tion curves indicate the presence of carboxylate ions that could be explained by the presence of Na salts of some organic acids as impurities.

Elementary analyses of the model substance gave the simplest empirical formula of $C_{18}H_{26}O_{11}N$, whereas the empirical formula of the isolate from barley roasted at 250° for 40 min was found to be $C_{18}H_{27}O_{11}N$. Elementary analysis of both the model substance and the isolate from roasted barley indicated that they are of similar composition. The manner by which nitrogen is chemically bound in the brown substance, produced by the process of nonenzymatic browning after the temperature treatment, is not understood. The concentration of the brown substance in roasted barley was unexpectedly high. However, it can be explained by the fact that nonenzymatic browning occurs in two ways. The first occurs from the reaction between amino acid and reducing sugar, and the second occurs from caramelization, which can, but need not, include nitrogen.

Isolation of the brown pigments from roasted barley by means of ion-exchange resin Permutit ES confirms that the substance is of an ionic nature and has a negative charge. According to the results obtained from uv and ir spectra (Figures 1 and 2) and from elementary analysis it was concluded that the isolate of the brown substance from roasted barley was of similar composition to the model substance.

ACKNOWLEDGMENT

The able technological assays of M. Glogovčan are gratefully acknowledged. The authors wish to thank P. Džadžić for his expert assistance.

LITERATURE CITED

Anet, E. F. L. J., *Aust. J. Chem.* **13,** 396 (1960).
Anet, E. F. L. J., *Aust. J. Chem.* **14,** 295 (1961).
Anet, E. F. L. J., *Chem. Ind. (London)* **9,** 262 (1962).
AOAC, "Official Methods of Analysis", 9th ed, Association of Official Agricultural Chemists, Washington, D.C., 1960.
Burton, H. S., McWeeny, D. L., *Chem. Ind. (London)* **11,** 462 (1964).
Grujić-Injac, B., Milić, B. Lj., Piletić, M. V., Lajšić, S., First Conference of Chemists and Technologists of Yugoslavia, Novi Sad, Dec 1971, Paper 16/III.
Holtermand, A., *Die Stärke* **18,** 319 (1966).
Kass, J. P., Palmers, L. S., *Ind. Eng. Chem.* **32,** 1360 (1940).
Knauf, A. E., Hann, R. M., Hudson, C. S., *J. Am. Chem. Soc.* **63,** 1447 (1941).
Lea, C. H., Hannan, R. S., *Nature (London)* **165,** 438 (1950).
Lee, C. M., Chichester, C. O., Lee, T.-C., IVth International Congress of Food Science and Technology, Madrid, Sept 1974, Topic 7a, Paper 11.
Milić, B. Lj., Vlahović, M. N., *J. Food Sci.* **36,** 828 (1971).
Reynolds, T. M., Anet, E. F. L. J., Ingles, D. L., Communication of the 1st International Congress of Food Science and Technology, London, 1962.
Underwood, C. E., Deatherage, F. H., *J. Food Res.* **17,** 419 (1952).
Wasserman, A. E., Spinelli, A. M., *J. Food Sci.* **35,** 328 (1970).
Wiseman, H. G., Mallack, J. C., Jackson, C. O., *J. Agric. Food Chem.* **8,** 78 (1960).
Wolfrom, M. L., *J. Am. Chem. Soc.* **67,** 53 (1945).
Wolfrom, M. L., Plunket, R. A., Liver, M. L., *J. Agric. Food Chem.* **8,** 58 (1960).

Received for review September 19, 1974. Accepted April 23, 1975.

Gas Chromatographic Determination of Thiofanox Residues in Soil, Plants, and Water

Wei-Tsung Chin,* Warren C. Duane, Mary B. Szalkowski, and Don E. Stallard

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, undergoes two-step oxidation in soil, plants, and animals to its sulfoxide (P_1), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)-carbonyl]oxime, and sulfone (P_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)-carbonyl]oxime. Procedures are presented for determining the total carbamate residues of the thiofanox (P_t) and some individual metabolites in soil, plants, and water.

