Quantitative Measurement of Combinations of Aramite, DDT, Toxaphene, and Endrin in Crop Residues

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Numerous types of insecticidal applications are used for pest control during the production of seed crops. As a result chaff, straw and the seed screenings usually have residues of the chemicals after harvest. Recommendations are usually made that the straw, chaff and seed screenings from fields treated with DDT, toxaphene, Aramite, chlordane or any other material not registered for use on alfalfa hay or forage crops not be fed to livestock or poultry. However, chaff and seed screenings have been used as animal feeds, either separately or in feedstuff mixtures.

Several procedures have been published for the detection of Aramite but very few data are available for its measurement in the presence of crop material. Mitchell (7) separated Aramite from several other pesticides qualitatively, using paper chromatography. Brokke et al. (4) determined Aramite colorimetrically. Moye and Winefordner (8) studied the phosphorimetric characteristics of several common pesticide standards, including Aramite. Bowman and Beroza (3) used gas-liquid chromatography with an electron affinity detector to

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study the extraction p-values of several pesticides and related compounds (including Aramite) in six binary solvent systems but did not study them in the presence of crop materials.

The present procedure for the detection of Aramite in the presence of DDT, toxaphene, and endrin was developed to provide a rapid, accurate, and sensitive method for the quantitative measurement of these pesticides in combinations in crop residues. The clean-up, separation and detection procedures provide for quantitative measurement of these pesticides in the presence of crop materials.

Experimental

Chemicals and Equipment. All chemicals used in these studies were of reagent grade. The pesticides were analytical standards supplied by the manufacturer or purified in our own laboratory; the reagent grade solvents were redistilled shortly before use. The gas chromatograph was the Varian Aerograph (Model 1200) equipped with an electron capture detector and a Leeds and Northrup Speedomax W 1-m.v. recorder with a chart speed of 1/2 inch per minute. Areas under the peaks were measured with a polar planimeter. The chromatographic column, made with an 8' x 1/8" stainless steel tube packed with 60/80 mesh DMCS-treated Chromosorb W, acid washed, was coated with 5 per cent Dow 710 silicone fluid and 5 per cent SE-30 silicone gum rubber. Nitrogen carrier gas (50 p.s.i.; 20 ml./min.) and a column temperature of 220°C. gave the best results and were used in these experiments. For DDT, toxaphene, and endrin an electrometer setting of 10 and an attenuator setting of 1 were used. For Aramite an electrometer setting of 1 and an attenuator setting of 4 were used except when extreme sensitivity was required, in which case the electrometer setting was 1 and the attenuator setting was 2.

DDT and endrin were analyzed both with and without dehydrohalogenation (5); toxaphene was analyzed with dehydrohalogenation only (1). Since Aramite is destroyed by alkali treatment, it was analyzed without dehydrohalogenation. All pesticides were cleaned-up on a florisil column. The dehydrohalogenation reagent was prepared fresh daily by dissolving (for each sample) 5g. of C.P. potassium hydroxide in 3 ml. of distilled water and adding, with stirring, 17 ml. of ethanol.

Recoveries of added pesticides were essentially quantitative. Extraction of the crop materials (15 grams crop material/250 ml. solvent) was made by pooling three benzene reflux extracts (2). With the exception of Aramite (where solvent extraction gave 85% or higher recovery), the total residues include the sum of that of the solvent extract plus the ethanolic potassium hydroxide reflux extract of the solvent-extracted plant material (which provided 90% or higher recovery). All results were calculated on a dry-weight basis.

Crop material clean-up was accomplished in the following manner: A 25 mm. x 250 mm. glass chromatographic column with a 300 ml. reservoir at the top was plugged loosely with glass wool. Fifteen grams of anhydrous sodium sulfate powder was placed in the column followed by approximately 21 grams of activated florisil (heated to 270°C. for 3 hours previously), the packing was tamped lightly and an additional 15 grams of anhydrous sulfate powder added. Fifty milliliters of pentane were then added to the column; the concentrated crop extract was added in with the last 10 ml. of pentane. The sample container was then rinsed into the column with two 25 ml. pentane portions. DDT and its analogs as well as toxaphene were eluted from the column with 100 ml. of 10% diethyl ether plus 90% pentane into the first receiver. Endrin (if present) was eluted with 520 ml. of 10% diethyl ether plus 90% pentane into

the second receiver. Aramite was eluted with 260 ml. of 2% isopropyl alcohol plus 98% pentane into the third receiver. The cleaned-up samples were then analyzed by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) either separately or in combination.

