form reagent, no iodoform response was observed. Bidrin was unaffected by similar treatment with sodium bisulfite.

Increasing the sensitivity of Bidrin or its derivatives to EC detection was the primary objective of this research. This was accomplished by converting Bidrin to iodoform which was approximately 3000 times more responsive to EC detection than Bidrin.

A second objective, the development of a suitable and practical cleanup procedure was accomplished by conversion of Bidrin to iodoform resulting in no discernable background from cabbage extracts (Figure 3).

To date, proposed tolerances for Bidrin on several crops are under FDA consideration. It is anticipated that a tolerance of 1.0 p.p.m. will eventually be requested on leafy crops such as cabbage. Therefore, no attempt was made to analyze for crop residues at the 0.01-p.p.m. level, although data from spiked crops indicated that field residues at this level could have been determined.

The described iodoform procedure should be readily adaptable to routine analysis. Cleanup for 12 crop extract samples should not require more than 4 hours and conversion to iodoform plus preparation prior to injection, not more than an additional 2 hours. The iodoform retention time ($1^1/_2$ minutes compared with 12 minutes for Bidrin under the described conditions) is sufficiently short to allow determination of 12 samples in 2 hours and, therefore, a dozen or more Bidrin samples could be analyzed in a normal working day. Unlike Bidrin, iodoform produces well-defined and symmetrical chromatograms which makes possible a direct relationship between peak heights and quantity of pesticide injected.

Of the common solvents investigated, methylene chloride consistently produced the highest recovery values for extracting Bidrin from cabbage. Hexane, acetone, and isopropyl alcohol were unsuitable because Bidrin selectively partitions from hexane into water, whereas acetone and isopropyl alcohol undergo the iodoform reaction. In the extraction step, magnesium sulfate was added to remove water from the macerate and, thereby, reduce the amount of Bidrin lost to the aqueous phase. Activated charcoal was added directly to crop samples, which were then blended and filtered through Celite producing a colorless filtrate. When the identical macerate, minus charcoal, was chromatographed on a charcoal column, plant pigments passed through the column with the eluent. Addition of charcoal before blending proved to be more convenient as well as more effective than column chromatography in the cleanup.

Acknowledgment

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RESIDUE DETERMINATION

Colorimetric Determination of Abate Residues from Several Environmental Conditions

ROGER C. BLINN and N. ROBERT PASARELA

Agricultural Division, American Cyanamid Co., Princeton, N. J.

A colorimetric method for residues of Abate insecticide, O,O,O′,O′-tetramethyl-O,O′-thiodi-p-phenylene phosphorothioate, is based on its hydrolysis to 4,4′-thiodiphenol and subsequent determination at 485 mμ after reaction with 4-aminoantipyrine and periodate. Procedures are described for natural waters, mud, oysters, rice grain, and rice foliage. Recoveries and apparent Abate insecticide concentrations in control tissues are given.

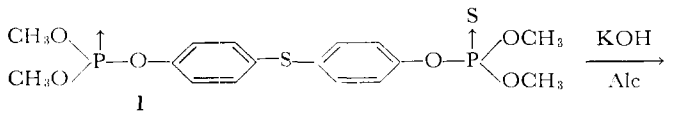
THE *O,O,O′,O′* - tetramethyl - *O,O′*-thiodi-*p*-phenylene ester of phosphorothioic acid (I) (Abate insecticide, compound 52,160, American Cyanamid Co.) is registered for the large-scale control of mosquito larvae. Before this compound could be used commercially, data concerning its residual behavior in natural waters and adjacent mud were required for registration purposes. Because of the possibility of exposure of oysters and rice during or following normal applications for mosquito control, information about residues on and in these tissues was also of interest.

Since the validity of a residue method depends upon its response to major toxic metabolites as well as to the parent compound, preliminary studies were conducted to investigate the extent and nature of metabolic degradation of this insecticide in simulated natural waters, mud, and bean plants. These studies showed evidence of only minor, if any, residues of postulated metabolic products; therefore, an analytical method responding to parent Abate insecticide should allow a realistic residue evaluation.

