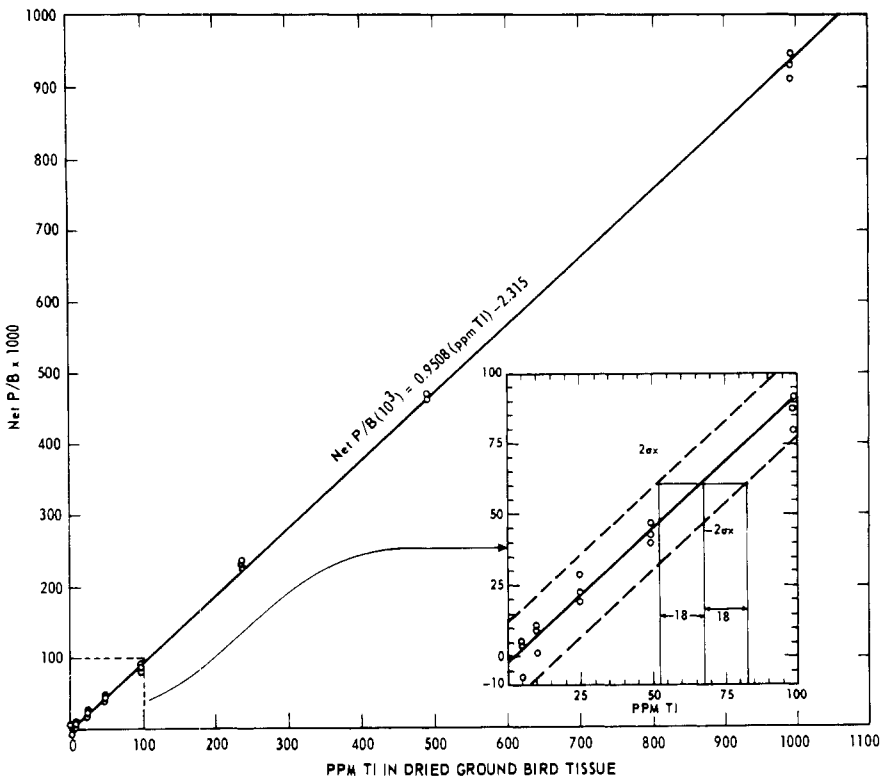

Figure 2. Thallium Lβ net peak to background ratio in dried ground bird tissue containing known additives of thallium

Linearity of response (net P/B × 1000) to increasing concentration of Tl (5 to 987 p.p.m.) is fit by the relation $y = a + bx$ where y = net P/B(1000), $a = -2.315$ P/B(1000), $b = 0.9508$ net P/B(1000)/p.p.m. Tl, x = p.p.m. Tl. The expanded inset replots the 0 to 100 p.p.m. data including the 95% confidence interval (2σ) on the P/B (1000) measurements which corresponds to ± 18 p.p.m.

weight basis would be computed using the appropriate desiccation value. Whereas the 3- to 5-gram samples of dry tissue indicated a detection limit of about 18 μg. Tl per gram, and were derived from 10 to 17 grams of fresh tissue, a lower detection limit of 5 μg. Tl per gram of fresh tissue would be expected from a 10-gram sample, dried and measured once. This suggests that at least 5 p.p.m. of Tl in a 10-gram sample of fresh tissue is required in order to prepare a dried sample with adequate Tl for the determination.

As the effective atomic number of most biologic materials does not vary markedly, it is probable that this method can be extended to other elements present in trace concentrations in biologic media if one uses the appropriate corrections for scattering within media of diverse composition in the region of the characteristic wave length of interest. For thallium determinations, a preliminary spectral scan in the region of the L-emission lines verified the absence of interfering elements. The proximity of the WLβ lines of the tube target to the thallium Lα line necessitated utilization of the less intense Tl Lβ emission. However, Tl sensitivity for the range of concentrations studied was sufficiently adequate and appears to equal or exceed most of the published wet-chemical methods (*1*, *2*).

Literature Cited

(1) Dyfverman, A., *Anal. Chim. Acta* **21**, 257–65 (1959).
(2) Korenman, I. M., "Analytical Chemistry of Thallium," Israel Program for Scientific Translations, Jerusalem, 1963.
(3) Rose, H. J., Flanagen, F. J., X-ray Fluorescence Determinations of Thallium in Manganese Ores, *U. S. Geol. Surv. Profess. Paper* 450-B, Art. **32**, p. B-82-83 (1962).
(4) Whittig, L. D., Buchanan, J. R., Brown, A. L., J. Agr. Food Chem. **8**, 419–21 (1960).

