG.	Added P.p.m.	Recovered[a] μG.	Recovered[a] %
Alfalfa	20	0	0.0	8, 10	
		200	10	188, 180	94, 90
	125	100	0.80	98, 92	98, 92
		25	0.20	23, 27	92, 108
		0	0.00	23, 19	
Celery	20	0	0.0	8, 7	
		200	10	178, 173	89, 87
	125	100	0.80	94, 99	94, 99
		25	0.20	24, 23	96, 92
		0	0.00	12, 12	
Lemon peel	20	0	0.0	10, 10	
		200	10	200, 208	100, 104
	125	100	0.80	94, 97	94, 97
		25	0.20	24, 26	96, 104
		0	0.00	25, 24	
Lettuce	20	0	0.0	10, 9	
		200	10	180, 184	90, 92
	125	100	0.80	98, 94	98, 94
		25	0.20	22, 20	88, 80
		0	0.00	8, 12	
Orange peel	20	0	0.0	13, 15	
		200	10	208, 204	104, 102
	125	100	0.80	97, 99	97, 99
		25	0.20	26, 25	104, 100
		0	0.00	25, 30	
Potato peel	20	0	0.0	4, 3	
		200	10	206, 211	103, 105
	125	100	0.80	92, 100	92, 100
		25	0.20	25, 27	100, 104
		0	0.00	4, 5	
Potato pulp	20	0	0.0	9, 8	
		200	10	173, 168	87, 84
	125	100	0.80	94, 88	94, 88
		25	0.20	23, 24	92, 96
		0	0.00	16, 16	
String beans	20	0	0.0	10, 11	
		200	10	196, 192	98, 96
	125	100	0.80	103, 94	103, 94
		25	0.20	24, 23	96, 92
		0	0.00	18, 20	
Tomato	20	0	0.0	13, 15	
		200	10	185, 175	92, 87
	125	100	0.80	91, 82	91, 82
		25	0.20	23, 19	92, 76
		0	0.00	16, 16	

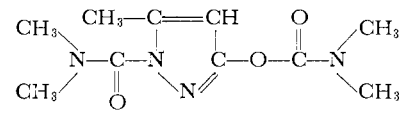
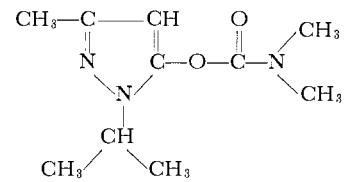
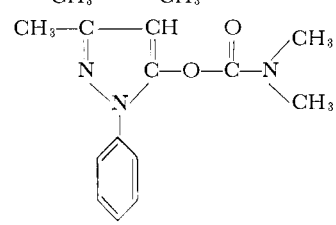
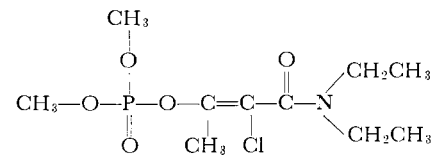
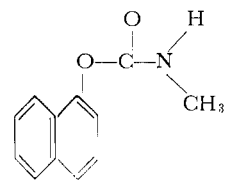
[a] Results of fortified samples corrected for "apparent residues" present in control sample to which no Bidrin was added.

Table III. Residue Values of Bidrin Found in Multiple Analyses of Laboratory-Fortified Samples of Lemon and Orange Peel Extractives

(125 grams analyzed)

	Bidrin, P.P.M. Added	Bidrin, P.P.M. Recovered	Dev. from Av., P.P.M.
Orange peel	0.80	0.76	0.00
		0.77	0.01
		0.74	0.02
		0.74	0.02
		0.72	0.04
		0.83	0.07
		0.75	0.01
		Av. 0.76	0.02
Lemon peel	0.77	0.75	0.02
		0.76	0.03
		0.74	0.01
		0.70	0.03
		0.72	0.01
		Av. 0.73	0.02

Table IV. Relative Intensity of Color Produced by Other Insecticides

Common Name	Chemical Structure	Molar Color Intensity[a]
Dimetilan		2.2
Isolan		1.6
Pyrolan		1.1
Phosphamidon		0.0
Sevin		0.0

[a] Relative to Bidrin = 1.

Gunther and Blinn (*2*), using 2 ml. of benzene per gram of peel. Two aliquots of each replicate were analyzed (Table I).

Control samples of alfalfa, celery, lemons, lettuce, oranges, potatoes, string beans, and tomatoes were obtained locally. These samples were processed as described above and aliquots were fortified with 10, 0.8, and 0.2 p.p.m. of Bidrin in the stripping solution stage. Results are in Table II.

Table III contains data to demonstrate the reliability of the method on laboratory-fortified stripping solutions of lemon and orange peels. Comparison of Table I with Table III shows less average deviation among laboratory-fortified samples than among field-treated samples.

The method was tried on standard solutions of five chemically related insecticides and the final molar color intensities (relative to Bidrin = 1) are listed in Table IV. As expected, the solutions of Dimetilan, Isolan, and Pyrolan, all of which yield dimethylamine upon hydrolysis, developed color and therefore residues of these compounds could be determined by this method. Phosphamidon, which should yield diethylamine upon hydrolysis, developed no color, indicating that the compound was not hydrolyzed under the conditions described. However, if the conditions were modified to cause hydrolysis, the cleanup and color development would be applicable to determinations of Phosphamidon residue. Sevin, which yields monomethylamine upon hydrolysis, developed no color and therefore cannot be determined by this method.

Discussion

Benzene is the stripping solvent rather than methylene chloride, because the latter resulted in excessively high background readings in the range of 0.175 absorbance unit for a 125-gram sample of control orange peel. Hexane should not be used for extractions because the Bidrin residue selectively partitions out of the hexane into the water phase of any water-containing samples.

Evaporation of the benzene stripping solutions on a steam bath without the use of a Snyder column caused no detectable loss of Bidrin residues. Conversely, the use of a Snyder column during the evaporation of the methylene chloride solution and also during the acid reflux step was essential for consistent results. The purpose of the ethyl alcohol in the acid reflux mixture was to lower its boiling point, so that vigorous boiling and subsequent mixing of the two layers occurred at steam-bath temperatures. A shorter reflux period caused higher control values, while longer reflux periods did not lower either control or blank values below those obtained in the described 90-minute reflux.

The amount of sodium hydroxide for the amine hydrolysis step is rather critical. Smaller than recommended amounts afforded low results from incomplete hydrolysis; larger amounts increased the tendency to bump. Gentle tapping until smooth boiling occurred was sometimes necessary, even when the described amount was used. Distillation of more than 3 ml. into the collection tube caused an increase in control sample readings. The rinses of the distillate in the collection tube with the carbon disulfide–benzene solution removed an oily substance that increased control readings. There was no detectable loss of dimethylamine in these rinsings so long as the distillate remained acidic.

Control samples representing 125 grams of orange peel showed an apparent residue of 25 to 30 μg. of Bidrin, which corresponds to a background of 0.20 to 0.24 p.p.m. Correction for this apparent background is most easily done by comparing results to a recovery curve rather than to a standard curve. The typical recovery curve, made by adding known amounts of insecticide to control samples and plotting the resulting absorbance values against micrograms added, is a straight line between 25 and 250 μg., having the same slope as the standard curve. As described by Gunther (*1*), results should not be considered quantitative unless the sample reading is at least twice the value of the control sample reading.

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