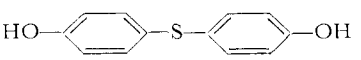
The analytical procedure used for the preliminary residue study incorporated thin-layer chromatography for separation of Abate insecticide from postulated metabolic products and either infrared spectrophotometry or total phosphorus determination by Schöniger flask combustion for quantitation. These procedures possessed adequate sensitivity and specificity for collecting the required residue data, but were not suited for mass-production efficiency so necessary for a large residue program. Abate insecticide and its hydrolysis product, 4,4′-thiodiphenol (II), possess low vapor pressure and lack of fluorescence, thus negating the utilization of the attractive features of vapor-phase chromatography and fluorescent spectrophotometry. Although Abate insecticide does absorb strongly in the ultraviolet, the very difficult inherent cleanup problems of ultraviolet residue procedures made this approach undesirable.

Parent Abate insecticide does not possess chemical properties suitable for the more conventional colorimetric pro-

cedures. However, the phosphorothioic acid ester moiety can be removed by hydrolysis to give 4,4′-thiodiphenol (II), and this reaction is used as the basis of a formulation analytical procedure, with ultraviolet spectrophotometric determination (*8*).



I



II

Because the cleanup problems are less difficult for colorimetric than for ultraviolet procedures, color reactions were investigated. The usual diazotization coupling color reactions are generally unsatisfactory for para-substituted phenols, and the close vicinity of the absorption peak of the colored solution resulting from the reaction of thiodiphenol with *p*-nitrobenzenediazonium fluoborate with that of the reagent made this reaction unsuitable. Hirano and Tamura (*3*) reported recently a colorimetric procedure in which 4-methylthio-*m*-cresol reacts with 4-aminoantipyrine under oxidizing conditions to give an intense yellow color. The reaction appears to be analogous to that of *p*-phenylenediamine with phenols, in which indophenols are formed. Emerson (*2*) and Johnson and Savidge (*5*) report that even phenols substituted in the para-position by halogen, alkoxy, sulfonic, and carboxylic acid groups give a positive color reaction, and that these para-substituents are eliminated in the indophenol reaction (*4, 6*). Thiodiphenol (II) reacted with 4-aminoantipyrine under oxidizing conditions to give an intense red color in alkaline aqueous solution ($\lambda_{max.}$ 510 mμ) (*8*). This color reaction was then adopted as the basis for a colorimetric residue analytical procedure.

Experimental

Preliminary Metabolic Study Procedure. Simulated Natural Pond. A large aquarium, containing natural pond water and mud, and supporting vigorous colonies of duckweed, hornwart, guppies, and a variety of algae, worms, etc., was treated with a formulated sample of technical Abate insecticide so as to give an initial concentration of 10 p.p.m. Samples of water and mud were taken periodically for 7 days for analysis. The water was extracted with chloroform, and the residue from the evaporated chloroform was chromatographed on an 8 × 8 inch fluorescent silica gel thin-layer plate. Development was accomplished by migration of toluene in one direction, turning the plate and developing with methanol–chloroform–toluene (10:95:95), then developing in the first direction again with nitromethane–acetonitrile–toluene (37:98:165). Abate insecticide and possible metabolic products were located on the chromatogram by viewing under ultraviolet light of 2540 A. Confirmation of identity was accomplished by eluting the areas of interest and determining the infrared characteristics.

The mud samples were air-dried, extracted with acetone, and treated as a chloroform solution with Nuchar C-190-N activated vegetable charcoal before chromatography and infrared characterization as described for water.

Bean Plants. Two-week sieva lima bean plants were sprayed with an emulsifiable formulation of Abate insecticide at the rate of 0.5 pound actual in 86 gallons of water per acre. The plants were kept in the greenhouse until removed for sampling. Samples were taken periodically for 16 days following treatment. The bean plants were macerated with chloroform, treated with activated charcoal, and passed through a column of polyethylene-coated alumina in aqueous acetonitrile solution before chromatography and infrared characterization as described for water. Quantitation of organophosphorus was accomplished by means of oxygen-flask combustion (*1*).

