Pease, H. L., Gardiner, J. A., *J. Agric. Food Chem.* **17**, 267 (1969).
Pease, H. L., Holt, R. F., *J. Assoc. Off. Anal. Chem.* **54**, 1399 (1971).
Peterson, C. A., Edgington, L. V., *J. Agric. Food Chem.* **17**, 898 (1969).
Reyes, A. A., *Phytopathology* **65**, 535 (1975).
Rouchaud, J. P., Decallonne, J. R., *J. Agric. Food Chem.* **22**, 259 (1974).
Steurbaut, W., Dejonckheere, W., Kips, R. H., *Meded. Fac. Landbouwwet. Rijksuniv. Gent* **38**, 917 (1973).
Upham, P. M., Delp, C. J., *Phytopathology* **63**, 816 (1973).

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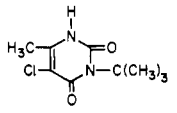
Determination of Terbacil and Metabolite Residues Using Microcoulometric Gas Chromatography

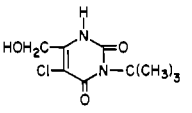
Richard F. Holt* and Harlan L. Pease

Residues of terbacil herbicide (3-*tert*-butyl-5-chloro-6-methyluracil) and three metabolites in plant and animal tissues and in soil are determined by initial extraction with chloroform, cleanup by liquid/liquid partitioning steps, and measurement by halogen-sensitive microcoulometric gas chromatography after formation of silyl derivatives of the metabolites. Method sensitivity is 0.04 ppm for all four compounds relative to a 25-g sample.

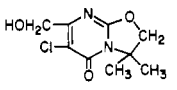
Terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) is the active ingredient in Du Pont's Sinbar terbacil herbicide. An analytical method for determining residues of terbacil has been published previously (Pease, 1968). The present paper describes a new procedure which detects not only the parent compound but three metabolites: 3-*tert*-butyl-5-chloro-6-hydroxymethyluracil (metabolite A); 6-chloro-2,3-dihydro-7-hydroxymethyl-3,3-dimethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one (metabolite B); and 6-chloro-2,3-dihydro-3,3,7-trimethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one (metabolite C). These metabolites were all detected in the urine of dogs maintained on diets containing terbacil (Rhodes et al., 1969). The same metabolites were found in [^{14}C]terbacil studies with alfalfa (Rhodes, 1977).



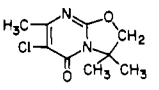
TERBACIL



METABOLITE A



METABOLITE B



METABOLITE C

This new residue method is based on gas chromatographic measurement after reaction of the extracted residues with Regisil-TMCS [bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane]. The derivatization step is necessary to convert metabolites A and B to more volatile compounds.

EXPERIMENTAL SECTION

Apparatus and Reagents. The Tracor Model MT-220 gas chromatograph (Tracor Instruments, Austin, Tex.) equipped with a Dohrmann microcoulometric titrating system, consisting of a C-200 microcoulometer, T-300 halide titrating cell, and a S-200 sample inlet combustion unit was used for the analyses. The chromatographic column was 6 ft glass, 0.25 in. o.d., 3/16 in. i.d. packed with 5% XE-60/0.2% Epon 1001 on 80–100 mesh Gas-Chrom Q (Applied Science Lab., Inc., State College, Pa.).

Homogenization and extractions were conducted using a blender–centrifuge bottle and adapter base as shown in Figures 1 and 2. It is not necessary to construct this specialized equipment unless desired. However, use of this equipment reduces the possibility of mechanical losses by blending and centrifuging in the same piece of apparatus. Considerable analyst time is also saved as there is less handling of the sample and less cleanup of equipment. These items were designed in this laboratory and have been in use for several years. Conventional bottles and centrifuge tubes may be used but are somewhat more time consuming. Centrifugation was carried out with an International size 1, type SB centrifuge capable of accommodating the 250-ml bottle shown in Figure 1 and with a standard clinical model bench centrifuge.