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INSECTICIDE DETERMINATIONS

Ultraviolet Spectrophotometric Method for Fenthion

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An improved analytical method has been developed for fenthion based on the UV absorption at 252 mμ after separating impurities on a Florisil column with heptane.

Fenthion, *O,O* - dimethyl *O* - [4-(methylthio)-*m*-tolyl] phosphorothioate, is also designated as BAY 29493, Baytex, Entex, or Tiguvon. Baytex formulations are used for mosquito control, Entex formulations for fly and roach eradication, and Tiguvon formulations for parasite control in animals.

A method for the determination of technical fenthion was developed by Farbenfabriken Bayer (*3*). This utilized a colorimetric measurement of the diazo coupling product of 4-amino-2-nitrobenzenesulfonic acid in alkaline buffer solution with 4-methylthio-*m*-cresol, an hydrolysis product of fenthion.

Hirano and Tamura (*5*) described a colorimetric method in which fenthion was hydrolyzed in a caustic alcohol solution, and the phenol produced was condensed with 4-aminoantipyrene to give an orange-colored pigment. This was extracted into chloroform and determined spectrophotometrically at 458 mμ.

An infrared - spectrophotometric method (*1*) is now used for manufacturing control at Chemagro for determining fenthion by measuring the thiono phosphorus (P=S) vibration peak at 12.0 microns.

Impurities in fenthion which were isolated and identified by Pavel (*7*) include *O,S*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate, *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl)]phosphate, 4-methyl-*m*-cresol, 3-methyl-4-(methylthioanisole) — (I), *O,O*-dimethyl chloridophosphorothioate-(II), and *O,O,O,O*-tetramethyldithiopyrophosphate - (III). As all the analytical methods cited are affected by the impurities present in technical fenthion, a more accurate method was needed.

Patchett and Batchelder (*6*) described a procedure which involved the chromatography of a synthetic mixture of Trithio [*S*-(*p*-chlorophenylthio)methyl-*O,O*-diethyl phosphorodithioate], its oxygen analog [*S*-(*p*-chlorophenylthio)-methyl-*O,O*-diethyl phosphorothioate], and its oxygen analog sulfone [*S*-(*p*-chlorophenylsulfonyl)methyl - *O,O* - diethyl phosphorothioate] on a Florisil column using a series of solvent mixtures of increasing polarity. Florisil was used because it was effective in purifying many types of experimental phosphate pesticides.

The improved procedure described in this paper involves the elution of fenthion from a Florisil column with heptane, dilution to the proper concentration, and measurement at 252 mμ. The polar components are retained on the column. The nonpolar components which are eluted simultaneously with fenthion are (II), (III), and (I). Only the latter compound interferes in the UV determination. However, it is determined independently by gas-liquid chromatography (GLC) or thin-layer chromatography (TLC) and a correction applied. The recovery of fenthion by this elution process is about 95%, and the 5% remaining on the column was determined by TLC.

Reagents

Adsorbent, Florisil, 60 to 100 mesh, 1200° F. activation (Floridin Co., Pittsburgh).

2,6 - Dibromo - *N* - chloro - *p* - quinoneimine (DCQ), 1% in acetone, reagent grade.

Standard Solutions. *O,O*-Dimethyl chloridophosphorothioate, (II), 0.01% in acetone.

O,O,O,O-Tetramethyldithiopyrophosphate, (III), 0.01% in acetone.

3-Methyl-4-methylthioanisole, (I), two needed, 10 p.p.m. in heptane and 0.1% in acetone.

These standards were purified by column chromatography (Florisil-Skelly B) and gave only one spot by two-dimensional TLC (heptane–acetone 7 to 1, and chloroform).

Equipment

Chromatographic plates, silica gel G (0.5-mm.) thin layer, prepared in the usual manner were activated at 110° C. for 90 minutes and stored in a desiccator (*4*).

Chromatographic tubes, Ultramax valve, 152 mm. long, 19-mm. i.d.

Gas chromatograph, Micro-Tek, GC 2500, equipped with flame-ionization detector and stainless steel column, 1/4 inch × 2 feet, packed with 3% silicone gum rubber SE-30 on Chromosorb W 60/80 mesh.

Hamilton microsyringe, 10 μl.

Spectrophotometer, ultraviolet.

