

COMMUNICATIONS

Thin-Layer Chromatographic Quantitation of Abate (*O,O,O',O'*-Tetramethyl-*O,O'*-thiodi-*p*-phenylene Phosphorothioate) Residues in Water

A thin-layer chromatographic procedure is described for quantitatively measuring Abate (*O,O,O',O'*-tetramethyl-*O,O'*-thiodi-*p*-phenylene phosphorothioate) concentrations in surface waters. Distilled water, surface water, and a 4-to-1

mixture of surface water and settled sewage samples were enriched with 33 to 107 $\mu\text{g.}$ of Abate per liter. After extraction and cleanup, average recovery was about 75%.

Abate, a pesticide used to kill mosquito larvae, is the thiophosphoric acid ester, *O,O,O',O'*-tetramethyl-*O,O'*-thiodi-*p*-phenylene phosphorothioate. Blinn and Pasarela (1966) published a colorimetric procedure for its quantitative determination. Wright *et al.* (1967) reported on a gas chromatographic procedure. As these analytical techniques are lengthy and time-consuming, this study was conducted to develop a rapid, simple analytical procedure for monitoring Abate residues in water samples by thin-layer chromatography.

Many thiophosphoric acid esters similar to Abate have been chromatographed on thin layers of Silica Gel G with mixtures of hexane and acetone (Randerath, 1963) and visualized by spraying with 1% *N,N*-dimethyl-*p*-phenylazoaniline in 95% 2-propanol (Ragab, 1967). However, no procedure has been published for quantitative determination of Abate by thin-layer chromatography.

EXPERIMENTAL PROCEDURES

Reagents and Apparatus. Chloroform, distilled from glass, was used as the extraction solvent. The purified reagent was obtained from Burdick and Jackson Laboratories, Inc., Muskegon, Mich.

Crystalline *O,O,O',O'*-tetramethyl-*O,O'*-thiodi-*p*-phenylene phosphorothioate (Abate) of 99.9% purity was obtained from American Cyanamid Co., Princeton, N. J. A standard, stock solution was prepared by dissolving 2.00 grams of Abate in 1 liter of acetone, and just before use, diluting 5.00 ml. to 1 liter with distilled water.

A slurry of 5.0 grams of Silica Gel G (Brinkmann Instruments, Inc., Cantiague Road, Westbury, L. I., N. Y.) and 10.0 ml. of water was spread on 20 \times 20 cm. thin-layer plates 250 microns deep with a rod which was treated for water resistance. The plates and details of the technique used are listed elsewhere (Kontes Glass Co., 1967).

A Hamilton PB-600 dispenser (Hamilton Co., P. O. Box 307, Whittier, Calif.) with 50- $\mu\text{l.}$ syringe, which was clamped 2 mm. above the plates, was used for stepwise spotting of samples. Lang-Levy pipets did not give satisfactory reproducibility.

Extraction and Cleanup. Desired amounts of the standard, stock Abate solution were diluted to 1500 ml. with distilled water, surface water, or a 4-to-1 mixture

of surface water and settled sewage. These samples were acidified with 5 ml. of 6*M* sulfuric acid, filtered through glass wool, and shaken for 1 minute with 500 ml. of chloroform. The chloroform layer was filtered through 50 grams of anhydrous sodium sulfate, and evaporated to dryness with a stream of dry nitrogen on a hot water bath at 60° to 70° C. The residues were dissolved in acetone just before the thin-layer plates were spotted. Table I gives volumes of acetone used to dissolve residues.

Thin-Layer Chromatography. The thin-layer plates were activated for 1 hour at 90° C., then allowed to cool to room temperature. Then 15 $\mu\text{l.}$ of acetone solutions containing Abate standards and sample residues were applied to the plates with the dispenser. The standard portions contained 9, 12, 15, 18, 21, and 24 $\mu\text{g.}$ of Abate, and the sample ones contained from 9 to 24 $\mu\text{g.}$ Each plate contained six standards and duplicate aliquots of the sample, respectively, as represented by viewing Figure 1 from left to right. The plates were developed for 1 hour in a tank presaturated with a 10-to-1 mixture of hexane and acetone, air-dried for 1 minute, exposed to bromine vapors for 1 minute, re-aerated for 3 minutes, and sprayed with 1% *N,N*-dimethyl-*p*-phen-

Table I. Recoveries of Abate Insecticide by Thin-Layer Chromatography

Abate Added, $\mu\text{g. per Liter}$	Dry Residue, $\mu\text{l. of Acetone}$ Added	Abate Found, $\mu\text{g. per Liter}$	Recovery, %
DISTILLED WATER			
33	50	30	91
53	50	39	73
53	50	39	73
SURFACE WATER			
53	50	41	78
53	50	37	71
4-TO-1 MIXTURE OF SURFACE WATER AND SETTLED SEWAGE			
0	50	0	0
33	50	28	85
53	50	39	74
53	50	36	68
80	75	48	60
107	100	78	73

