capsule wall concentration of 1.6 ppm) and the mixture was subjected to the analysis procedure, a fluorescence reading of 3.5 was obtained. Background fluorescence from uncontaminated lettuce subjected to the analysis procedures was negligible. From the standard curve the amount of recoverable ethylenediamine was found to be 1.9 μg. Therefore, the total weight of ethylenediamine from the sample before dilution was 3.8 μg. The control value for the wall formulation was 0.0051 μg of ethylenediamine/μg of cell wall material.

Thus, the amount of capsule wall residue in the lettuce sample in parts per million was then calculated to be 1.5, a value well within the range of experimental error for this technique.

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Manganese Ethylenebis(dithiocarbamate) (Maneb)/Ethylenethiourea (ETU) Residue Studies on Five Crops Treated with Ethylenebis(dithiocarbamate) (EBDC) Fungicides

Harlan L. Pease* and Richard F. Holt

Analysis of tomatoes, potatoes, cucumbers, summer squash, and cantaloupes taken from 17 different locations throughout the United States where maneb was applied according to label directions showed no residual ETU (<0.05 ppm) on the raw agricultural commodities, even in the presence of up to 4 ppm of maneb. Maneb residues were determined by the conventional CS_2 evolution method. ETU was measured by a gas chromatographic (GC) method based on the butyl derivative of ETU (reaction with bromobutane) and measurement by sulfur-sensitive flame-photometric detection. Average ETU recovery was 90% in the range of 0.05 to 0.2 ppm.

Manzate, Manzate D, and Manzate 200 fungicides are used to control a wide variety of important fungal diseases on vegetable, fruit, and ornamental crops. Maneb [(manganese ethylenebis(dithiocarbamate)] is the active ingredient in Manzate and Manzate D. The active ingredient in Manzate 200 is a coordination product of zinc ion and manganese ethylenebis(dithiocarbamate). Ethylenethiourea (ETU), a degradation product of the EBDC fungicides under certain conditions, has been reported to be carcinogenic to rats (Graham and Hansen, 1972; Graham et al., 1973). In a more recent paper (Graham et al., 1975), these FDA workers confirmed carcinogenicity at higher dietary rates but concluded that ETU was "not biologically deleterious to the rat" at feeding levels of 5 and 25 ppm in 2-year studies.

Although ETU itself is readily degraded to ethyleneurea, glycine, and other materials (Rhodes, 1977), trace amounts of ETU residues have been reported on EBDC sprayed crops (Lyman, 1971; Lyman and Lacoste, 1975; Newsome et al., 1975; Nash, 1974, 1975, 1976; Yip et al., 1971). The present studies were conducted to provide additional information on this question. This paper reports results of extensive field tests on tomatoes, potatoes, cucumbers, summer squash, and cantaloupes that had been treated with recommended rates of Manzate products. These tests were conducted at 17 different locations throughout the United States. The treated crops were harvested at various times (1, 3, 5, and 7 days) after the last application, frozen immediately, and shipped frozen to our laboratory in Wilmington, Dela. The samples were held frozen until analyzed for both maneb and ETU residues. No ETU residues (<0.05 ppm) were detected on any of these raw agricultural products even in the presence of up to 4 ppm of maneb. Details of the method employed for determining ETU residues are presented along with an analysis of the limitations (0.05 ppm practical sensitivity) encountered when ETU residues are determined on substrates containing maneb.

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EXPERIMENTAL DETAILS

Crop Samples. Tomatoes, potatoes, cucumbers, summer squash, and cantaloupe for residue studies were collected by qualified investigators from 17 different locations throughout the United States where EBDC products had been used as fungicides. The products, Manzate Maneb Fungicide, Manzate D Maneb-Fungicide, and Manzate 200 Fungicide, were applied usually on a regular weekly spray application schedule and usually at 2 or 3 lb of product/acre (2.24 or 3.36 kg/ha). Additional details as to location and number of applications and treatment rates are given in Tables I through V.