Analytical Procedure

Special Apparatus. Omni-Mixer. Ivan Sorvall, Inc., Norwalk, Conn.

Snyder Column, one-ball type. Dohrmann Instrument Co., Palo Alto, Calif.

Special Reagents. Methanol Solution. Add 25 ml. of 6*N* sulfuric acid to 350 ml. of redistilled methanol and dilute to 400 ml. with water.

Propylene Glycol Solution. Dissolve 1.0 ml. in 100 ml. of chloroform.

Buffer Solution, pH 10. Dissolve 6.2 grams of boric acid and 3.5 grams of sodium hydroxide in water and dilute to 1000 ml. with water.

4-Aminoantipyrine Solution. Dissolve 0.5 gram in 100 ml. of water just before use.

Sodium Metaperiodate Solution. Dissolve 0.9 gram in 25 ml. of water just before use.

Butanol Solution. Saturate butanol with pH 10 buffer solution.

Abate Insecticide. A recrystallized sample was used for preparing standard solutions.

Colorimetry. Hydrolysis. Dissolve Abate insecticide from either a standard solution or from a cleaned-up residue in the tube of a Kuderna-Danish apparatus in 5 ml. of redistilled methanol. Add boiling chips and 1 ml. of 15*M* potassium hydroxide solution, then place a one-ball Snyder column in the top of the tube and heat on a steam bath. After the methanol has all evaporated, remove the Snyder column and continue to heat on a steam bath for an hour. Cool and dissolve the residue in 5 ml. of water. Transfer the hydrolyzate to a 60-ml. separatory funnel with the aid of an additional 5 ml. of water and two 5-ml. portions of chloroform. Shake the mixture thoroughly and discard the chloroform. Repeat with an additional 10 ml. of chloroform. Acidify the aqueous layer with 5 ml. of 6*N* sulfuric acid and extract with two 20-ml. portions and one 10-ml. portion of chloroform, drying by passage through sodium sulfate into a Kuderna-Danish apparatus. Add 1.0 ml. of propylene glycol solution and evaporate the extract to dryness on a steam bath.

Colorimetric Determination. Add two drops of thymolphthalein solution to the residue in the concentrator tube, followed by 3.0 ml. of pH 10 buffer solution. If the solution is not blue at this point, add 0.1*N* sodium hydroxide solution dropwise until the solution just turns blue. Add 0.8 ml. of 4-aminoantipyrine solution followed by 1.0 ml. of sodium metaperiodate solution and mix thoroughly. After 10 minutes, add either 5.0 or 25.0 ml. of butanol solution, depending on the size of cuvette to be used, and shake vigorously for 20 seconds. Filter the upper colored solution through a pledget of cotton into a cuvette. Heat the cuvette in warm water until the colored solution is optically clear, and determine the absorbance at 485 mμ *vs.* a color-step reagent blank. A typical standard curve prepared from parent Abate insecticide, using a $^1/_2$-inch Spectronic 20 test tube and 5 ml. of butanol, had a slope of 7.6 ± 0.7 μg. per 0.1 absorbance unit; using a 5-cm. cuvette and 25 ml. of butanol, the curve had a slope of 57.5 ± 5.3 μg. per 0.1 absorbance unit.

Extraction and Cleanup. Natural Water. Filter 1500 ml. of natural water, acidified with 5 ml. of 6*N* sulfuric acid, through a 90-mm. coarse-porosity fritted-glass funnel into a 2000-ml. separatory funnel. Rinse the container and funnel with two 15-ml. portions of redistilled acetone and filter into the separatory funnel. Add 100 ml. of chloroform via the filter into the separatory funnel and shake the funnel vigorously for 45 seconds. If emulsification persists, break it by filtration through a pad of diatomaceous earth. Filter the separated chloroform extract through a pad of activated vegetable carbon overlying a pad of diatomaceous earth. Wash the cleaned-up chloroform extract with 25 ml. of 0.1*N* sodium hydroxide solution and dry the washed extract by passage through anhydrous sodium sulfate into a Kuderna-Danish apparatus. Repeat the extraction and cleanup steps with two additional portions of fresh chloroform and combine the extracts in the concentrator. Evaporate the solution to dryness on a steam bath for hydrolysis and colorimetric determination.

