Kittredge, J. S., Hughes, R. R., *Biochemistry* **3**, 991 (1964).
Kittredge, J. S., Isbell, A. F., Hughes, R. R., *Biochemistry* **6**, 289 (1967).
Korn, E. D., Dearborn, D. G., Fales, H. M., Sokolosk, E. A., *J. Biol. Chem.* **248**, 2257 (1973).
Liebman, A. A., Mundy, B. P., Rapoport, H., *J. Am. Chem. Soc.* **89**, 664 (1967).
Mahler, H. R., Cordes, E. H., "Biological Chemistry", Harper and Row, New York, N.Y., 1966.
Marvel, J. T., Brightwell, B. B., Malik, J. M., Sutherland, M. L., Rueppel, M. L., 172nd National Meeting of the American Chemical Society, San Francisco, Calif., Aug 1976, Abstract No. PEST 02.
Marvel, J. T., Brightwell, B. B., Malik, J. M., Sutherland, M. L., Rueppel, M. L., *J. Agric. Food Chem.*, submitted for publication, 1977.
Marvel, J. T., Rueppel, M. L., Schaefer, J. F., 168th National Meeting of the American Chemical Society, Atlantic City, N.J., Sept 1974, Abstract No. ORGN 28.
Marvel, J. T., Suba, L. A., Brightwell, B. B., Sutherland, M. L., Colvin, L. B., Miller, J. A., Curtis, T. G., Ho, C., Chen, H., 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975, Abstract No. PEST 137.
Parr, J. F., Smith, S., *Soil Sci.* **107**, 271 (1969).
Peterson, J. I., *Anal. Biochem.* **31**, 204 (1969).
Peterson, J. I., Wagner, F., Siegel, S., Nixon, W., *Anal. Biochem.* **31**, 189 (1969).
Quilty, S. P., Geoghegan, M. J., University College, Dublin, Ireland, private communication, 1976.
Rieck, C. E., Wright, T. H., Harger, T. R., *Weed Sci. Soc. Am. Abstr.*, 119 (1974), *Abstr.* 277.
Roberts, E., Simonsen, D. G., Horiguchi, M., Kittredge, J. S., *Science* **159**, 886 (1968).
Rueppel, M. L., Marvel, J. T., *Org. Magn. Reson.* **8**, 19 (1976).
Rueppel, M. L., Marvel, J. T., Brightwell, B. B., 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975a, Abstract No. PEST 24.
Rueppel, M. L., Marvel, J. T., Schaefer, J., 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975b, Abstract No. PEST 25.
Rueppel, M. L., Marvel, J. T., Suba, L. A., 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975c, Abstract No. PEST 26.
Rueppel, M. L., Marvel, J. T., Suba, L. A., Schaefer, J., 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975d, Abstract No. PEST 27.
Rueppel, M. L., Suba, L. A., Marvel, J. T., *Biomed. Mass Spectrom.* **3**, 28 (1976).
Schaefer, J., *Macromolecules*, **4**, 98 (1971a).
Schaefer, J., *Macromolecules* **4**, 105 (1971b).
Schaefer, J., *Macromolecules* **5**, 427 (1972).
Sharp, D. B., Monsanto Co., St. Louis, Mo., private communication, 1974.
Sprankle, P., Meggitt, W. F., Penner, D., *Weed Sci. Soc. Am. Abstr.*, 119 (1974), *Abstr.* 276.
Sprankle, P., Meggitt, W. F., Penner, D., *Weed Sci.* **23**, 224 (1975a).
Sprankle, P., Meggitt, W. F., Penner, D., *Weed Sci.* **23**, 229 (1975b).
Stahl, E., Ed., "Thin Layer Chromatography", Springer-Verlag, New York, N.Y., 1969, p 886.
Stejskal, E. O., Schaefer, J., *J. Magn. Reson.* **13**, 249 (1974a).
Stejskal, E. O., Schaefer, J., *J. Magn. Reson.* **15**, 173 (1974b).
Tiedje, J. M., Mason, B. B., *Soil Sci. Soc. Am., Proc.* **38**, 278 (1974).

Received for review August 13, 1976. Accepted January 18, 1977.