Table I. Residue Analyses; Tomato Fruit

Location	No. of applctns	Days after last applctn	Residue, ppm: Manzate D 3 lb/acre[a] Maneb	Manzate D 3 lb/acre[a] ETU	Manzate 200 3 lb/acre[a] Maneb	Manzate 200 3 lb/acre[a] ETU	Manzate 3 lb/acre[a] Maneb	Manzate 3 lb/acre[a] ETU
Robbins, Calif.	3	1	4.0	<0.05	2.8	<0.05		
		3	1.8	<0.05	3.0	<0.05		
		5	2.5	<0.05	3.3	<0.05		
Niles, Mich.	6	1	0.35	<0.05	1.2	<0.05	0.44	<0.05
		3	0.35	<0.05	0.68	<0.05	0.56	<0.05
		5	1.0	<0.05	1.1	<0.05	0.68	<0.05
Bradenton, Fla.	7	1	0.72	<0.05	1.3	<0.05	0.61	<0.05
		3	0.58	<0.05	0.70	<0.05	0.98	<0.05
		5	0.75	<0.05	1.3	<0.05	0.79	<0.05
		7	0.65	<0.05	0.54	<0.05	0.98	<0.05
Salisbury, Md.	8	1	2.5	<0.05	2.5	<0.05	3.9	<0.05
		3	3.5	<0.05	1.6	<0.05	1.3	<0.05
		5	2.3	<0.05	2.5	<0.05	1.8	<0.05
Clayton, Dela.	10	1	2.5	<0.05	2.1	<0.05	1.9	<0.05
		3	1.9	<0.05	1.3	<0.05	1.2	<0.05
		5	1.6	<0.05	1.2	<0.05	1.4	<0.05
Felda, Fla.	18	2	2.3	<0.05				
		4	3.4	<0.05				

[a] 3 lb/acre is equivalent to 3.36 kg/ha.

Table II. Residue Analyses; Potato Tubers

Location	No. of applctns	Days after last applctn	Residue, ppm: Manzate D 2 lb/acre[a] Maneb	Manzate D 2 lb/acre[a] ETU	Manzate 200 2 lb/acre[a] Maneb	Manzate 200 2 lb/acre[a] ETU	Manzate 2 lb/acre[a] Maneb	Manzate 2 lb/acre[a] ETU
Painter, Va.	4	6	<0.1	<0.05	<0.1	<0.05	<0.1	<0.05
Wendell, Idaho	4	9			<0.1	<0.05		
Glyndon, Minn.	5	7	<0.1	<0.05	<0.1	<0.05		
Bradenton, Fla.	6	1	<0.1	<0.05	<0.1	<0.05	<0.1	<0.05
Presque Isle, Maine	6	6	<0.1	<0.05	<0.1	<0.05	<0.1	<0.05
Wendell, Idaho	6	6	<0.1	<0.05	<0.1	<0.05		
Twin Falls, Idaho	6	6			<0.1	<0.05		
Glyndon, Minn.	7	14	<0.1	<0.05	<0.1	<0.05		
Bradenton, Fla.	8	1	<0.1	<0.05	<0.1	<0.05	<0.1	<0.05
Painter, Va.	8	7	<0.1	<0.05	<0.1	<0.05	<0.1	<0.05
Twin Falls, Idaho	10	7	<0.1	<0.05	<0.1	<0.05		

[a] 2 lb/acre is equivalent to 2.24 kg/ha.

Table III. Residue Analyses; Cucumbers

Location	No. of applctns	Days after last applctn	Residue, ppm: Manzate D 3 lb/acre[a] Maneb	Manzate D 3 lb/acre[a] ETU	Manzate 200 3 lb/acre[a] Maneb	Manzate 200 3 lb/acre[a] ETU	Manzate 3 lb/acre[a] Maneb	Manzate 3 lb/acre[a] ETU
Charleston, S.C.	4	1	1.1	<0.05	1.5	<0.05		
		5	<0.1	<0.05	<0.1	<0.05		
		7	<0.1	<0.05	0.20	<0.05		
Niles, Mich.	5	1	0.40	<0.05	0.35	<0.05	0.68	<0.05
		3	0.33	<0.05	0.40	<0.05	0.63	<0.05
		5	0.20	<0.05	0.35	<0.05	0.26	<0.05
Greenfield, Calif.	5	1	0.26	<0.05	0.63	<0.05		
		3	0.33	<0.05	0.51	<0.05		
		5	0.10	<0.05	0.21	<0.05		
Bradenton, Fla.	5	1	0.23	<0.05	0.32	<0.05	0.23	<0.05
		3	0.21	<0.05	<0.1	<0.05	0.20	<0.05
		7	0.20	<0.05	0.19	<0.05	<0.1	<0.05

[a] 3 lb/acre is equivalent to 3.36 kg/ha.

These crop samples were taken at various times (1, 3, 5, and 7 days) after the last application of fungicide, frozen immediately, and shipped frozen to our Residue Laboratory in Wilmington, Dela. They were held frozen until residue analyses were conducted.