Procedure

Determination of Fenthion. Three grams of Florisil are added to a 152 × 19 mm. chromatographic tube containing 20 ml. of heptane, A.R. The column is tamped with a broad-tipped glass rod until the Florisil is firm. A 1/2-inch plug of glass wool is placed on the top of the column to avoid disturbing the surface. The column is washed with 25 ml. of heptane, and about 1 mm. of heptane is left on the top of the Florisil. A disposable pipet is used to transfer about 500 mg. of accurately weighed sample to the column. The sample is allowed to pass slowly into the Florisil. The sides of the columns are washed four times with 5-ml. portions of heptane, and each portion is eluted separately at one drop per second or 3 ml. per min. The tube is filled repeatedly with heptane and eluted at four drops per second (12 ml. per min.), until the eluate fills a 500-ml. volumetric flask (Solution A). A 10-ml. aliquot of Solution A is pipetted into a 100-ml. beaker and evaporated to dryness under an air dryer. The residue is dissolved in heptane, transferred quantitatively to a 100-ml. volumetric flask, and diluted to volume with heptane (Solution B). A 10-ml. aliquot of Solution B is pipetted into a 100-ml. volumetric flask and diluted to volume with heptane (Solution C). The absorbances of Solution C and a standard similarly prepared are read at 252 mμ using matched silica cells and a heptane blank.

Determination of the Methyl Ether. The (I) is determined in fenthion by gas-liquid chromatography using the following settings: column temperature, 170° C.; injector port and transfer line, 190° C.; nitrogen flow, 100 ml. per min.; standard or sample injection volume, 5 μl.; input resistance, 10^9 ohms; attenuation for concentrations less than 0.5%, X16 and for higher concentration, X32 or X64. A 10% solution of fenthion to be analyzed is prepared in acetone. Three standard solutions of (I) in acetone are prepared—0.01, 0.05, and 0.1%—and chromatographed. The peak heights found at an elution time of about 10 minutes are proportional to the concentrations used. None of the other constituents interferes in this region. The (I) is also determined by thin-layer chromatography by spotting 10 μl. of the 10% acetone solution of fenthion and 4-, 6-, 8-, 10-, 12-, and 14-μl. spots of 0.1% standard (I) in acetone, on a silica gel G plate. The plate is developed in heptane-acetone 7 to 1, sprayed with DCQ reagent, heated in a 110° C. oven for 10 minutes, and the concentration in the sample estimated by visual comparison with the standard spots at R_f 0.48.

Discussion

Florisil effects good separation of the polar impurities. The less polar impurities remain with fenthion. Other resins such as polyamide used by Endres and Hörmann (*2*), although well-suited for separation of phenols and acids, gave poor separation of the components in technical fenthion. When fenthion was applied to a silica gel G column, poor separation and more tailing were observed than with a Florisil column.

To avoid the use of chromatographically purified heptane throughout the procedure, the first heptane aliquot was evaporated to dryness before further dilutions were made in heptane. Figure 1 shows the elution profile of fenthion and the four nonpolar components collected in 1-ml. fractions from the Florisil column. Reducing the column flow rate to 0.3 ml. per min. did not significantly improve the component separation. Four minor spots together with fenthion were revealed. Three of these four spots, identified by comparison with known standards having the same R_f's, were: (I), purple, R_f = 0.48; (II), light pink, R_f = 0.43; unknown, pink, R_f = 0.41; and (III), light pink, R_f = 0.23. The highest purity obtainable as a standard contained 98.2% fenthion, and this was used in the present work.

R_f values on TLC indicated that the unknown pink spot at R_f 0.41 was a mixture of *O,O,O*,-trimethyl phosphorothioate and *O,O,S*-trimethyl phosphorodithioate. However, neither IR nor GLC scans on extracts of the isolated spot confirmed this, and further work is needed to identify this spot.

To determine when fenthion had eluted completely from the column, about 500 mg. of material was chromatographed, and 10 100-ml. fractions were collected. The recoveries are shown in Table I, and indicate that 500 ml. is a convenient volume in which to collect the major portion (95%) of

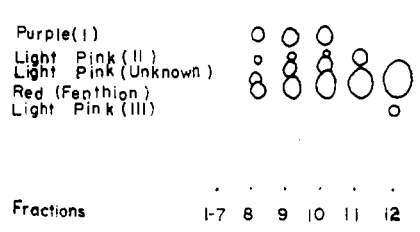

Figure 1. Thin-layer chromatogram (heptane–acetone 7:1, DCQ reagent) of technical fenthion fractions