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, is a potent systemic and contact insecticide developed by Diamond Shamrock Corporation. Metabolic studies in soils (Duane, 1974), plants (Whitten and Bull, 1974), and animals (Sullivan and Tallant, 1974) show that P undergoes rapid oxidation to its sulfoxide (P_1), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)carbonyl]oxime, and sulfone (P_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)carbonyl]oxime. In most cases, only P_2 hydrolyzes to its oxime (O_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime. A preferred metabolic pathway of P is outlined in Figure 1 and the LD_{50} values of the residues involved are given in Table I. Judging from the relative toxicities of these six compounds listed in Table I and their quantities determined during metabolic studies, P_2 and P_1 were found to be the major and P and O_2 the minor residues in thiofanox-treated samples. For routine residue analysis, all P and P_1 are first oxidized quantitatively to P_2 which is determined as the total carbamate residues of thiofanox (P_t). This is based on the fact that of these three carbamates, P_2 is the major residue and has the highest toxicity. In this paper, procedures are presented for gas chromatographic determinations of P_t, P, P_1, P_2, and O_2 in soil, potatoes, sugar beets, cottonseeds, cotton gin-trash, foliage, and water.

Diamond Shamrock Corporation, T. R. Evans Research Center, Painesville, Ohio 44077.

EXPERIMENTAL SECTION

Apparatus and Reagents. The instrument used was a Tracor 550 gas chromatograph equipped with a flame photometer detector and a 394-mμ sulfur filter. The chromatographic columns were 6 ft long glass tubing, 0.25 in. o.d. and $^3/_{16}$ in. i.d. Two columns were used: column no. 1 was packed with 1.5% OV-17 and 1.95% OV-210 on Chromosorb W DMCS 60–80 mesh; column no. 2 was packed with 6% DC 200 on 80–100 mesh Gas Chromosorb Q (Tracor, Inc., Austin, Tex.). Florisil (Floridin Co., Pittsburgh, Pa.) was activated at 95° for 10 hr and maintained at this temperature. Cleanup columns were glass tubes, 19 mm i.d. × 400 mm, with a Teflon stopcock. Peracetic acid (FMC, Inorgan-

Table I. LD_{50} of Thiofanox and Its Residues (mg/kg)

Compounds	Oral (rat)	Dermal (rabbit)
P, 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)-carbonyl]oxime	8.5	38.9
P_1, 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)carbonyl]oxime	3.8	178.0
P_2, 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)carbonyl]oxime	1.9	60.0
O, 3,3-dimethyl-1-(methylthio)-2-butanone oxime	813	
O_1, 3,3-dimethyl-1-(methylsulfinyl)-2-butanone oxime	2000	
O_2, 3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime	734	

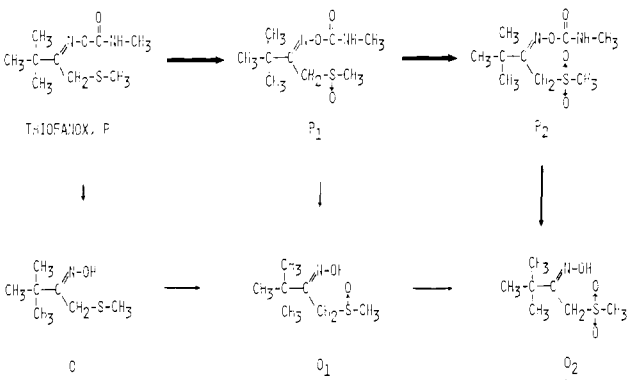

Figure 1. Probable metabolic pathway for thiofanox.