Analytical Methods. Analyses to determine maneb residues were made using the modified carbon disulfide evolution method of Keppel (1971). ETU residues were determined using modification of the original residue method published by Onley and Yip (1971). The principal differences between our ETU method and the original are that: (a) our procedure utilizes a partitioning step into water from the chloroform phase for added clean-up, and (b) a gel filtration column is used to purify the extracts,

Table IV. Residue Analyses; Squash

Location	No. of applctns	Days after last applctn	Residue, ppm: Manzate D 3 lb/acre[a] Maneb	Manzate D 3 lb/acre[a] ETU	Manzate 200 3 lb/acre[a] Maneb	Manzate 200 3 lb/acre[a] ETU	Manzate 3 lb/acre[a] Maneb	Manzate 3 lb/acre[a] ETU
Watsonville, Calif.	2	1	0.20	<0.05	0.21	<0.05		
		3	0.21	<0.05	0.20	<0.05		
		5	0.10	<0.05	0.21	<0.05		
Charleston, S.C.	3	1	0.51	<0.05	1.2	<0.05		
		5	0.26	<0.05	0.25	<0.05		
		7	<0.1	<0.05	<0.1	<0.05		
Bradenton, Fla.	4	1	0.32	<0.05	0.32	<0.05	0.25	<0.05
		3	0.18	<0.05	0.21	<0.05	0.23	<0.05
		7	<0.1	<0.05	<0.1	<0.05	<0.1	<0.05
Niles, Mich.	6	1	0.44	<0.05	0.30	<0.05	0.23	<0.05
		3	0.10	<0.05	0.12	<0.05	0.10	<0.05
		5	0.10	<0.05	<0.1	<0.05	<0.1	<0.05

[a] 3 lb/acre is equivalent to 3.36 kg/ha.

Table V. Residue Analyses; Cantaloupe

Location	No. of applctns	Days after last applctn	Residue, ppm: Manzate D 3 lb/acre[a] Maneb	Manzate D 3 lb/acre[a] ETU	Manzate 200 3 lb/acre[a] Maneb	Manzate 200 3 lb/acre[a] ETU	Manzate 3 lb/acre[a] Maneb	Manzate 3 lb/acre[a] ETU
San Juan, Tex.	7	1	1.4	<0.05	0.63	<0.05	0.56	<0.05
		3	1.2	<0.05	0.44	<0.05	0.84	<0.05
		5	0.40	<0.05	0.32	<0.05	0.89	<0.05
Tifton, Ga.	10	1	1.5	<0.05	0.74	<0.05		
		3	2.3	<0.05	0.58	<0.05		
		5	0.91	<0.05	1.0	<0.05		

[a] 3 lb/acre is equivalent to 3.36 kg/ha.

again for additional clean-up. As in the original method, ETU is measured as the *S*-butyl derivative, after reaction of ETU with 1-bromobutane, by sulfur-sensitive flame photometric gas chromatography.

ETU Residue Procedure. *Reagents* used included the following. (a) Solvents: Distilled-in-glass chloroform and benzene; spectro-grade methanol, ethyl alcohol. (b) Chemicals: reagent NaOH; reagent NaCl, Sephadex G-10, Celite 545, reagent cuprous chloride. (c) Washed aluminum oxide: Blend 100 g of aluminum oxide (basic activity I) 10 min with 150 mL of methanol. Filter with suction and wash with 75 mL of methanol. Dry for 1 h at 130 °C and store in a closed jar. (d) 1-Bromobutane: Add 2 to 3 g of cuprous chloride to 1-bromobutane (Eastman Organic Chemicals) and thoroughly mix. Slowly filter 40 mL through a column (22 mm i.d.) with a fritted exit containing 20 g of dried aluminum oxide. Store in a brown bottle and purge with nitrogen. (e) Reference Standards: Purified samples of ethylenethiourea (ETU) and *S*-butylethylenethiourea (*S*-Bu-ETU).

Apparatus used included the following. (a) Centrifuge: International Model FS or equivalent and International Model BE50 or equivalent. (b) Mills tube: 10-mL capacity (Kontes Glass Co.). (c) Gel-Filtration Chromatographic Column: 250 mm length, 25 mm i.d., reservoir capacity 500 mL (Kontes Glass Co.). (d) Gas Chromatograph: Model MT-220 (Micro-Tek Instruments, Inc.) equipped with a flame photometric detector with interference filter for spectral isolation of sulfur emission at 394 nm. (e) Gas Chromatographic Column: 1.8 m × 4 mm i.d. glass column, 5% Carbowax 20M plus 2.5% KOH on 80–100 mesh Chromosorb W (HP) preconditioned at 210 °C for 2 days. (f) Micro-syringe Filter Holder: 25-mm diameter, 0.45 μm thick Millipore filters (Millipore Corp.).