The reference standards of terbacil and metabolites A, B, and C were obtained from the Biochemicals Department, Agrichemicals Marketing Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. The solvents used were distilled-in-glass purchased from Burdick and Jackson Laboratories, Inc., Muskegon, Mich. The silylation reagent used was Regisil-TMCS purchased from Regis Chemical Co., Chicago, Ill.

Isolation. Weigh 25 g of a representative sample (use 50 g when analyzing milk, urine, or aqueous solution) into the blender–centrifuge bottle, add 150 ml of chloroform, cover, place in the adapter base, and blend at high speed for about 5 min. (Note: Chloroform should be used only in a well-ventilated hood. Skin contact should be avoided. Use of neoprene gloves is suggested.) Centrifuge at 1500 rpm for 10 to 15 min and carefully pass the chloroform through cotton into a 500-ml round-bottomed flask. For liquid samples, the lower chloroform layer may be withdrawn from the blender bottle using a 200-ml syringe. Repeat the extraction two more times using additional 100-ml portions of chloroform. Add 10 ml of water to the combined extracts and evaporate the chloroform in a vacuum rotary evaporator at 60 °C.

Biochemicals Department, Research Division, Experimental Station, E. I. du Pont de Nemours & Co., Inc., Wilmington, Delaware 19898.

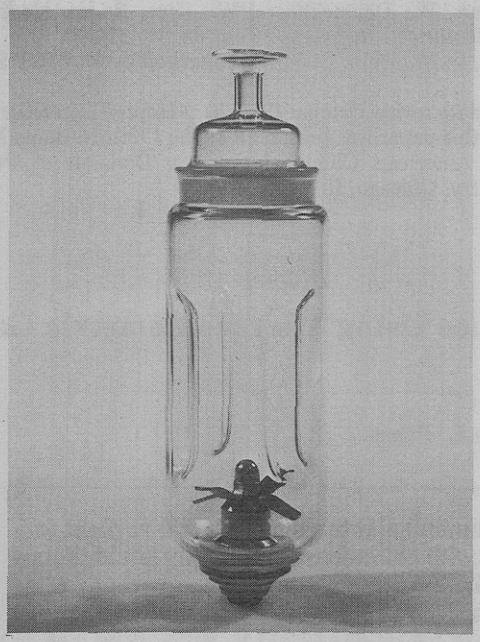


Figure 1. Blender–centrifuge bottle. Reprinted by permission of copyright owners (Pease and Holt, 1971).

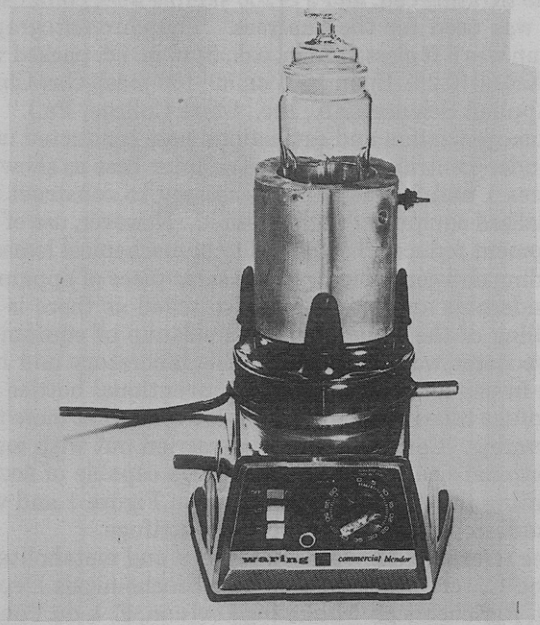


Figure 2. Combined extraction unit: Waring blender base, adapter base, blender–centrifuge bottle. Reprinted by permission of copyright owners (Pease and Holt, 1971).