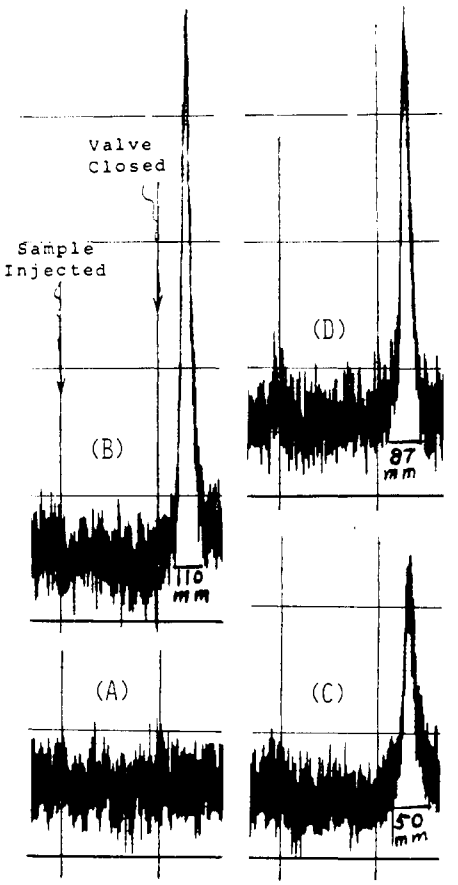

Figure 2. Gas chromatographic determinations of P_t: (A) extract of a check soil sample, 5.0 μl/1.0 ml per 50 g; (B) 96% recovery of a check soil sample fortified with 100 ppb of P and 1.0 ppm of T, 5.0 μl/2.5 ml per 50 g; (C) this peak represents 0.73 ppb of P_t in a run-off collected 36 in. away from a plot sprayed with 1.5 lb/acre of EC P, 5.0 μl/1.0 ml per 1100 g; (D) this peak represents 16.0 ppb of P_t in a potato sample harvested from a treatment of 1.5 lb/acre of 10-G P in infurrow at planting, 5.0 μl/2.0 ml per 200 g, column no. 1, attenuator: 16×10^3.

ic Division, Buffalo, N.Y.) and other reagents and solvents were all laboratory grade.

Determination of P_t in Soil. *Extraction of P, P_1, P_2, and O_2.* Extract 50 to 100 g of air-dried, 12 mesh soil by macerating with 500 ml of acetone for 2 min at high speed with a sealed Waring Blendor. Filter by suction and wash with 100 to 200 ml of acetone. Evaporate the acetone extract in a 1-l. round-bottomed flask at 45° with a flash evaporator.

Oxidation of P and P_1 to P_2. When the volume of the extract is reduced to approximately 50 ml, remove the flask and add 40 ml of 0.5 *N* HCl, 2 ml of 30% H_2O_2, and 2 ml of 40% peracetic acid (Roberts and Caserio, 1964). After stirring for 5 min, continue the evaporation for 20 more min. Transfer the residue extract quantitatively to a 125-ml separatory funnel. Wash the flask with 2 × 20 ml of 0.2 *N* HCl and extract P_2 with 3 × 20 ml of $CHCl_3$. Mix 0.05 g of active carbon with the $CHCl_3$ extract which is then dried by adding 10 to 20 g of Na_2SO_4.

Cleanup by Column Chromatography. Pack 15 g of activated Florisil and 10 g of granular Na_2SO_4 on the top of florisil in a chromatographic column. Prewet the column with 20 ml of $CHCl_3$ and quantitatively transfer the $CHCl_3$ extract onto the column through a folded filter paper. When the transfer is completed, elute the column with 30 ml of fresh $CHCl_3$ followed by 50 ml of anhydrous diethyl ether. Change receivers and elute P_2 with 100 ml of acetone. Evaporate the acetone eluate to dryness in a 250-ml flat-bottomed flask at 45° using the flash evaporator. Finally, dissolve P_2 in 1000 μl of benzene for gas chromatographic determination.