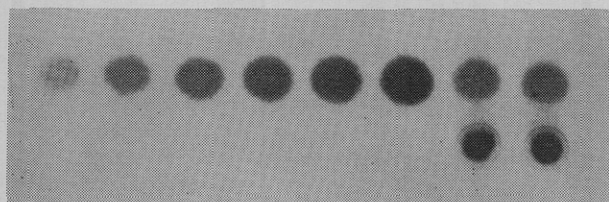


Figure 1. Thin-layer chromatogram of Abate standards (first 6 spots viewed from left to right) and samples (last 2 spots at far right) separated from a 4-to-1 mixture of surface water and settled sewage

ylazoaniline in 95% 2-propanol. (Caution! Bromine vapor is hazardous. Use a ventilated hood.) Areas of sharp red spots with R_f values of 0.11 ± 0.01 on a bright yellow background indicated the amount of Abate. Spot areas were outlined, transferred to tracing paper, and measured by superposition on millimeter graph paper.

RESULTS AND DISCUSSION

The per cent recovery of Abate from the samples was determined by averaging the spot areas from the duplicate aliquots and comparing this area to the curve derived from the six standards on the same plate. The typical spot area for 9 μg . of Abate applied in 15 μl . of acetone was 51 sq. mm., the area for 24 μg . was 172 sq. mm., and areas for microgram values between were directly proportional. Assuming no error in areas of six spots measured for standard spot area vs. microgram curves, the deviation of points from the straight line was never greater than 0.5 μg . A typical standard curve for 9 to 24 μg . of Abate applied in 15 μl . of acetone gave a straight line with a slope of 1.2 μg . per 10 sq. mm. Best results were obtained by using freshly prepared plates and allowing sufficient time for dissipation of bromine.

Figure 1 shows a typical chromatogram of Abate. The first six increasing spot areas represent Abate standards of 9, 12, 15, 18, 21, and 24 μg . spotted in 15 μl . of acetone, and the last two spots represent

duplicate 15- μl . aliquots of an Abate residue dissolved in 50 μl . of acetone. Referring to Figure 1, comparison of the last two spot areas with those of the standards led to an average Abate recovery of 68% from a 4-to-1 mixture of surface water and settled sewage spiked with 53 μg . per liter of Abate. Examination of Figure 1 clearly shows that interfering materials in the sewage mixture are either left behind or chromatographed away from Abate.

Table I presents recoveries of Abate from distilled water, surface water, and a 4-to-1 mixture of surface water and settled sewage. It also gives volumes of acetone used to dissolve dry residues prior to spotting duplicate 15- μl . portions onto the thin layers. An average recovery of 75% with an average deviation of 6% was obtained. A freshly prepared 4-to-1 mixture of surface water and settled sewage did not have a background value. Recoveries from distilled water or surface water appear to be the same as from a 4-to-1 mixture of surface water and settled sewage. Thus, impurities in either surface water or a 4-to-1 mixture of surface water and settled sewage do not appear to affect Abate recoveries appreciably.

LITERATURE CITED

- Blinn, R. C., Pasarella, N. R., *J. AGR. FOOD CHEM.* **14**, 152-6 (1966).
 Kontes Glass Co., Vineland, N. J., "Kontes General Catalog TG-30," pp. 101, 103, 1967.
 Ragab, M. T. H., Division of Agricultural and Food Chemistry, 154th Meeting, ACS, Chicago, Ill., September 1967.
 Randerath, K., "Thin-Layer Chromatography," p. 205, Academic Press, New York, 1963.
 Wright, F. C., Gilbert, B. N., Riner, J. C., *J. AGR. FOOD CHEM.* **15**, 1038-9 (1967).

L. H. Howe III
 C. F. Petty

Tennessee Valley Authority
 Chattanooga, Tenn. 37401

Received for review June 13, 1968. Accepted January 10, 1969. Division of Agricultural and Food Chemistry, 156th Meeting, ACS, Atlantic City, N. J., September 1968. Mention of specific manufacturers and models is illustrative and does not imply endorsement by the Tennessee Valley Authority.

Nature of Compounds Uniformly Labeled with Radiocarbon in a Benzene Ring

Mass spectrometric measurements show that a majority of the labeled molecules present in ring-UL- ^{14}C benzoic acid ring-UL- ^{14}C diphenamid contain more than one radiocarbon atom per

molecule. The possible implications of these results for experimentalists using ring-labeled compounds are discussed.

It is now common practice in residue studies to employ compounds uniformly labeled with radiocarbon in a benzene ring. All these compounds are made ultimately from the same starting material, radiocarbon-labeled benzene. Benzene- ^{14}C is prepared commercially by procedures that involve the trimerization

of symmetrical two-carbon reaction intermediates, customarily carried out at very high specific activities. Unlabeled benzene is then added to reduce the specific activity to usable levels. From these considerations, it can be predicted that both radiobenzene itself and compounds prepared from it will contain radioactive