Transfer the residue (~5 ml of H_2O) using several volumes of acetonitrile to a 250-ml separatory funnel (final volume not to exceed 100 ml). Add 50 ml of *n*-hexane and shake for 1 min. Allow the phases to separate; centrifuge if necessary to obtain a clean separation. Discard the hexane. Repeat the wash two more times with additional 50-ml portions of hexane. Discard the hexane after each wash. Quantitatively transfer the acetonitrile to a 250-ml round-bottomed flask. Evaporate the solvent to dryness on a vacuum rotary evaporator at 60 °C.

Totally dissolve all residues using several volumes of 0.1% NaOH and transfer to a 250-ml separatory funnel (final volume not to exceed 80 ml). Add 75 ml of ethyl acetate and shake for 2 min. After phase separation, filter the ethyl acetate through a 1.5-in. bed of anhydrous sodium sulfate contained in a 45° funnel into a 500-ml

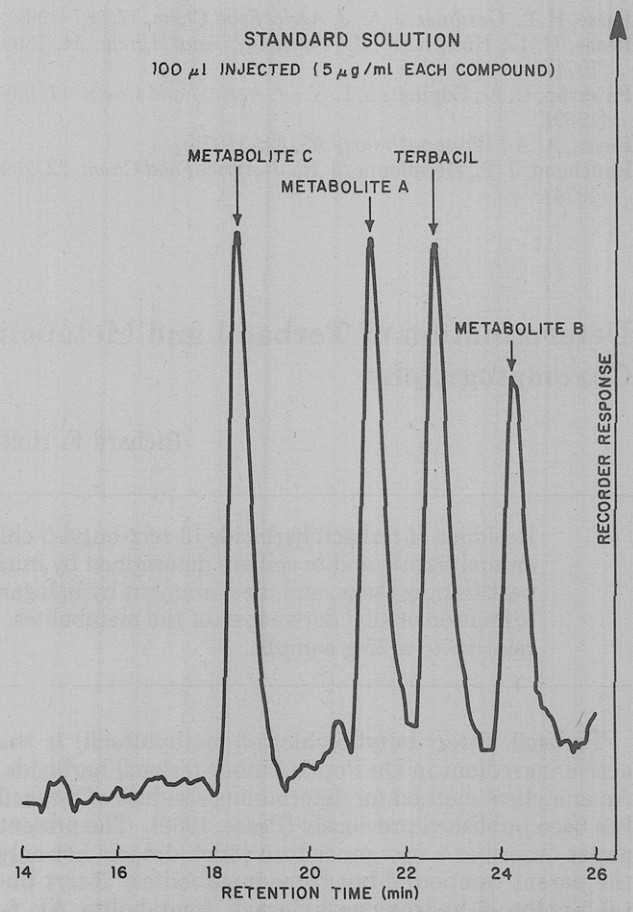


Figure 3. Standard solution of terbacil and metabolites A, B, and C.

round-bottomed flask. Repeat the extraction three more times, each time using 75 ml of ethyl acetate. Concentrate the combined extracts to about 5 ml using a vacuum rotary evaporator at 60 °C.

Using several small ethyl acetate washes, quantitatively transfer the sample to a 10-ml centrifuge tube (total volume approximately 10 ml). Concentrate the sample in a water bath (60 °C) under a N_2 stream to a volume of 1 ml.

Add 300 μl of Regisil-TMCS, cap, and shake vigorously for approximately 20 s. Let the sample stand at room temperature for approximately 16 h, overnight is convenient, before chromatographic determination.