Determination of P_t in Other Samples. *Potatoes and Sugar Beets.* Extract 200 g of chopped sample with 2 × 500 ml of acetone. Evaporate the acetone extract at 45° with two drops of Dow Corning Antifoam A until approximately 150 ml of aqueous solution is left. Add 40 ml of acetone, 10 ml of 5 *N* HCl, 2 ml of 30% H_2O_2, and 2 ml of 40% peracetic acid. After stirring for 5 min, continue the evaporation for 20 more min. Filter the residue extract through a folded filter paper into a 250-ml separatory funnel. The remaining procedures for extraction and cleanup are the same as for soil.

Foliage. Extract 50 g of chopped sample with 500 ml of acetone in a manner similar to that used for soil.

Water. Filter the water sample and acidify to approximately 0.2 *N* HCl. Extract the residues with $CHCl_3$ and evaporate the extract to dryness at 45°. Add 40 ml of acetone, 40 ml of 0.5 *N* HCl, 2 ml of 30% H_2O_2, and 2 ml of 40% peracetic acid for oxidation. The remaining procedures are the same as for soil.

Cottonseed. Extract 50 g of finely ground delinted cottonseeds with 500 ml of dichloromethane. For 50 g of undelinted, ground cottonseeds, use 2 × 500 ml of dichloromethane for extraction. Evaporate the dichloromethane to dryness at 45° and add 40 ml of acetone, 40 ml of 0.5 *N* HCl, 2 ml of 30% H_2O_2, and 2 ml of 40% peracetic acid. Stir for 5 min and continue the evaporation for 20 more min. Transfer the residue extract into a 125-ml separatory funnel and separate the lower aqueous fraction into a second separatory funnel (through a folded filter paper if emulsion

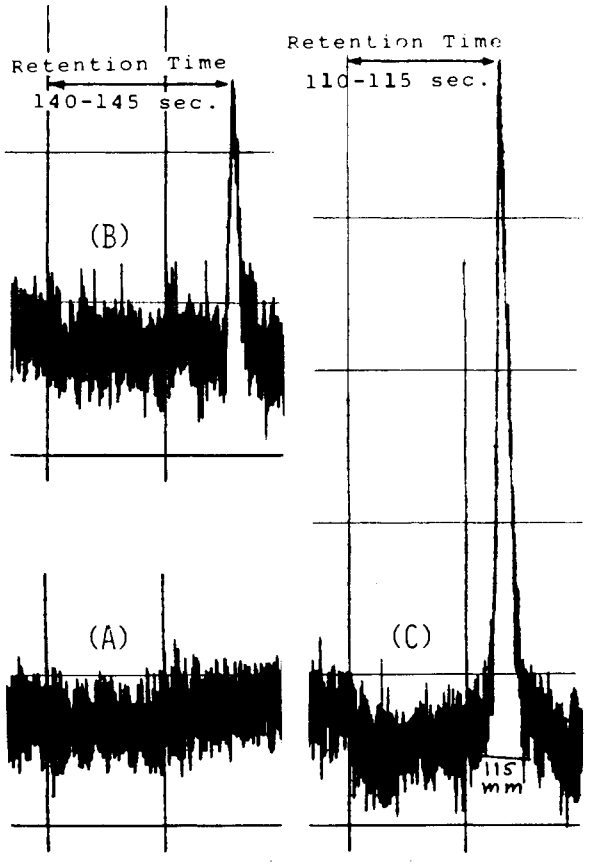

Figure 3. The insensitivity of T and different retention times of T_2 and P_2: (A) 100 ng of T; (B) 100 ng of T_2; (C) 10.0 ng of P_2, column no. 1, attenuator: 16×10^3.

is formed). Wash the reaction flask with 2 × 20 ml of 0.2 *N* HCl. Transfer the washings into the top oily fraction contained in the first separatory funnel and mix well. Separate the lower aqueous layer into the second separatory funnel. Extract the aqueous fraction with 50 ml of cyclohexane followed by 3 × 20 ml of $CHCl_3$. Discard the cyclohexane fraction. Mix the $CHCl_3$ extract with 0.05 g of active carbon and 10 to 20 g of Na_2SO_4 and cleanup by column chromatography.