Mud. Spread the mud in a thin layer

on aluminum foil and allow it to air-dry overnight. Pulverize the dried mud with a mortar and pestle and screen out rocks, sticks, etc. Place 75 grams in a jar with 300 ml. of redistilled acetone, and blend with an Omni-mixer. Filter the mixture through a pad of diatomaceous earth, then evaporate a 200-ml. aliquot of the filtrate to dryness in a Kuderna-Danish apparatus on a steam bath. Dissolve the residue in 100 ml. of methylene chloride, treat with about a gram of activated vegetable carbon, and filter through a pad of diatomaceous earth into a Kuderna-Danish apparatus, washing with 100 ml. of fresh methylene chloride. Evaporate the filtered extract on a steam bath. Dissolve the yellow residue in 20 ml. of hexane and extract the solution with four 20-ml. portions of aqueous methanol solution. Wash the combined extracts with 20 ml. of hexane and extract the hexane wash with 20 ml. of aqueous methanol solution. Add 25 ml. of water to the combined alcoholic extracts and extract twice with 100-ml. portions of hexane. Wash the hexane extracts with 25 ml. of 0.1*N* sodium hydroxide solution, then dry by passage through anhydrous sodium sulfate into a Kuderna-Danish apparatus. Rinse glassware with 25 ml. of hexane and add to the concentrator, then evaporate the solution to dryness on a steam bath. Dissolve the residue in 5 ml. of chloroform, treat the solution with about 0.1 gram of activated vegetable carbon, filter through a pad of diatomaceous earth overlaid with activated vegetable carbon, and wash the filter pad with 35 ml. of fresh chloroform. Evaporate the combined chloroform solution in a Kuderna-Danish apparatus on a steam bath for hydrolysis and colorimetric determination.

Oysters. Place 150 grams of drained, fresh oysters in a jar with 600 ml. of methylene chloride and blend with an Omni-mixer. Filter the macerate through cheesecloth into a 500-ml. separatory funnel and allow the aqueous phase to rise to the surface. Drain the methylene chloride phase into a 1000-ml. Erlenmeyer flask and treat with about 1 gram of diatomaceous earth and about 3 to 4 grams of activated vegetable carbon. Filter the mixture through a pad of diatomaceous earth and evaporate to dryness a 400-ml. aliquot in a Kuderna-Danish apparatus on a steam bath. Dissolve the yellow, oily residue (ca. 1 ml.) in 20 ml. of hexane and extract with four 20-ml. portions of aqueous methanol solution. Wash the combined extracts with 20 ml. of hexane and extract the hexane wash with 20 ml. of aqueous methanol solution. Add 25 ml. of water to the combined alcoholic extracts and extract them twice with 100-ml. portions of hexane; wash the hexane extracts with 25 ml. of water, then dry the washed extracts by passage through anhydrous sodium sulfate onto a 40 × 20 mm. column of Florisil chromatographic adsorbent and allow the solution to percolate through the column. Rinse the column with 25 ml. of fresh hexane. Discard the hexane percolate, and elute the column with 150 ml. of 5% ethyl acetate in hexane into a Kuderna-Danish apparatus. Evaporate the eluate to dryness on a steam bath for hydrolysis and colorimetric determination.