Gas Chromatographic Calibration. Equilibrate the gas chromatograph under the following conditions: inlet temperature, 230 °C; detector temperature, 200 °C; column temperature, 190 °C; helium carrier gas flow, 80 cm^3/min; oxygen flow, 20 cm^3/min; air flow, 50 cm^3/min; hydrogen flow, 180 cm^3/min. After conditioning the chromatographic column by maintaining the temperature at 190 °C with carrier gas flowing for at least 24 h, inject aliquots (1 to 5 μL) of standard solutions of *S*-Bu-ETU prepared in benzene. Concentration ranges should be 0.5, 1, 5, and 10 μg/mL, and the peak responses should not exceed full scale. The retention time for *S*-Bu-ETU is about 3.5 min.

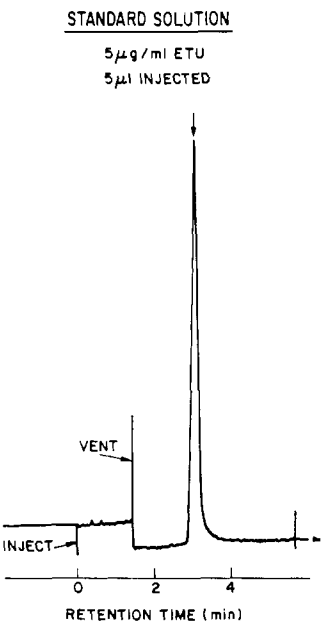

Figure 1. Standard solution of *S*-Bu-ETU equivalent to 5 μg/mL ETU.

A typical gas chromatographic scan for the standard solution is shown in Figure 1. Construct calibration curves for the different attenuations by plotting micrograms of *S*-Bu-ETU injected, calculated as ETU, vs. peak height. Use log–log paper to obtain a straight line. Chromatograph one or more calibration solutions daily to ensure that the calibration curve remains accurate.

Isolation. Weigh 50 g of a representative crop sample into a Waring Blendor jar, add 200 mL of ethyl alcohol, cover, and blend at high speed for 2 min. Reduce blendor speed; add 100 mL of chloroform and 10 g of Celite 545. Blend for an additional 2 min at high speed. Filter the sample with suction through ca. 0.5 in. Celite on top of Whatman No. 42 filter paper in a 12-cm Buchner funnel.

Quantitatively transfer 250 mL of the filtrate (representing about a 35-g sample) to a 500-mL separatory funnel and add 200 mL of distilled water. Shake for 2 min and allow phases to separate. Centrifuge if necessary to obtain complete separation. Transfer the water extract to a 1000-mL separatory funnel and re-extract the organic phase with an additional 80 mL of distilled water. After complete separation, combine the water phases and wash with 200 mL of chloroform by shaking for 1 min. Allow the phases to separate. Centrifuge if necessary. Filter the water phases through cotton into a 1000-mL round-bottomed flask. Back extract the chloroform with 50 mL of distilled water. Shake for 2 min and allow phases to separate. Centrifuge if necessary.

Combine the aqueous phases and concentrate to 10 mL using a rotary evaporator at 60–65 °C. Transfer to a 30-mL beaker using several small water washes (total volume ca. 25 mL). Concentrate in a water bath (80–90 °C) with a constant nitrogen flow to ca. 3 mL.

Filter the sample, plus several water washes (total volume not to exceed 5 mL), through a 25 mm diameter, 0.45 μm thick Millipore filter onto the surface of a 25 mm i.d., 14 cm long column of Sephadex G-10. This column is prepared according to the manufacturer's recommendations from beads swollen 24 h before packing in a 0.05 M NaCl buffer. The column is calibrated using standard ETU samples.

Elute the sample from the Sephadex column using a 0.05 M NaCl buffer as the carrier. The proper elution increment (generally between 100 and 150 mL) is collected and transferred to a 250-mL round-bottomed flask. The total volume is concentrated on a rotary evaporator at 60–65 °C to approximately 15–20 mL.