Gas Chromatographic Calibration. Equilibrate the chromatograph under the following conditions: inlet temperature, 235 °C; transfer temperature, 255 °C; furnace temperature, 850 °C; column temperature, 230 °C; helium carrier-gas flow, 100 cm^3/min; helium purge flow, 40 cm^3/min; oxygen flow, 40 cm^3/min. After conditioning the chromatographic column by maintaining the temperature at 230 °C with carrier gas flowing for at least 48 h, set the initial column temperature at 100 °C. Set the coulometer sensitivity at 100 Ω using the Hi-Gain mode. Inject aliquots (10–100 μl) of 1.0, 2.0, 5.0, 10, and 20 μg/ml silanized and water-washed calibration solutions (refer to section on Gas Chromatographic Analysis) of terbacil, metabolite A, metabolite B, and metabolite C, either separately or in mixtures, so that the peaks do not exceed full-scale deflection. All standard solutions are silanized in the same manner as are the treated samples (300 μl of Regisil + 16 h). Wait 2 min and increase the column temperature at 5 °C/min to a maximum of 230 °C. Hold the column at this temperature for about 10 min. The approximate retention times from the initiation of the programming are:

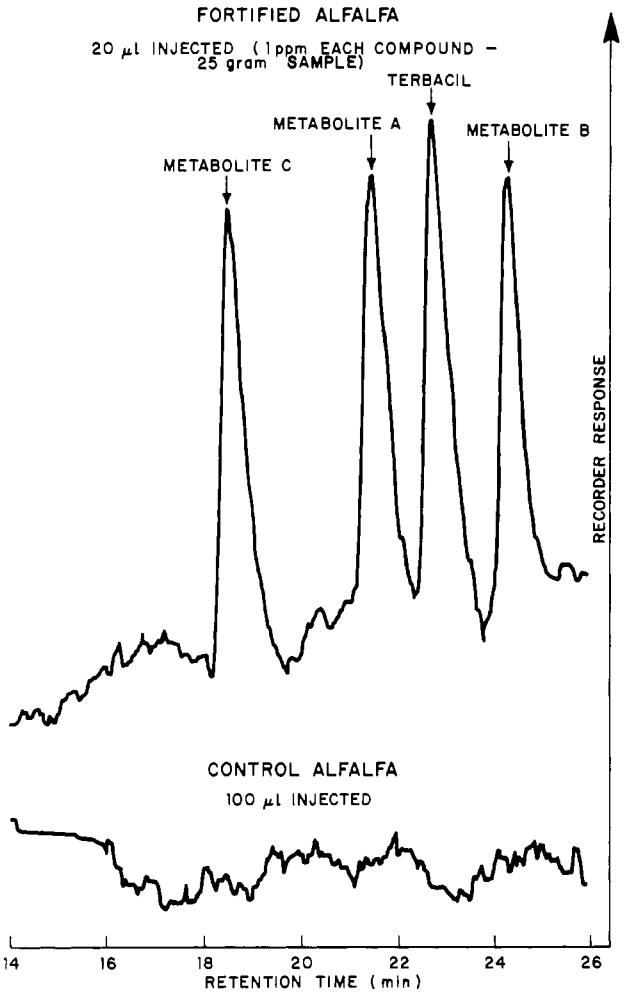


Figure 4. Extract of alfalfa.