Table I. Recovery of Fenthion from Florisil Column

Fraction	Weight, %	Fraction	Weight, %
1	79.7	6	0.9
2	7.8	7	0.8
3	3.5	8	0.6
4	2.1	9	0.5
5	1.8	10	0.2

Total = 97.9%

Table II. Reproducibility Study Given in Per Cent

Day 1	Day 2	Day 3	Day 4
91.1	89.8	89.9	89.1
90.7	90.0	89.3	91.3

Average = 90.0
Standard deviation = 0.8
95% confidence limits = ±1.9

fenthion. The over-all recovery of fenthion was determined by chromatographing replicate 500-mg. samples of standard fenthion as described. The four 500-mg. samples yielded: 95.7, 94.6, 94.7, and 95.8% for an average of 95.2%. Each batch of Florisil is standardized and a recovery factor (usually 1.05) applied to all subsequent determinations. Thus, essentially 100% of the fenthion added was recovered.

A recovery of 94.7% was obtained when 50 mg. of standard fenthion was chromatographed using 1 gram of Florisil in a 6-mm. column. This shows that the reduction in the column size and sample size gave the same recovery. Thus, if limited sample is available, smaller columns of proportionate size and capacity can be used.

Replicate determinations on one sample by two people on 4 days are shown in Table II. Nonreproducible results may be caused by incomplete adsorption of the polar impurities owing to improper packing and/or a high flow rate. Florisil should be protected from moisture at all times in order to maintain reproducibility.

(I) is the only nonpolar compound which interferes at 252 mμ. However, it can be determined independently by GLC or TLC then applied as a correction to the fenthion value.

Acknowledgment

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Literature Cited

(1) Chemagro Corp., Kansas City, Mo., "Determination of Fenthion by Infrared Spectrophotometry," Test Method No. C-8.3.
(2) Endres, H., Hörmann, H., *Angew. Chem. Intern. Ed. Engl.* **2,** 254 (1963).
(3) Farbenfabriken Bayer A. G., Leverkusen, Germany, "Analytical Method for S-1752," Le 59 165/144/3, February 13, 1959.
(4) Gordon, A. H., Eastoe, J. E., "Practical Chromatographic Techniques," p. 188, Van Nostrand, Princeton, N. J., 1964.
(5) Hirano, Y., Tamura, T., *Anal. Chem.* **36,** 800 (1964).
(6) Patchett, G. G., Batchelder, G. H., J. AGR. FOOD CHEM. **9,** 395 (1961).
(7) Pavel, K., Farbenfabriken Bayer A.G., private communication, 1963.

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ANALYTICAL PROCEDURE

Colorimetric Determination of Dimethoate Residues in Plants and Milk

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An improved colorimetric method for the quantitative estimation of dimethoate (Cygon, Rogor) residues in plants and milk is based on treatment of dimethoate residues with methanolic sodium hydroxide and 1-chloro-2,4-dinitrobenzene to form a colored product, which is measured spectrophotometrically. The calibration curve conforms to Beer's law at the peak wavelength of 505 mμ. No interference was encountered from 38 other pesticides or from the organic solvents used in the method. Residue data were obtained on lima beans, green beans, cabbage, range forage, turnip greens, and tomatoes.

PUBLICATIONS devoted to the quantitative determination of residues of dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate, Cygon, Rogor] have been discussed by Giang and Schechter (*1*). These authors have developed and discussed in detail an analytical procedure for residues of this compound based on the reaction between thioglycolic acid and phospho-18-tungstic acid.

In the colorimetric method described herein, dimethoate is treated with methanolic sodium hydroxide and 1-chloro-2,4-dinitrobenzene to form a colored product. The color development procedure is a modification of the amine test reported by Snell and Snell (*4*), based upon the work of McIntire, Clements, and Sproull (*3*). Kolbezen, Eckert, and Bretschneider (*2*) reported a modification to eliminate the high background absorbance due to the formation of 2,4-dinitrophenetole. The procedure appears to be less subject to interference from different contaminants that may be present in sample extracts than the method of Giang and Schechter.

The oxygen analog of dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothioate] also reacts to form the color by the present method, but it tends to get lost in the cleanup procedure, so that both dimethoate and its oxygen analog cannot be determined in the same crop sample. Walker and Beroza (*7*) report separation of the two by using thin-layer chromatography, and suggest that it could be used as the differentiation procedure for cleanup of residue samples. Van Middelem and Waites (*6*) compared gas chromatography results with the colorimetric method.

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