Using several small water washes, quantitatively transfer the sample to a 250-mL Erlenmeyer flask. Total volume must not exceed 25 mL. Add 5 g of NaOH, and a stirring bar, and place on a combination hot plate–stirrer. Attach a water-cooled condenser. Start stirrer and after the NaOH has gone into solution turn on heat. Add dropwise, over a 5-min period, 50 mL of an 8% 1-bromobutane/92% methanol solution and reflux for 10 min. Cool to room temperature and quantitatively transfer to a 250-mL separatory funnel. Extract the aqueous phase with three 100-mL portions of benzene. Filter the benzene through anhydrous sodium sulfate into a 500-mL round-bottomed flask. Using a rotary evaporator at 50 °C, concentrate to ca. 5 mL. Using several small benzene washes, quantitatively transfer the sample to a 10-mL graduated Mills tube. Continue concentrations in a water bath (50 °C) under a stream of nitrogen to a 1-mL volume. Remove and cool to room temperature.

Gas Chromatographic Analysis. Equilibrate the gas chromatograph and inject aliquots of the prepared sample extracts as described under the section on Calibration.

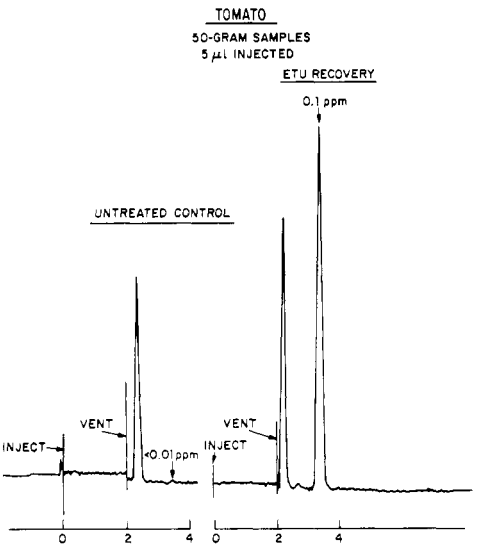

Figure 2. Extracts of tomato; untreated control; ETU recovery at 0.1 ppm.

Table VI. Summary of Recovery Data

	Residue level, ppm	No. of determ.	Recovery, % Av	Recovery, % Range
	Maneb			
Tomato fruit	0.20-5.0	6	103	89-125
Potato tubers	0.20-0.50	5	94	83-105
Squash	0.20-5.0	9	91	72-114
Cucumber	0.20-5.0	8	92	78-105
Cantaloupe	0.40-2.0	4	87	80-93
	ETU			
Tomato fruit	0.06-0.20	5	96	88-116
Potato tuber	0.06-0.20	6	79	66-99
Squash	0.05-0.20	6	74	65-90
Cucumber	0.05-0.20	8	103	88-128
Cantaloupe	0.05-0.20	6	89	68-102

Determine the micrograms of ETU in the aliquot using the calibration curve previously prepared. Calculate the concentration of ethylenethiourea in parts per million by dividing the micrograms of ETU found, corrected for injection aliquot, sampling aliquot (1.36), and recovery factor, by the sample weight in grams.

RESULTS AND DISCUSSION

The selectivity and sensitivity of the residue method for ETU are of prime importance to these studies. The method employed for the work reported herein makes use of a special gel filtration clean-up column during the isolation steps. Separations on this column are based on molecular size. In addition, the final selective gas chromatographic readout is based on a detector sensitive only to sulfur-containing compounds. Figure 2 shows the GC scan on an untreated control tomato sample plus the GC scan on 0.1 ppm of ETU added to the same sample. Table VI gives a summary of our ETU recovery data to verify the method.

Examination of Figure 2 shows that this procedure is capable of detecting ETU at levels down to 0.01 to 0.02 ppm. However, when applied to substrates containing finite residues of maneb, traces of ETU are formed from the maneb during extraction, isolation, and workup. For example, Figure 3 shows a control tomato sample fortified with 4 ppm of Manzate D. There was essentially no ETU in the Manzate D used to spike this test sample, yet 0.03 ppm of ETU was found. As shown in Table VII, the ETU

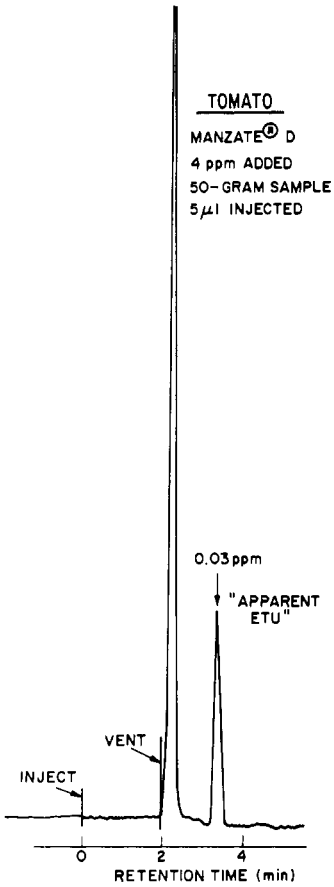

Figure 3. Extract of Tomato, Manzate D added.