Rice Foliage. Place 150 grams of chopped rice foliage in a jar with 600 ml. of methylene chloride and blend with an Omni-mixer. Filter the macerate through cheesecloth and treat the filtrate with about 1 gram of diatomaceous earth and about 3 to 4 grams of activated vegetable carbon. Filter the mixture through a pad of diatomaceous earth and evaporate a 400-ml. aliquot of the filtrate to dryness in a Kuderna-Danish apparatus on a steam bath. Dissolve the light greenish residue in 20 ml. of hexane and extract with an 80-ml. and a 20-ml. portion of aqueous methanol solution. Add 25 ml. of water to the combined extracts and extract twice with 100-ml. portions of hexane. Wash the hexane extracts with 25 ml. of water, then dry by passage through anhydrous sodium sulfate into a 40 × 20 mm. column of Florisil and allow the solution to percolate through the column. Rinse the column with 25 ml. of fresh hexane. Discard the hexane percolate and elute with 150 ml. of 5% ethyl acetate in hexane into a Kuderna-Danish apparatus. Evaporate the eluate to dryness on a steam bath. Dissolve the yellowish residue in 5 ml. of chloroform, treat with about 0.1 gram of activated vegetable carbon, and wash the filter pad with 35 ml. of fresh chloroform. Evaporate the combined solutions to dryness in a Kuderna-Danish apparatus on a steam bath for hydrolysis and colorimetric determination.

Whole Rice Grain. Grind the whole rice grain in a Wiley mill, coffee grinder, or other suitable device. Place 150 grams in a jar with 600 ml. of methylene chloride and blend with an Omni-mixer. Filter the macerate through cheesecloth and treat the filtrate with about 1 gram of diatomaceous earth and 3 to 4 grams of activated vegetable carbon. Filter the mixture through a pad of diatomaceous earth and evaporate a 400-ml. aliquot of the filtrate to dryness in a Kuderna-Danish apparatus on a steam bath. Dissolve the 3 to 4 ml. of yellow, oily residue in 20 ml. of hexane and extract it with five 20-ml. portions of aqueous methanol solution. Wash the combined extracts with 20 ml. of hexane, and extract the hexane wash with 20 ml. of aqueous methanol solution. Add 30 ml. of water to the combined extracts and extract twice with 100-ml. portions of hexane. Wash the hexane extracts with 25 ml. of 0.1*N* sodium hydroxide solution, then dry the hexane layer by passage through anhydrous sodium sulfate onto a 40 × 20 mm. column of Florisil and allow it to percolate through the column. Rinse the column with 25 ml. of fresh hexane. Discard the hexane percolate and elute the column with 150 ml. of 5% ethyl acetate into a Kuderna-Danish apparatus. Evaporate the eluate to dryness on a steam bath for hydrolysis and colorimetric determination.

Results and Discussion

Preliminary Metabolic Study. No organophosphorus compounds other than Abate insecticide were found in any of the substrates tested. The phenolic hydrolyzates of the postulated toxic metabolic products, 4,4′-thiodiphenol, sulfonyl diphenol, and monoesters from these phenols, were also shown to be absent from the water; these phenolic products would not have survived the cleanup procedures necessary for mud or bean plants. The chromatographic system was capable of separating the oxygen analogs, sulfoxides, and sulfone derivatives of Abate insecticide which were postulated as the expected metabolic products. A typical chromatogram of this separation is shown in Figure 1.

It appears, therefore, that Abate insecticide undergoes little or none of the postulated metabolic degradation under the biotic conditions described. Thus a residue procedure responding to parent Abate insecticide should give a realistic evaluation of its residual behavior.

Analytical Procedure. Hydrolysis. Although the procedure for the determination of Abate insecticide in formulations involves a hydrolysis step using 1*N* sodium hydroxide solution in 10% methanol (*8*), attempts to use this hydrolytic procedure as an integral portion of the colorimetric determination resulted in 70 to 80% recovery. When inert extractives from the sample were present, even lower and erratic recoveries were encountered. Using the more rigorous hydrolyzing conditions described in the present procedure, complete hydrolysis is achieved as is evident from the data in Table I.