Cotton Gin-Trash. Extract 50 g of dried and finely ground gin-trash with 500 ml of acetone in a manner similar to that used for soil.

Gas Chromatographic Determination of P_2. The gas chromatograph was operated in the oxidative mode as follows: column no. 1, 185° for column, inlet, and outlet, 160° for detector; column no. 2, 160° for column, 190° for inlet and outlet, 165° for detector. Identical flow rates were applied to both columns: 150, 40, 20, and 80 ml/min for the hydrogen, air, oxygen, and nitrogen, respectively. Depending on the concentration of P_t in a sample, adjust benzene to an optimum volume. For each injection, 5.0 μl of sample solution is recommended. The standard curve is prepared by extracting and cleaning a check sample according to the procedure described above. Evaporate the extract to dryness and add 500–1000 μl of a standard P_2 solution (5.0 ng of P_2/μl of benzene). Aliquots of this mixture are taken for constructing standard curves by plotting peak heights against the nanograms of P_2 injected. Examples of P_2 determinations in soil, water, and potato samples are shown in Figure 2.

An Alternative Procedure for Determining P_t in Soil. Evaporate the acetone extract at 45° completely. Wash the flask with 3 × 20 ml of 0.2 *N* HCl and transfer the washings quantitatively through a filter paper into a 125-ml separatory funnel. Extract the residues with 3 × 20

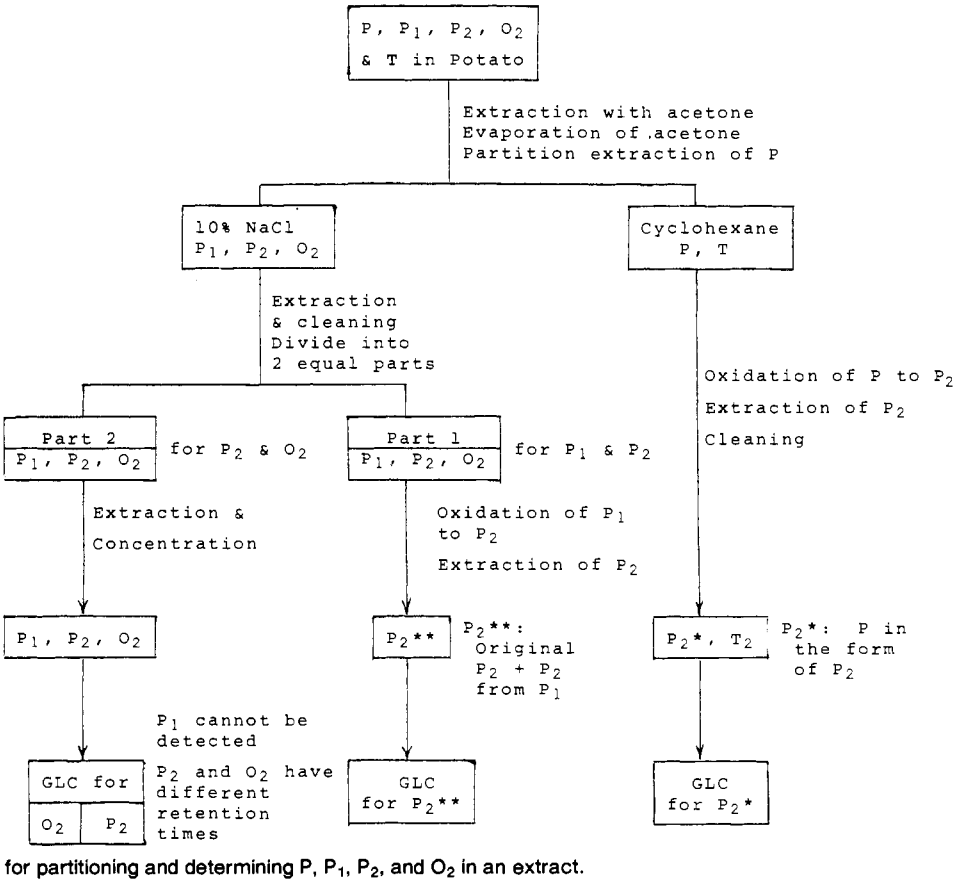

Figure 4. An outline for partitioning and determining P, P_1, P_2, and O_2 in an extract.