Table VII. ETU Conversion from Samples Fortified with EBDC

Crop	EBDC added, ppm	ETU found, ppm	% conversion (by wt)
Tomato fruit	0.3	<0.01	<3.3
Tomato fruit	1.0	<0.01	<1.0
Tomato fruit	2.0	<0.01	<0.5
Tomato fruit	2.0	0.02	1.0
Tomato fruit	4.0	0.03	0.8
Tomato fruit	5.0	0.02	0.4
Tomato juice	2.0	0.01	0.5
Tomato juice	4.0	0.02	0.5
Tomato foliage	20	0.04	0.2
Tomato foliage	52	0.07	0.1
Squash	8.0	0.04	0.5
Squash	8.0	0.05	0.6
Lettuce	60	0.30	0.5
Bean foliage	1.7	<0.01	<0.6
Bean foliage	5.3	<0.01	<0.2

method employed in this study converts no more than 1% of the existing maneb residues to ETU. Even then, however, if 4-ppm maneb residues are present on a crop, the artifact of the analytical method would show an apparent ETU residue of 0.04 ppm.

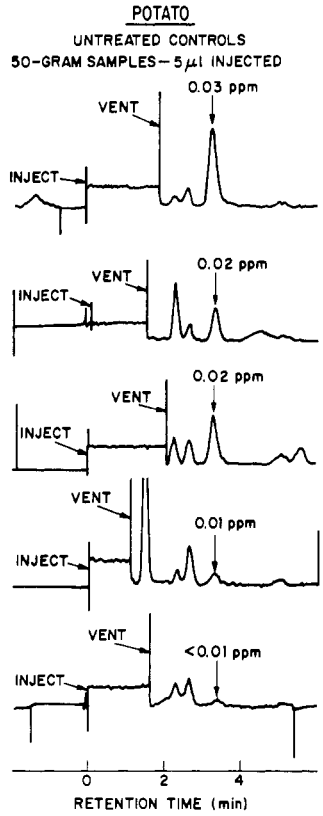

Figure 4. Extracts of potato, untreated controls.

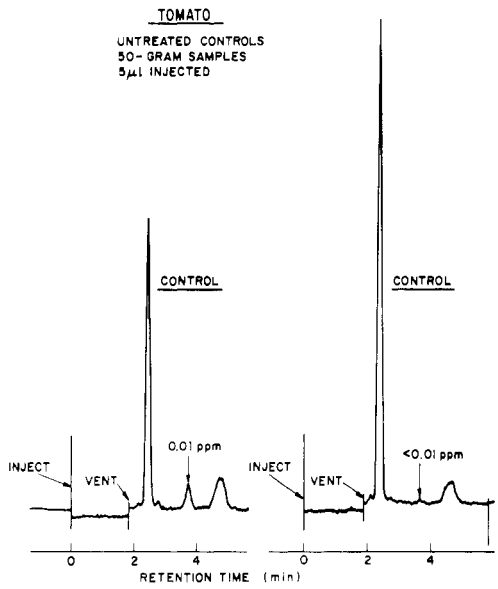

Figure 5. Extracts of tomato, untreated controls.

In addition to the conversion problem, variable background levels were encountered with untreated crop samples. On a particular untreated potato control sample, which was analyzed five times by the technique described, "apparent" ETU residues were <0.01, 0.01, 0.02, 0.02, and 0.03 ppm. The gas chromatographic scans obtained on these samples are shown in Figure 4. Similar variation was encountered on other untreated crops also. For example, Figure 5 shows chromatograms obtained on a sample of untreated control tomatoes (duplicate extractions). One time we found <0.01 ppm of ETU; the next time we found 0.01 ppm of ETU, a positive "apparent" residue. We do not believe these "apparent" residues in untreated crops are due to analyst contamination. We

Table VIII. ETU Conversion from Samples Fortified with EBDC (Alternate ETU Residue Procedures)