TLC was employed for screening purposes and in combination with GLC, as an analytical tool. Silica gel H absorbent (0.5 mm. thick) and the indophenol color test (6) were employed. The solvent systems used for spot separation as well as the R_f values for a solvent travel of 15 cm. are shown in Table I.

TABLE I

TLC R_f Values for Several Pesticides
Using Various Solvent Systems

	R_f Values					
Solvent Systems	DDE	DDT	DDD	Toxaphene	Endrin	Aramite
100% Benzene	0.95	0.95	0.95	0.95	0.39	0.76
100% Pentane	0.41	0.26	0.14	0.10 to 0.20	0	0
5% Diethyl ether + 95% Benzene	0.98	0.98	0.98	0.98	0.98	0.81

DDT, its analogs, and toxaphene were separated (together) using 100% pentane; Aramite was separated using 5% diethyl ether plus 95% benzene; and endrin was separated using 100% benzene. Although toxaphene was not completely resolved on TLC from DDD, final separation was achieved by GLC. For quantitative work, the chromatogram areas (containing the unknowns) were quantitatively extracted from the silica gel by a

5 min. shaking in benzene or pentane after comparing parallel R_f values with developed standard pesticide tracers. The solvent extracts were analyzed by GLC.

A sample of seed screenings obtained from ladino clover seed crops was analyzed. The known pesticide applications for the crops from which the sample was taken was as follows: 1 lb. DDT plus 2 lbs. toxaphene per acre May 28 and 1 1/2 lbs. DDT plus 3 lbs. toxaphene plus 1 1/2 lbs. Aramite per acre on July 5. The crop sample was treated in 1965 and received by us in May 1966. Two commercially prepared pellet samples with the residues of an unknown spray history were also obtained for analysis. These pellets consisted of 75% alfalfa seed crop threshings and 25% alfalfa seed crop screenings.

Results and Discussion

At the GLC apparatus settings stated above, the electron capture detector showed a linear response (within the range tested) (Fig. 1). At a setting of 1 on the electrometer and 2 on the attenuator the background noise was high but 10 ng. of Aramite were still easily detected; the Aramite response was linear in this range. If extractives equivalent to 200 mg. of crop material were injected into the GLC column, a sensitivity of 0.05 p.p.m. was theoretically possible.

Tests involving injection of twenty-one Aramite standards onto the GLC column resulted in a mean precision of 4% for the method. Aramite recoveries in samples of seed screenings fortified before extraction at the 1 p.p.m. level resulted in a mean accuracy of 96% with a standard deviation of $\pm$ 11%. Other experiments showed that the error of the method was almost equally divided between sampling-extraction-clean-up and chromatography measurement steps.

The Aramite standard gave two reproducible peaks with GLC, a minor peak at 1 1/4 minutes and a major peak at 6