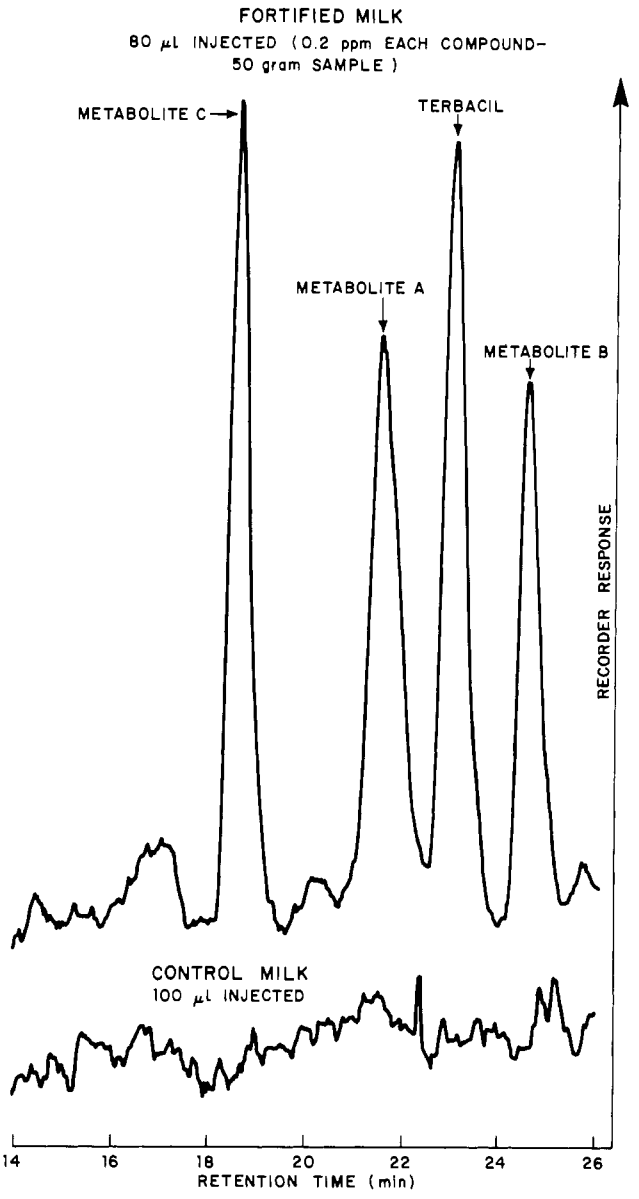


Figure 5. Extract of milk.

18 min for metabolite C, 21 min for metabolite A, 23 min for terbacil, and 24 min for metabolite B. A typical gas chromatographic scan of a standard solution is shown in Figure 3. Construct a calibration curve of micrograms of each individual compound injected vs. peak height. Chromatograph one or more calibration solutions daily to ensure that the calibration curve remains accurate.

Gas Chromatographic Analysis. Approximately 10 min before chromatography, add 1 ml of water to the sample in the centrifuge tube, cap, and shake for 20 s. Let the sample stand for 10 min. After separation (centrifuge if necessary), chromatograph aliquots of the top phase. Samples must be chromatographed within 1 h after the addition of water. After this period, the silanized derivatives tend to decompose to the parent compound.

Measure the peak heights of each compound and individually determine the micrograms of each in the aliquot, using the calibration curves previously prepared. Calculate the concentrations in parts per million by dividing the micrograms found, corrected for aliquot and recovery factors, by the sample weight in grams. Since both standards and samples are identically silanized, all calculations are relative to the parent compounds.

RESULTS AND DISCUSSION

The gas chromatographic method described herein is sensitive to about 1.0 μg of each compound or 0.04 ppm based on a 25-g sample (100 ng injected). Recoveries have been demonstrated from 0.04 to 10 ppm. The results of recovery studies on a variety of substrates are summarized in Table I. The recoveries were conducted by adding known amounts of the compounds to the untreated control samples contained in the blender–centrifuge bottle. After evaporation of the solvent, analyses were then initiated by addition of the first portion of chloroform. In Figure 4, typical gas chromatographic scans obtained on extracts of a control sample of alfalfa (lower scan) and on a control sample of alfalfa fortified with 1 ppm of terbacil and metabolites A, B, and C (upper scan) are illustrated. Figure 5 shows scans representing control milk (lower scan) and milk fortified with terbacil and metabolites A, B, and C at the 0.2-ppm level (upper scan).

Chloroform was selected as the primary extracting solvent in this analytical procedure because of the excellent partitioning coefficients of terbacil and all metabolites. Its applicability as an initial crop extractant was determined by reextracting a series of terbacil field-treated crop samples, which had displayed metabolite residue via the proposed residue procedure, with a more polar solvent (methanol). In each case, no additional terbacil or metabolite residues were detected, indicating total initial extraction with chloroform.