Studies with Manganese [^{14}C]Ethylenebis(dithiocarbamate) ([^{14}C]Maneb) Fungicide and [^{14}C]Ethylenethiourea ([^{14}C]ETU) in Plants, Soil, and Water

Robert C. Rhodes

Total radiochemical residues on tomato and bean plants decreased rapidly with time following foliar field applications of either [^{14}C]maneb or [^{14}C]ETU (ethylenethiourea). No accumulation or persistence of intact ETU residues was observed from either treatment. In soil treated with [^{14}C]ETU or with [^{14}C]maneb, the half-life of total radiochemical ^{14}C-labeled residues under field conditions was less than 4 weeks for ETU and between 4 and 8 weeks for maneb, whereas the half-life for intact ETU itself was less than 1 week. In plant uptake tests, only trace amounts of total radiochemical residues were taken up by tomato plants grown in field soil treated with [^{14}C]maneb or [^{14}C]ETU. No intact ETU residues were detected in plants from these treatments, and ripe tomatoes from these plants contained no detectable ^{14}C-labeled residues (<0.01 ppm). In water, glycine was confirmed as the major photodegradation product of [^{14}C]ETU. Ethyleneurea, hydantoin, and Jaffe's base were also identified by mass spectrometry. Overall, these ^{14}C data indicate very little likelihood for the appearance of significant amounts of ETU in the environment or on maneb-treated crops.

The ethylenebis(dithiocarbamate) (EBDC) fungicides are used extensively for the control of a variety of fungus diseases of certain vegetable, fruit, and ornamental crops. Ethylenethiourea (ETU), a possible degradation product of the EBDC fungicides, 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 workers confirmed carcinogenicity at higher dose rates but concluded that ETU was "not biologically deleterious to the rat" at 5- and 25-ppm dietary levels in 2-year studies.

Trace amounts of ETU 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). In general, several of these workers and others have shown that ETU disappears rapidly from treated plants and soil (Blazquez, 1973; Hoagland and Frear, 1976; Kaufman and Fletcher, 1973). Other workers, however,

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

have felt that ETU was stable in plants and soils (Dekhuijzen et al., 1971). A very recent paper (Pease and Holt, 1977) gives extensive and direct residue data on 5 crops treated in the field with EBDC fungicides. No ETU residue (<0.05 ppm) was present on these crops even in the presence of up to 4 ppm of maneb.

Described herein are studies with [^{14}C]ETU on field-treated crops, in soil and in water, plus other studies with one of the EBDC fungicides, [^{14}C]maneb (manganese EBDC), on field-treated crops and in soil. These tests show that ETU is readily biodegradable, that accumulation of ETU in plants, soils, or water is very unlikely, and that little, if any, ETU (<0.05 ppm) is likely to be present on raw agricultural commodities treated with maneb under normal use conditions.

EXPERIMENTAL SECTION

Radiolabeled Materials. The radiolabeled materials, [^{14}C]maneb [manganese ethylenebis(dithiocarbamate)] and [^{14}C]ETU (ethylenethiourea), were synthesized from ^{14}C-labeled ethylenediamine dihydrochloride (New England Nuclear Corp.). The specific activity of [^{14}C]maneb was 5.72 μCi/mg and [^{14}C]ETU was 9.82 μCi/mg. The radiochemical purity of [^{14}C]ETU, determined by TLC, was 99%. Very few impurities could be extracted from [^{14}C]maneb which is highly insoluble; therefore, its radiochemical purity was estimated to be >98%.

Portions of the [^{14}C]maneb were formulated as 80% active wettable powders for the crop test studies. One formulation of [^{14}C]maneb had a specific activity of 4.57 μCi/mg while another had 1.52 μCi/mg specific activity. The [^{14}C]ETU was used unformulated in aqueous solutions.

Crop Studies. *Crop Treatment.* Foliar Spray Application. Two rows of tomato plants (*Lycopersicum esculentum*, var. Manalucie) (19.5 ft per row, plants spaced 6 in. apart) and two rows of snapbean plants (*Phaseolus vulgaris*, var. Harvester) (19.5 ft per row, plants spaced 4 in. apart) were grown in Bradenton, Fla. The soil type was Leon Immokalee fine sand: pH 6.3; cation exchange capacity, 3.9 mequiv/100 g; organic matter content, 2.4%; clay, 1%; silt, 2%; and sand, 97%.

One row of tomato plants was treated with [^{14}C]maneb at 2 lb/100 gal (120 mg, 548 μCi) by a single spray application when the tomato plants were in a pre-bloom stage, about 12–15 in. high. The second row of tomato plants was treated with [^{14}C]ETU at 0.2 lb/100 gal (12 mg, 118 μCi) by a single spray application when the plants were in first bloom stage, about 18 in. high.

The bean plants were treated with three spray applications on a 7-day schedule when the bean plants were in a pre-bloom stage, about 8–10 in. high. The first row was treated three times with [^{14}C]maneb (2 lb/100 gal, 120 mg, 182 μCi) with each spray and the second row with [^{14}C]ETU at 0.2 lb/100 gal (12 mg, 118 μCi). Identical rows of untreated bean and tomato plants were maintained for controls.