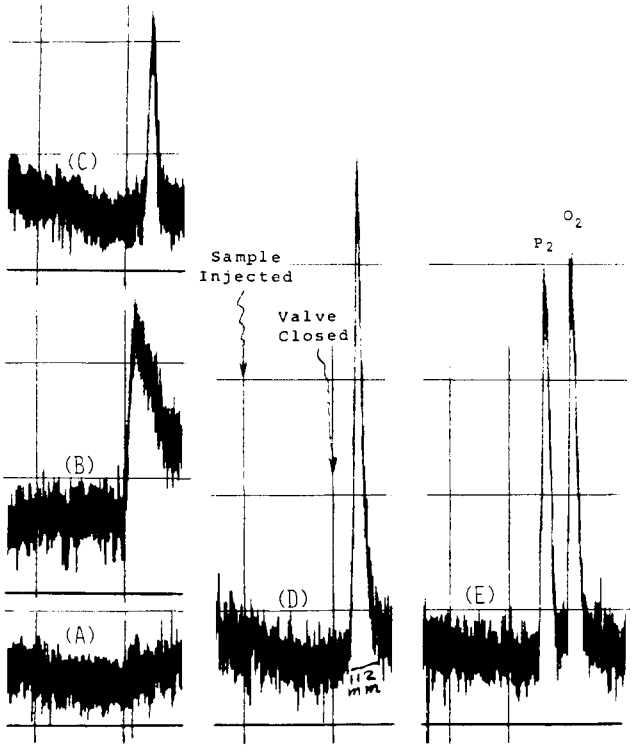

Figure 5. Recoveries of a check potato sample fortified with 100.0 ppb of P, P_1, P_2, and O_2 and 1.0 ppm of T: (A) the check sample without fortification; (B) the insensitivity of 100 ng of P_1; (C) 96% recovery of P_2 in part 2, no oxidation, P_2 = 96.0 ppb, 5.0 μl/10 ml per 100 g; (D) sum of P_1 and P_2 in the form of P_2 in part 1, oxidized, P_2 = 189.0 ppb, P_1 can be evaluated by 189 − 96 = 93 ppb, 93% recovery of P_1, 5.0 μl/10 ml per 100 g. All the above determinations were made with column no. 1; (E) using column no. 2 to determine P_2 and O_2 at the same time; O_2 = 94.0 ppb, 94% recovery, 5.0 μl/10 ml per 100 g; attenuator, all at 16 × 10^3.

ml of $CHCl_3$ and evaporate the $CHCl_3$ extract to dryness at 45° in a 250-ml flat-bottomed flask. Add 5 ml of $CHCl_3$ and 1 ml of 40% peracetic acid to the flask. Stir for 20 min and evaporate the $CHCl_3$ completely again. Add 50 ml of saturated $NaHCO_3$ solution and swirl until all gas escapes. Transfer the mixture into a 125-ml separatory funnel quantitatively and extract P_2 with 3 × 20 ml of $CHCl_3$. The $CHCl_3$ extract is dried over Na_2SO_4 and evaporated to dryness. Dissolve P_2 in benzene for gas chromatographic determination.

Determination of Pt in Other Samples by the Alternative Procedure. This simplified procedure for soil can be applied to foliage and water without modification. In the case of potatoes and sugar beets, 10 ml of 5 *N* HCl is added to the remaining aqueous extract. Transfer the residue extract through a folded filter paper into a 250-ml separatory funnel and do the same extraction and oxidation as for soil. This alternative procedure, however, has not been successfully applied to cottonseeds.