Color Development. The colorimetric reaction for phenols, using 4-aminoantipyrine and an oxidant (*2*), proved a sensitive and reliable means for determining the phenolic hydrolysis product, thiodiphenol, of Abate insecticide. The

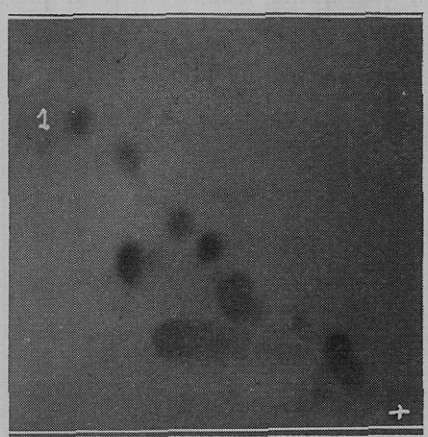


Figure 1. Silica gel thin-layer chromatogram of some possible metabolic products of Abate insecticide

Viewed under ultraviolet light of 2540 A. Solvents: toluene ↑, methanol–chloroform–toluene (10:95:95)→, nitromethane–acetonitrile–toluene (37:98:165) ↑. Abate insecticide is spot 1

Table I. Effect of Heating Time on Hydrolysis[a]

Heating Time, Min.	% Hydrolysis
0	55
5	66
10	87
20	100
40	98

[a] Add two pellets of potassium hydroxide to 77 μg. of Abate insecticide in 4 ml. of methanol. Heat on a steam bath until alcohol is evaporated and continue heating for the indicated time period.

Table II. Recovery and Background Values for Abate Insecticide

Abate Insecticide, μg. Added	Abate Insecticide, μg. Found[a]	Abate Insecticide, P.P.B. Added	Abate Insecticide, P.P.B. Found[a]	% Recovery[a]
		From Natural Stream Water		
0	7, 6	0	5, 5	...
0	7, 9	0	5, 6	...
37	29	25	19	79
37	30, 28	25	20, 19	81, 76
68	56	45	37	82
68	56, 60	45	37, 40	82, 88
		From Stream Bed Mud		
0	3[c]	0	0.06[b,c]	...
0	4[c]	0[b]	0.08[b,c]	...
22.5	18, 21	0.45[b,c]	0.36, 0.42[b,c]	80, 93
45	37, 31, 32	0.9	0.74, 0.62, 0.64[b,c]	82, 69, 71
		From Store-Purchased Oysters		
0	8, 8, 3[d]	0	0.08, 0.08, 0.05[d]	...
0	8, 8[d]	0	0.09, 0.08[d]	...
10	6	0.1	0.06	60
22	17, 15	0.22	0.17, 0.15	77, 68
66	51, 54, 46	0.66	0.51, 0.54, 0.46	77, 82, 70
		From Whole Rice Grain		
0	6, 5[d]	0	0.06, 0.05[d]	...
0	4, 6[d]	0	0.04, 0.08[d]	...
66	44, 45	0.66	0.44, 0.45	67, 68
		From Rice Foliage		
0	6, 6, 5[d]	0	0.06, 0.06, 0.05[d]	...
22	18	0.22	0.18	82
66	40, 47	0.66	0.40, 0.47	61, 71

[a] Fortified samples corrected for control values.
[b] Based on air-dried weight.
[c] Not corrected for averaged reagent blank value of 4-μg. apparent Abate insecticide (0.08 p.p.m.).
[d] Not corrected for averaged reagent blank value of 4-μg. apparent Abate insecticide (0.04 p.p.m.).