Plants (three plants per sampling) were taken for analysis at various intervals after the last treatment by cutting at ground level. All plants were immediately frozen and stored in a freezer until analyzed. The frozen plants were placed in a metal tray and the tray placed in another tray containing dry ice to keep the sample frozen while being finely chopped. The finely chopped plant material was thoroughly mixed and aliquots of each sample were used for the analyses.

Soil Application for Uptake Studies. Small individual soil plots at Stine Farm, Newark, Dela., were isolated from the surrounding soil by inserting stainless steel boxes (1 ft × 1 ft × 1 ft, open at top and bottom) into the ground, leaving about 1 in. of rim protruding above ground level to minimize run-off. The soil type was Keyport silt loam: pH 5.4; cation exchange capacity, 8.9 mequiv/100 g; organic matter, 2.3%; clay, 21%; silt, 70%; and sand, 9%.

Three tomato plants (Bonnie Best variety, ca. 12 in. high) were planted in each isolated area. Duplicate plots were treated as follows.

[^{14}C]Maneb (20.8 mg, 119 μCi) in 15 mL of water was applied at 2 lb/acre by pouring the mixture as uniformly as possible over the soil surface. The sample container was washed 5 times with 5-mL portions of water and the washings were poured evenly over the soil surface.

[^{14}C]ETU (11.5 mg, 113 μCi) was applied at ca. 1.1 lb/acre by pipetting the [^{14}C]ETU on the soil surface in 5 mL of water.

[^{14}C]ETU (11.5 mg, 113 μCi) was thoroughly incorporated into the 0–4-in. layer of soil just prior to planting the tomato plants.

One plant was taken from each of the three duplicated test plots 1, 3, and 10 weeks after treatment by cutting at ground level. Ripe tomatoes were also picked from each plant at the time of the 10-week sampling. Each sample was analyzed for total radiochemical residues and specifically for ETU.

Total Radiochemical Residue Analysis. Twenty grams of sample and 100 mL of water were placed in a blender and blended at high speed for 5 min, and the mixture was freeze-dried. Aliquots of each sample (equivalent to 0.25–0.40 g, fresh weight) were analyzed for total ^{14}C residues by the combustion–liquid scintillation counting method using a Packard Model 305 sample oxidizer.

ETU Analysis. Plant samples were analyzed for ETU by a procedure similar to that reported by Yip et al. (1971). Fifty grams of sample and 200 mL of ethanol were blended for 2 min at high speed in a blender. Chloroform (100 mL) and Celite (10 g) were added and the mixture was blended for 2 min. The mixture was filtered with suction through a 0.5-in. layer of Celite on filter paper in a Buchner funnel and the volume measured. The corrected sample weight was calculated according to Yip et al. (1971).

The filtrate was extracted with 200 mL of water and then 100 mL of water. The aqueous extracts were combined and washed with 100 mL of $CHCl_3$. The $CHCl_3$ wash was extracted with 100 mL of water and this extract added to the previous aqueous extract. One milliliter of 50% NaOH was added to the aqueous extract and the volume reduced to ca. 5 mL in vacuo at 60 °C. The solution was adjusted to pH 7 with 6 N HCl, quantitatively transferred to a 10-mL volumetric flask, and made to volume with water. Aliquots of each extract were counted to determine the total radioactivity in the extract.

An aliquot of each extract (0.5 mL) was applied to a TLC plate (250-μm silica gel 60F-254, E. M. Laboratories, Inc.) as a streak and 20 μg of ETU standard spotted next to the sample. The plate was developed for 15 cm with a mixture of ethanol, chloroform, and benzene (1:5:10, v/v/v). The R_f of ETU (0.15–0.20) was determined by viewing the plate under short-wavelength UV light. The plate was scanned on a Varian Aerograph/Berthold Model 6000-2 TLC radioactivity scanner to determine the location of radiolabeled compounds. The area of the plate containing each ^{14}C-labeled material, including that for ETU, was removed from the plate and each was added to a scintillation vial containing 3.5 mL of water. Aquasol (11.5 mL) scintillation cocktail was added and the vial shaken. The resulting suspension was counted to determine the percent of total radioactivity for each compound.