Extraction of P, P_1, P_2, and O_2 from a Fortified Check Potato Sample. It is known that temik (T), 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime, has a similar chemistry, activity, and metabolic pathway as thiofanox (Andrawes et al., 1973). For this reason, during the routine analysis of P and its metabolites, the possible interference of T and its sulfone (T_2), 2-methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime, must be considered. Under the working conditions specified in this paper, the interferences of T and T_2 are eliminated by taking advantage of their insensitivity and different retention times compared with P_2, as shown in Figure 3.

The gas chromatographic determinations of P, P_1, P_2, and O_2 in an extract are demonstrated by recovering these four residues fortified in a check potato sample as follows. Extract 200 g of a check potato sample fortified with 100 ppb of P, P_1, P_2, and O_2 and 1.0 ppm of T with 2 × 500 ml of acetone. After evaporating the acetone completely at 45°, dissolve 15 g of sodium chloride in the remaining aqueous extract. Partition P and T with 3 × 50 ml of cyclohexane as outlined in Figure 4. Divide the sodium chloride fraction into two equal parts for further partitions.

Determination of P. Evaporate the cyclohexane fraction to dryness at 50–55° using a flash evaporator. The remaining processes for oxidizing P to P_2 and subsequent cleanup and determination are identical with the determination of P_t.

Determination of the Sum of P_1 and P_2 in the Form of P_2. Extract part 1 of the sodium chloride fraction with 3 × 20 ml of $CHCl_3$. Evaporate the $CHCl_3$ extract to dryness at 45°. The remaining processes for oxidizing P_1 to P_2 and subsequent determination of P_2 are identical with the determination of P_t.

Determination of P_2 and O_2. Extract part 2 of the sodium chloride fraction with 3 × 20 ml of $CHCl_3$. The $CHCl_3$ extract is dried over Na_2SO_4 and evaporated to dryness at 45°. Dissolve the residues in benzene and determine P_2 and O_2 directly without oxidation using column no. 2, as shown in Figure 5.

Evaluation of P_1. Under the working conditions specified, P_1 cannot be detected by both columns, as shown in Figure 5. The actual amount of P_1 fortified, however, can be quantitatively evaluated by subtracting the amount of P_2 (the second part, no oxidation) from the sum of P_1 and P_2 (the first part, with oxidation), as demonstrated in Figure 5.

RESULTS AND DISCUSSION

The procedures presented are simple and sensitive enough for routine residue analysis of thiofanox. Recoveries averaging 95% are routinely obtained with fortifications of 50 to 500 ppb of P or its metabolites. The possible interferences of temik and its sulfone are eliminated. In actual samples treated with thiofanox, both P and O_2 are in trace or negligible quantities compared with P_1 and P_2. Because of the relative low toxicity and quantity of O_2 in actual samples, O_2 is usually neglected for routine residue analysis. However, if O_2 is particularly studied, column no. 2 is good for its determination.

ACKNOWLEDGMENT

The authors thank Thomas A. Magee for his help in developing this method.

LITERATURE CITED

Andrawes, N. R.. Romine, R. R., Bagley, W. P., *J. Agric. Food Chem.* **21**, 379 (1973).
Duane, W. C., Diamond Shamrock Corporation, Cleveland, Ohio, Technical Bulletin, 1974.
Roberts and Caserio, Ed., "Basic Principles of Organic Chemistry", W. A. Benjamin, New York, N.Y., 1964, p 756.
Sullivan, L. J., Tallant, M. J., 168th National Meeting of the American Chemical Society, Division of Pesticide Chemistry, Atlantic City, N.J., 1974, paper 56.
Whitten, C. J., Bull, D. L., *J. Agric. Food Chem.* **22**, 234 (1974).

Received for review March 19, 1975. Accepted June 25, 1975.