conditions used by Hirano and Tamura (*3*) provided valuable guidelines for this study, but were not optimum for this problem. The 15 mg. of 4-aminoantipyrine recommended (*3*) resulted in an excessively intense yellow reagent blank, which absorbed strongly at 485 mμ. It was found that 4 mg. was sufficient for the concentrations of Abate insecticide normally expected to be encountered. The extraction of the colored product from aqueous solutions into chloroform yielded a light orange solution with the absorption maximum at 450 mμ; however, the yellow color from reagent blanks interfered. The aqueous dye solution is red with the absorption maximum at 505 mμ, but difficulty in clearing cloudy solutions without loss precluded the use of this medium. Extraction with butanol saturated with pH 10 buffer resulted in a colored solution with absorption maximum at 485 mμ. The colored product was stable for at least an hour after extraction into butanol. Because of the sensitivity of the color reaction to pH (*2*, *3*), pH 10 buffer was used as the reaction medium in order to minimize variation. However, extraneous extractives which survived the cleanup procedures always caused variation in the pH of the final colored solution. Therefore, thymolphthalein indicator was used to allow visual adjustment of pH just prior to color development. Interferences resulting from the use of nonredistilled reagent-grade acetone and methanol amounted to background values of 10 to 20 μg. of apparent Abate insecticide. Redistilling these solvents reduced this interference to about 4 to 6 μg. of Abate insecticide. Another serious interference encountered was due to the use of a Waring-type blender. Apparently, the methylene chloride extracted a lubricant from the bearings which responded strongly to the colorimetric portion of the procedure. Use of the Omni-mixer blender eliminated this source of interference.

Extraction. A favorable partition coefficient allowed the large water sample to be extracted with a relatively small volume of chloroform, thus effecting a considerable degree of concentration. Losses of Abate insecticide during the filtration were avoided by washing the filter with acetone and chloroform.

Acetone was the extraction solvent of choice for dried mud to minimize absorption on clay particles. For all other substrates, methylene chloride possessed favorable partition properties and allowed a more efficient cleanup of extractives by treatment with activated vegetable carbon than was the case with chloroform.

Cleanup. Activated Vegetable Carbon. Recoveries from the treatment of methylene chloride solutions with activated vegetable carbon were about 85%; those from chloroform were essentially quantitative. The advantage of the superior cleanup from methylene chloride outweighed the relatively poor recovery.

Partition Distribution. The use of 87.5% methanol solution in water for partitioning with hexane resulted in excellent cleanup of oily and waxy extractives. Dilution of the methanol phase with water to 70% and extraction of Abate insecticide with hexane allowed further cleanup from more polar extractives. The recovery obtained by the four extractions with the 87.5% alcohol is over 98%, as can be calculated from the partition coefficients in Figure 2, while recovery obtained by the two extractions from 70% methanol into hexane is essentially quantitative (Figure 2). Because of the higher oil content of the whole rice grain (about 3 to 4%), five extractions into 87.5% alcohol were necessary to increase recovery.

Florisil Column. The Florisil chromatographic adsorbent column was essential for the cleanup of the more polar extractives surviving the other cleanup procedures. Abate insecticide was strongly adsorbed onto Florisil from hexane and was eluted completely by 150 ml. of 5% ethyl acetate in hexane.

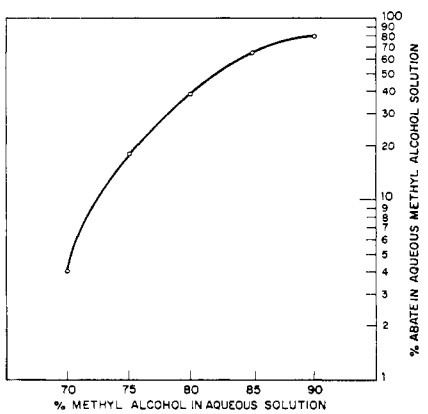


Figure 2. Partition rate of Abate insecticide between hexane and aqueous methanol

Use of 70 to 100 ml. of 10% ethyl acetate for elution, while complete, eluted a portion of the extractives which interfered in the colorimetry portion of the procedure.

Recoveries. Natural Waters. Table II presents the recovery and background values from control and fortified natural stream water accrued by the method. Background values averaged 5 p.p.b., and recoveries averaged 81%. In addition, background values from water taken from several locations in California and New Jersey ranged from 2 to 11 p.p.b. (7).