Crop	Method	EBDC added, ppm	ETU found, ppm	% conversion (by wt)
Tomato	Onley and Yip (1971)	3.2	0.09	2.8
Tomato	Onley and Yip (1971)	3.2	0.29	9.1
Squash	Onley and Yip (1971)	3.2	0.10	3.1
Squash	Onley and Yip (1971)	3.2	0.09	2.8
Tomato	Onley and Storherr (1975)	3.4	0.06	1.8
Tomato	Onley and Storherr (1975)	3.4	0.05	1.5
Tomato	Newsome (1972)	6.4	0.17	2.7
Tomato	Newsome (1972)	6.4	0.11	1.7
Squash	Newsome (1972)	6.4	0.04	0.62
Squash	Newsome (1972)	6.4	0.07	1.1
Tomato	Haines and Adler (1973)	8.0	0.13	1.6
Tomato	Haines and Adler (1973)	8.0	0.40	5.0
Tomato	BASF (1976)	4.0	0.12	3.0
Tomato	BASF (1976)	4.0	0.24	6.0

Table IX. Purchased Commercial Tomato Products

Brand	ETU residue, ppm: Tomato juice	Tomato soup	Canned tomatoes	Ketchup
A	<0.05	<0.05		
B	<0.05	<0.05		
C	<0.05	<0.05	<0.05	
D			<0.05	
E			<0.05	
F				<0.05
G				<0.05
H				<0.05

have been able to handle reagent blank samples in our laboratories routinely at the <0.01-ppm detection level.

Based on these two practical considerations, variable background interferences and partial conversion of maneb residues, we firmly believe that apparent levels of ETU below 0.05 ppm are not significant. Alternate residue methods for ETU were also evaluated in our laboratory (Onley and Yip, 1971; Onley and Storherr, 1975; Newsome, 1972; Haines and Adler, 1973; the BASF method, 1976). Each method appears to have certain characteristic advantages and disadvantages. In general, we find these methods to be less time consuming and as good as or better than our method for minimizing natural interferences from untreated control crop samples. However, in our laboratory, these methods have caused measurably higher amounts of conversion (up to 10%) of added maneb to "apparent" ETU. We did not try the method of Nash (1974) as his extraction and derivatization procedures were similar to that of Newsome (1972). Nash did report that 1 to 2% of maneb and zineb may degrade to ETU during the benzylation process of his method. This is consistent with what we found using Newsome's procedure. Table VIII shows our conversion study data. These considerations cause us to question reported ETU residues below the 0.05-ppm level when conducted in the presence of maneb residues.

Data obtained in our studies for both maneb and ETU residues are summarized in Tables I through VI. Table VI shows the results of recovery study data for both maneb and ETU on all sample types analyzed. Overall recoveries for the five crops analyzed averaged 96% for maneb and 90% for ETU. All recovery study results were based on addition of maneb or ETU directly to excised plant substrate followed by immediate analyses. [Note: if the ETU "spikes" are allowed to stand in contact with plant tissue for 1 h prior to solvent addition, the recoveries decrease to only 15–20% because of the reactivity of ETU. This information is in agreement with the results found in the ^{14}C studies of Rhodes (1977)].

Table I shows our results obtained on tomato fruit from six locations. The maneb residue ranged as high as 4.0 ppm, the tolerance limit. No ETU residue (<0.05 ppm) was detected in any of the samples analyzed. Results obtained on potato tubers are shown in Table II. No maneb (less than 0.1 ppm) and no ETU (<0.05 ppm) was detected in any of these samples taken from 11 locations. Both immature and mature tubers were analyzed from Bradenton, Fla. Cucumber data are summarized in Table III. These were samples from four locations. The maximum maneb residue found on these samples was 1.5 ppm on the Manzate 200 treated fruit collected 1 day after the last spray application at Charleston, S.C. No ETU residue (<0.05 ppm) was detected in this sample or in any of the other samples analyzed from the four test sites.

Similarly, no ETU residue (<0.05 ppm) was detected in any of the summer squash samples. Maneb residue was as high as 1.2 ppm. The squash were taken from tests at four locations. Results are shown in Table IV. As shown in Table V, cantaloupes were analyzed from only two locations. Results were similar to those obtained on the other crops studied; no ETU residue was detected.

As a supplement to the work described above, several grab samples of commercial tomato products (a crop where EBDC's are used extensively) were purchased at random from grocery stores in Wilmington, Dela. These samples were analyzed for possible ETU residue. None, i.e. less than 0.05 ppm, was found (Table IX).