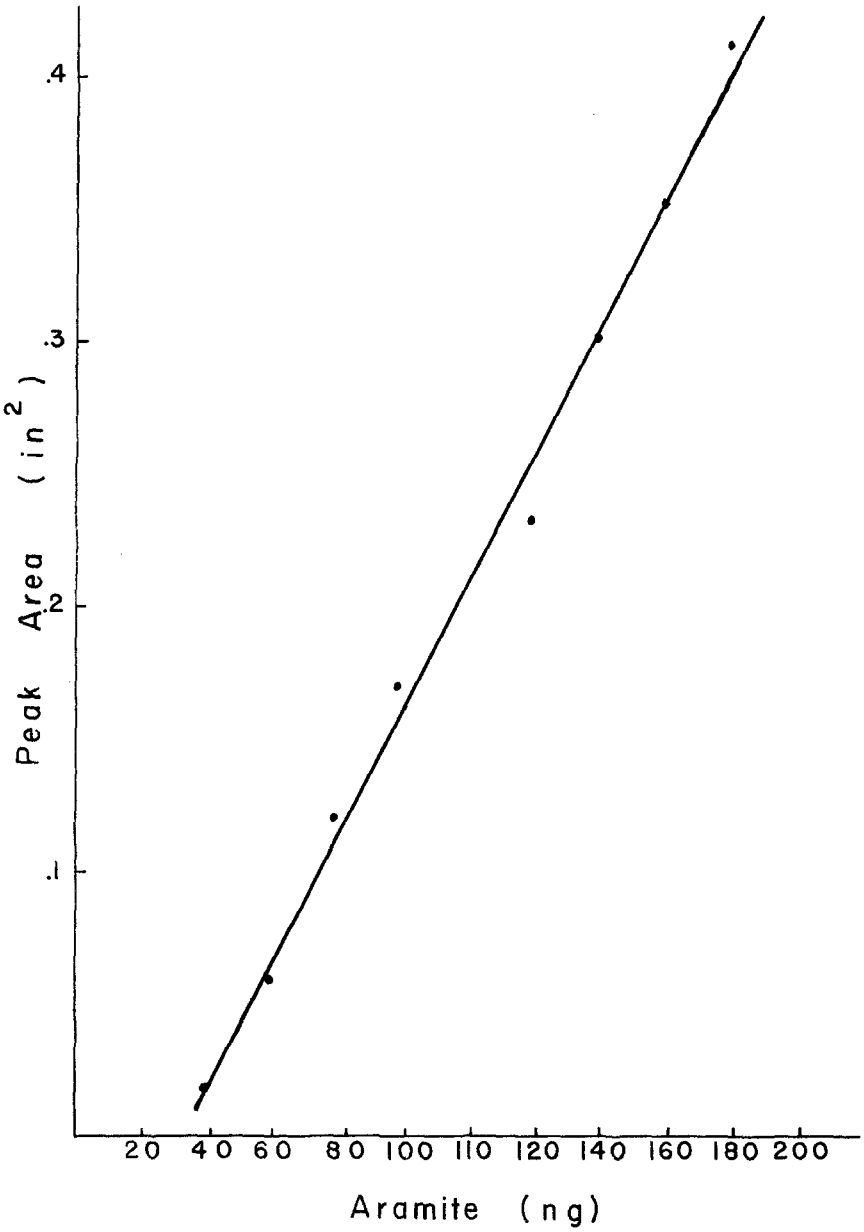

Figure I. Aramite Standard Curve

minutes, after sample injection. The area under the major peak was used for the quantitative measurements of this chemical. The GLC characteristics of DDT and its analogs and toxaphene have been previously reported (5,1). Aramite was cleanly separated from the above mentioned pesticides on TLC giving a single distinctive spot using the conditions described above.

The residues found on the seed crop screenings described above are shown in Table II.

TABLE II

Residues on Seed Screenings Sample [1]

Sample No.	DDT, p.p.m.	Toxaphene, p.p.m.	Aramite, p.p.m.
1	20.6	65.7	Not detectable

[1]Pooled three benzene solvent refluxes plus residues in extracted plant material (with exception of Aramite).

Both DDT and its analogs and toxaphene residues were very high which would definitely limit the quality of the seed screenings as an animal feed. Aramite residues were not detected in the sample when it was analyzed, (qualitatively and quantitatively by TLC and GLC). Endrin was not applied and of course was not found.

Two samples (Table III) of pellets of an unknown spray history were analyzed. The pellets were produced from 75% alfalfa seed crop threshings and 25% alfalfa seed crop screenings. The residue levels ranged from 3.8 p.p.m. to 6.1 p.p.m. for DDT and 6.3 p.p.m. to 16.0 p.p.m. for toxaphene. No detectable residues of Aramite were found. At the present time, the maximum recommended level for dairy animal feed is 0.5

p.p.m. DDT. It is obvious therefore that these pellets are unfit for dairy cattle feed.

TABLE III

Pesticide Residues in Pellets from

Alfalfa Seed Crop Threshings and Screenings

Sample	Benzene Solvent Extract		Ethanolic-Alkali Extract of Remaining Plant Material		Total		
	DDT p.p.m.	Tox. p.p.m.	DDT p.p.m.	Tox. p.p.m.	DDT p.p.m.	Tox. p.p.m.	Aramite p.p.m.
Pellets No. 1 (unknown history)	5.6	15.7	0.5	0.3	6.1	16.0	N.D.[1]
Pellets No. 2 (unknown history)	3.3	6.1	0.5	0.2	3.8	6.3	N.D.

[1]N.D. signifies no detectable residue.

The principal advantages of the procedure described herein is that it is relatively simple and rapid. The high sensitivity of the electron capture detector can be utilized; and the multiple techniques of column chromatography (with florisil), TLC, and GLC can be used for the pesticide separations. The cleanup procedure is efficient and reproducible for removing foreign crop extractives from the pesticides.

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