Since metabolites A and B are difficult to chromatograph directly, it was necessary to form volatile derivatives by reaction with Regisil-TMCS [bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane]. This

Table I. Summary of Terbacil and Metabolites A, B, and C Recovery Data

Crop	Recovery levels, ppm	No. of detns.	Av recovery, %				Recovery range, %			
			Terbacil	A	B	C	Terbacil	A	B	C
Alfalfa	0.08–1.2	5	89	98	75	94	78–120	73–113	53–100	70–120
Almond hulls	0.20–2.0	3	91	119	93	93	76–102	104–138	74–120	76–108
Almond nuts	0.08–0.20	3	109	112	81	105	105–114	95–125	68–90	94–115
Apples	0.08–0.40	3	100	100	98	99	85–110	82–110	96–100	82–115
Asparagus	0.08–1.0	7	90	86	87	81	80–95	64–105	60–115	72–90
Cabbage	0.20–0.60	2	85	112	90	88	80–90	104–120	80–100	82–93
Caneberries	0.08–0.40	9	102	100	87	89	80–134	72–133	65–130	65–120
Cherries	0.08–0.40	5	104	100	100	100	81–140	72–130	86–110	94–108
Grass	0.04–1.0	9	106	83	77	80	84–140	74–120	64–90	70–124
Mint hay	0.08–1.0	5	109	76	103	118	97–132	67–84	86–130	100–144
Mint oil	0.50 (duplicate)	2	56	47	67	69	52 and 60	58 and 36	98 and 36	64 and 74
Peaches	0.08–1.0	4	131	111	81	97	114–145	93–129	64–111	88–110
Pecans	0.20–1.0	2	90	111	60	78	72–108	92–130	60–60	72–84
Red beet roots	0.04–0.60	3	100	106	94	96	87–120	70–128	71–107	70–110
Red beet tops	0.20–0.60	2	125	109	69	74	113–138	100–118	61–76	67–80
Strawberries	0.04–0.40	5	93	101	111	73	65–120	72–120	76–140	60–100
Sugarcane	0.04–0.40	6	77	88	87	60	60–92	74–110	74–100	50–79
Soil	0.10–1.0	7	106	109	70	110	72–136	92–144	56–85	90–116
Milk	0.04–0.40	8	112	94	80	96	94–120	81–120	50–130	80–110
Eggs	0.04–0.40	6	98	106	74	93	66–125	77–135	48–120	68–130
Kidney	0.08–0.20	4	99	107	89	101	80–120	72–128	72–120	82–120
Liver	0.04–2.0	6	83	61	81	89	62–96	40–88	50–108	85–104
Muscle	0.04–0.40	6	107	95	75	98	92–140	50–130	52–120	81–130
Fat	0.08–5.0	5	74	73	97	88	45–133	52–100	76–133	58–120
Urine	0.04–10	5	104	105	74	106	94–130	83–135	55–100	92–128
Feces	0.04–0.40	5	86	92	64	79	65–110	60–120	50–70	65–100

reaction requires approximately 16 h for completion, and the derivatives are stable, in the absence of water, for 2–3 days. However, before chromatography, it is necessary to remove excess silyl reagent by the addition of water. After this, the derivatives remain stable for approximately 1 h. Therefore, chromatography of the sample must be accomplished in this period for optimum results.

Limited studies were conducted using the electron-capture detector but the possibility of making more stable derivatives with greater electron-capturing ability was not fully explored. If an alternate chromatographic approach ever becomes necessary, this will be an area worthy of further investigation. However, in our laboratory, we have found more general selectivity with the microcoulometric detector than with the EC detector. The Coulson conductivity detector was also tested and found to be less selective.

Due to the relatively large injection volumes (100 μl) necessary to obtain the required sensitivity, the use of small amounts of silylated glass wool in the glass inlet port of the chromatograph is recommended. This tends to retain nonvolatiles which would otherwise contaminate the inlet portion of the column. This material is changed daily, along with the septum, prior to initiation of chromatography.