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Determination of Residues of Karbutilate and Its Major Metabolites in Water, Soil, and Grass by High-Pressure Liquid Chromatography

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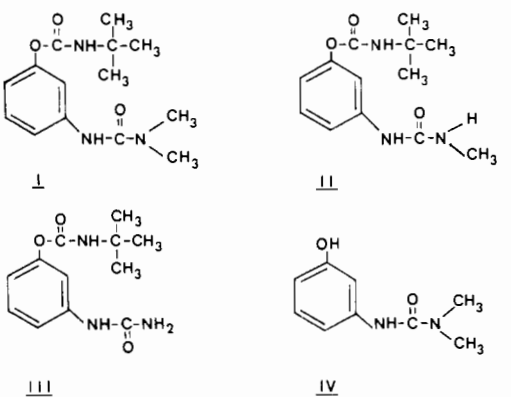
A high-pressure liquid chromatographic (HPLC) method was developed for the determination of residues of karbutilate (3-[[(dimethylamino)carbonyl]amino]phenyl (1,1-dimethylethyl)carbamate), its hydrolysis product *N'*-(3-hydroxyphenyl)-*N,N*-dimethylurea and its demethylated metabolites monomethyl karbutilate (3-[[(methylamino)carbonyl)]amino]phenyl (1,1-dimethylethyl)carbamate) and demethyl karbutilate (3-[(aminocarbonyl)amino]phenyl (1,1-dimethylethyl)carbamate), in water, soil, and grass. Recoveries of karbutilate and its degradation products from water ranged from 89 to 103%. The lower limit of sensitivity of the method is 0.01 ppm for karbutilate and *N'*-(3-hydroxyphenyl)-*N,N*-dimethylurea and 0.02 ppm for monomethyl karbutilate. The recovery of karbutilate and its carbamate metabolites from soil ranged from 84 to 95%. For residues in grass, the carbamates are converted to the corresponding phenols, which are analyzed by HPLC. The recovery of karbutilate and its carbamate metabolites from grass ranged from 80 to 87%. The lower limit of sensitivity of the method for residues in soil and grass is 0.1 ppm for karbutilate and 0.2 ppm for monomethyl karbutilate and demethyl karbutilate.

Karbutilate (I) (3-[[(dimethylamino)carbonyl]amino]-phenyl (1,1-dimethylethyl)carbamate), the active ingredient of Tandex weed and brush killer (FMC Corp.), is a nonselective broad spectrum herbicide. It is especially suited for the control of annual and hard-to-kill perennial broad-leaved weeds, and grasses, and woody species on noncrop land. Its most common uses are on railroad rights-of-way, airports, runways, industrial sites, and along fence lines.

Karbutilate is useful in controlling mesquite (*Prosopis* spp.) and other perennials that occur on extensive areas of grazing land. The applied karbutilate is carried by rainfall into the soil under the immediate area of application. Once in the soil, karbutilate is not susceptible to lateral movement. The brush will be killed when its extensive root system comes in contact with karbutilate. Only the grass in the immediate area of treatment will be affected. Before Tandex weed and brush killer could be used for the control of brushy species on rangeland, residue levels of karbutilate and its breakdown products available in the soil and in grass for ingestion by grazing livestock following application to rangeland had to be determined.

Karbutilate is metabolized in soil and grass to monomethyl karbutilate (II) (3-[[(methylamino)carbonyl]-amino]phenyl (1,1-dimethylethyl)carbamate) and demethyl karbutilate (III) (3-[(aminocarbonyl)amino]phenyl (1,1-dimethylethyl)carbamate) (Brandau and Robinson, 1974; Munger and Robinson, 1974). In water it is hydrolyzed to *N'*-(3-hydroxyphenyl)-*N,N*-dimethylurea (IV).

Karbutilate is the only urea carbamate herbicide commercially available. Gas chromatographic conditions for urea carbamates are not available. The gas chromatographic analysis of carbamates has been investigated by many and was recently reviewed (Magallona, 1975). Most workers agree that the gas chromatography of many carbamates cannot be carried out on the intact carbamates because of their thermal instability. Many authors have studied the direct gas chromatographic analysis of urea herbicides (McKone and Hance, 1968; Reiser, 1964; Henkel, 1966; Katz and Strusz, 1969; Spengler and Hamroll, 1970; Buser and Grolimund, 1974). Reiser states that only alkyl-substituted ureas can be chromatographed without decomposition. Henkel, Spengler, and Hamroll are of the opinion that most *N*-phenylurea compounds cannot be chromatographed undecomposed without previous chemical alteration. These compounds undergo thermal decomposition at the necessarily high temperatures in the injector block or the column.



I

II

III

IV

Work has been reported on the gas chromatographic analysis of certain carbamates and urea herbicides by derivatization of their aniline moieties after hydrolysis (Kirkland, 1962; Gutenmann and Lisk, 1964, 1966) and by bromination (Thier, 1971; Harris and Whiteoak, 1972). Direct methylation of phenylurea herbicides has recently been reported (Tanaka and Wien, 1973; Cochrane and

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