Mud. In Table II are collated the recovery and background values from control and fortified stream bed mud found by the method. Background values, not corrected for reagent blanks, averaged 0.07 p.p.m., and recoveries averaged 79%. In addition, background values from mud taken from various locations in California and New Jersey were less than 0.1 p.p.m. (7).

Oysters. Table II shows the recovery and background values from control and fortified oysters purchased in local markets. Background values, not corrected for reagent blanks, averaged 0.07 p.p.m., and recoveries averaged 72%. In addition, background values from Florida oysters ranged from 0.05 to 0.14 p.p.m. (7).

Rice Grain and Foliage. Table II also presents the recovery and background values from control and fortified whole rice grain and rice foliage. Background values, not corrected for reagent blanks, averaged 0.06 p.p.m. for whole rice grain and 0.06 p.p.m. for rice foliage; recoveries for whole rice grain averaged 67% and for rice foilage averaged 71%. In addition, background values for rice grain and foliage from California ranged from less than 0.01 to 0.07 p.p.m. and less than 0.01 p.p.m., respectively (7).

Acknowledgment

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ANALYTICAL METHODOLOGY

Colorimetric Determination of Potasan in Coumaphos

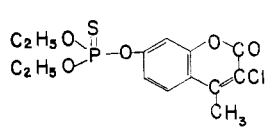
D. M. WASLESKI

Research Department, Chemagro Corp., Kansas City, Mo.

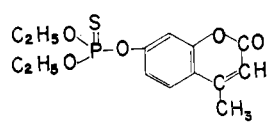
Potasan is separated from coumaphos by thin-layer chromatography on silica gel with chloroform. The Potasan spot, detected under ultraviolet light, is excised under vacuum and eluted from the silica gel with methanol. The residue after stripping is hydrolyzed with sodium carbonate and coupled with diazotized *p*-nitroaniline giving an intensely colored dye (λ_{max}. 500 mμ) which is compared with pure Potasan subjected to the same procedure.

Coumaphos, *O,O* - diethyl *O* - 3- (chloro - 4 - methyl - 7 - coumarinyl) phosphorothioate, is the product of the reaction of diethyl thiophosphoryl chloride and 3-chloro-4-methyl-7-hydroxycoumarin. Coumaphos has been formulated as a 25% wettable powder, Co-Ral, for control of animal parasites. Technical 3-chloro-4-methyl-7-hydroxycoumarin contains 3 to 5% of the unchlorinated analog, 4-methyl-7-hydroxycoumarin, as an impurity which reacts with diethyl phosphoryl chloride to form Potasan.

The structures of coumaphos and Potasan are similar, the only difference being the 3-chloro substituent:



Coumaphos



Potasan

Coumaphos is analyzed by measuring the ultraviolet absorption in methanol at 290 mμ (1), but Potasan, which has an ultraviolet maximum at 277 mμ, interferes. An accurate analysis for coumaphos requires a correction for the Potasan present. Also, as Potasan exhibits insecticidal properties, it is desirable to know the amount present. The ultraviolet and infrared spectra of these compounds do not show sufficient differences to make the determination of Potasan in coumaphos at the 3 to 5% level practical. Therefore, thin-layer chromatography was investigated.

Potasan can be separated cleanly from coumaphos by thin-layer chromatography using silica gel with chloroform as the solvent. Visual determinations on the thin-layer plate, after treatment with various color reagents, gave poor accuracy and reproducibility, so a method was developed involving separation, detection, extraction, and colorimetric determination (2) of the Potasan.

Experimental

Preparation of Standards. All standards were prepared from the purest Potasan—prepared by the reaction of diethyl thiophosphoryl chloride and 4-methyl-7-hydroxycoumarin and recrystallized from methanol—(m.p. 37–38° C.; one spot by TLC) and coumaphos (m.p. 94.5–95.0° C., two spots by TLC: coumaphos plus a trace of Potasan) (available from Chemagro Corp.). Solution A was a 5.00% w./v. solution of coumaphos in acetone. Solution B was a 0.500% w./v. solution of Potasan in acetone. Aliquots of 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml. of Solution B were