LITERATURE CITED

Pease, H. L., *J. Agric. Food Chem.* **16**, 54 (1968).
Pease, H. L., Holt, R. F., *J. Assoc. Off. Anal. Chem.* **54**, 1399 (1971).
Rhodes, R. C., *J. Agric. Food Chem.*, in press (1977).
Rhodes, R. C., Reiser, R. W., Gardiner, J. A., Sherman, H., *J. Agric. Food Chem.* **17**, 974 (1969).

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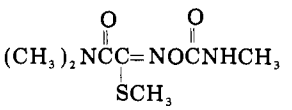
Spectrophotometric Determination of Oxamyl as Copper Dithiocarbamate

J. P. Singhal,* Samiullah Khan, and O. P. Bansal

A spectrophotometric method for the determination of oxamyl, a powerful nematocide, in aqueous solutions has been developed. Reaction variables for the formation of a characteristic colored system have been investigated. Average recoveries of oxamyl varied from 88.6 to 97.3% in different kinds of fortified samples in the 2–10-ppm range after a contact of 12 h. The method can be applied with practical utility in the field of soils, plant products, and water.

In view of its qualities, oxamyl (methyl *N',N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate) (Biochemicals Department, Experimental Station, E. I. DuPont de Nemours & Co., Inc., Wilmington, Dela.), a newly formulated product, is finding wide application as a broad spectrum pesticide for the effective control of nematodes and other plant pests in soils, and as a foliar spray (Timmer, 1974). A gas chromatographic method for its estimation has recently been reported by Holt and Pease (1976). No published data are, however, available on its spectrophotometric analysis.

The structure of the compound



is indicative of the presence of carbamoyl and thiooxamimidate as functional groups. Because of the relative chemical inertness of its functional groups, quantitative analysis of this nematocide is difficult.

Our investigations revealed that when oxamyl was subjected to hydrolysis, its products gave a precipitate with CS_2 and Cu^{2+}, which produced an intense brownish yellow color in immiscible organic solvents.

The aim of this work was, therefore, to propose a sensitive, accurate, and reproducible method for the quantitative estimation of the chemical in aqueous solutions down to a few parts per million. Optimum conditions necessary for the spectrophotometric analysis have been investigated through a study of the reaction variables involved. The method is expected to be especially useful for field use or in areas where more modern analytical instrumentation is not available.

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EXPERIMENTAL SECTION

Apparatus. Spectrophotometric measurements were made with a Bausch and Lomb spectronic 20 spectrophotometer and pH was measured with an Elico pH meter, Model L1-10.

Reagents. The chemicals used in this study were of BDH analytical grade. Solutions used were prepared as follows.

Standard Oxamyl Solution. The solution was prepared by dissolving 25 mg of oxamyl in water and diluting to 1 l. with redistilled water.

Basic Copper Solution. A solution of 0.2 g of copper sulfate pentahydrate and 20 g of ammonium acetate in 30 ml of distilled water was mixed with 25 ml of 40% sodium hydroxide and 20 ml of ammonium hydroxide. The volume was made up to 100 ml with distilled water.

Carbon disulfide solution: 5% in benzene.

Potassium hydroxide: 10% in distilled water.

Acetic acid: 30% in distilled water.

Diverse Interfering Ions. Aqueous solutions of Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Cl^-, NO_3^-, SO_4^{2-}, acetic acid, benzoic acid, and dimecron, alcoholic solutions of pyridine, diphenylamine, methylamine, trimethylamine, nemagon, and telone, and carbon tetrachloride, acetaldehyde, and methyl alcohol as such were used for interference studies.

Procedure. For preparation of a calibration curve 0.1 to 2.0 ml of a 25 μg/ml solution of oxamyl were refluxed with 5 ml of 10% potassium hydroxide solution for 10 min