Identification of ^{14}C-Labeled Residues. Fifty-gram samples of tomato plant from the [^{14}C]ETU foliar spray treatment were extracted three times in a blender by blending 5 min at high speed with 100-mL portions of methanol. The combined extracts were filtered with suction through a funnel with a fine fused fritted disk. Water (100 mL) was added to the combined extract and the volume of the resulting solution was reduced to ca. 75 mL in vacuo at 60 °C. The aqueous solution was washed 3 times with 100-mL portions of hexane and the hexane washings were discarded. The volume of the aqueous extract was reduced to 5.0 mL in vacuo at 60 °C. A 50-μL aliquot of each extract was counted to determine the total ^{14}C extracted.

A 0.5-mL aliquot of each extract was analyzed by TLC (250-μm silica gel GF) using a mixture of methanol, acetic acid, and water (10:1:1, v/v/v) as the developing solvent. The TLC plates were scanned and the radioactive areas counted as before.

The remainder of one extract (7-day exposure) was separated by TLC as above. The gel corresponding to ethyleneurea and Jaffe's base were removed from the plates and the individual compounds washed from the gel with 100 mL of methanol. Each radiolabeled compound was purified by several additional TLC steps. Infrared (KBr disk) and mass spectra were obtained on both isolated fractions.

Soil Studies. *Exposure of [^{14}C]ETU and [^{14}C]Maneb under Field Soil Conditions.* During the spring, ten 12-in. sections of 4-in. diameter stainless steel tubing were driven into the ground (Keyport silt loam) on a test site in Delaware to isolate undisturbed columns of soil. About 0.5 in. of each cylinder was left protruding above the ground surface to protect against run-off. Five cylinders were each treated with [^{14}C]ETU at 2 lb/acre by pipetting 1.0 mL of an aqueous solution of [^{14}C]ETU, containing 1.82 mg of [^{14}C]ETU/mL (14.0 μCi), on the soil surface. Cylinders were dug up for analysis after 0-, 1-, 4-, 12-, and 52-week exposures. The remaining five cylinders were each treated with [^{14}C]maneb at 2 lb/acre by washing 1.82 mg of [^{14}C]maneb (10.4 μCi) into the soil surface with 5 mL of water. Cylinders were dug up for analysis after 0-, 1-, 4-, 8-, and 52-week exposures.

The soil from each cylinder was removed for analysis and divided into the following increments as measured from the surface: 0–1, 1–3, 3–5, 5–8, and 8–12 in. All increments were spread on large trays to dry and then dry ballmilled for 48 h. Duplicate 1-g aliquots of each soil increment were analyzed for total ^{14}C residues by the combustion–scintillation counting method.

Soil samples (Keyport silt loam, 500 g each) were fortified with [^{14}C]maneb (4.16 ppm) and [^{14}C]ETU (1.07 and 2.14 ppm) to check recoveries of [^{14}C]ETU and [^{14}C]maneb using the combustion procedure described above. The fortified soil samples were air-dried, dry ballmilled for 48 h, and analyzed as described above. Recoveries of added ^{14}C by combustion analyses were all >90%, averaging between 93 and 94%.

Soil Metabolite Identification. Soil from the 0–1-in. increment from the 1-week exposure of [^{14}C]ETU (2 lb/acre) treated soil was analyzed specifically for [^{14}C]ETU and to determine the identity of the ^{14}C-labeled residues. The soil sample (25 g) and 100 mL of methanol were placed in a 200-mL centrifuge tube and the tube placed in a Cole-Parmer Ultrasonic Cleaner (Model 8845-3) for 5 min. The mixture was centrifuged at 3000 rpm for 5 min and then the methanol extract was decanted into a 1-L round-bottomed flask. The extraction was repeated twice more with methanol and 3 times with 100 portions of water. The extracts were combined and the volume reduced to 10.0 mL in vacuo at 60 °C (extract 1). The soil was then extracted 3 times with 100-mL portions of 0.1 M sodium hydroxide. The basic extracts were combined and the resulting solution was adjusted to pH 7. The solution was centrifuged for 30 min at 12000 rpm. The aqueous solution was decanted into a 1-L flask and the volume reduced to 10 mL in vacuo at 60 °C (extract 2). Extracts 1 and 2 contained 34.1 and 23.7% of the total residual ^{14}C, respectively.

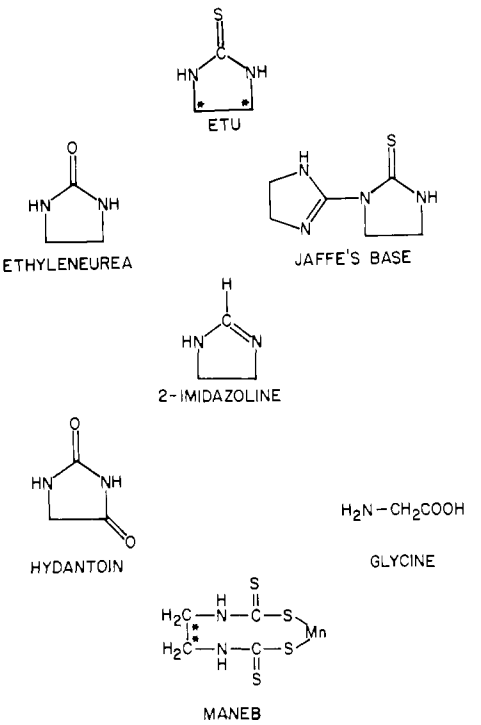

Figure 1. ETU degradation products. The asterisk denotes position of ^{14}C label.

Aliquots (0.5 mL) of each extract were analyzed by the TLC procedure previously described. The remainder of the extracts was separated by preparative TLC procedures as described above. The radioactivity corresponding to ethyleneurea was isolated and purified for mass spectral analysis as before.

Water/UV Studies. Aqueous solutions containing ca. 100 ppm of [^{14}C]ETU were exposed to a mercury vapor UV light for 8 h. Solutions of [^{14}C]ETU were in distilled water and in 0.1 M acetone in water. The volumes of the final solutions (250 mL) were reduced to 10 mL in vacuo at 60 °C. A 5-mL aliquot of each was treated with a 2% barium chloride solution to precipitate sulfate ion formed from ETU during UV exposure.

Aliquots (0.25 mL) of the resulting solutions were streaked directly on 1-mm silica gel GF plates next to 20 μg each of appropriate reference standards. The plates were developed to 15 cm with methanol–acetic acid–water (10:1:1). The plates were dried in a hood and then scanned on a TLC radioactivity scanner. Five ^{14}C compounds were detected; the area of gel containing each compound was removed from the plate and counted as previously described. Larger aliquots (1 mL) of the solutions were separated by the same TLC procedure. The gel corresponding to each ^{14}C compound was removed from the plates and the ^{14}C washed from the gel with ca. 100 mL of methanol. Each ^{14}C-labeled compound was purified by several additional TLC steps. Mass spectra were obtained on all isolated fractions on a Bendix Model 12-107

Table I. Analysis of Tomato and Bean Plants Treated with [^{14}C]Maneb

Days after treatment	Cumulative[a] rainfall, in.	Tomato foliage and stems: Total ^{14}C, ppm[b]	Tomato foliage and stems: ppm of ETU[c]	Bean foliage and stems: Total ^{14}C, ppm[b]	Bean foliage and stems: ppm of ETU[c]
0		34.4	0.17	91.6	0.57
1		24.9	0.28		
3	0.52	8.9	0.16	29.2	0.47
7		4.2	0.04	12.2	0.16
10		2.7	0.02	6.3	0.08
14		1.8	0.01	2.4	0.05
21	0.65	0.46	<0.01	3.1	0.02
28	3.50	0.33	<0.01	2.8	0.01
35		0.11	<0.01	0.20	<0.01

[a] Total rainfall (inches) from treatment to sampling; tomato plants had one [^{14}C]maneb treatment; bean plants had three treatments. [b] Calculated as parts per million of maneb; ^{14}C analysis based on combustion data. [c] Based on specific analysis for intact ETU.

Table II. Analysis of Tomato and Bean Plants Treated with [^{14}C]ETU

Days after treatment	Cumulative[a] rainfall, in.	Tomato foliage and stems: Total ^{14}C, ppm[b]	Tomato foliage and stems: ppm of ETU[c]	Bean foliage and stems: Total ^{14}C, ppm[b]	Bean foliage and stems: ppm of ETU[c]
0		2.2	0.08	5.5	0.66
1		2.1	0.06	6.2	0.21
3		0.95	0.01	3.9	0.05
7	1.26	0.75	0.03	3.0	0.03
10	2.98	0.62	0.01	1.6	0.03
14		0.25	<0.01	2.0	0.04
21		0.14	<0.01	1.9	0.01
28		0.16	<0.01	1.9	<0.01
35	3.09	0.06	<0.01	1.5	<0.01

[a] Total rainfall (inches) from treatment to sampling; tomato plants had one [^{14}C]ETU treatment; bean plants had three treatments. [b] Calculated as ETU; ^{14}C analysis based on combustion data. [c] Based on specific analysis for intact ETU.

Time-of-Flight mass spectrometer.

RESULTS AND DISCUSSION

Figure 1 gives the chemical structures of ETU and its breakdown products that will be discussed in this section. The structure of maneb itself is shown at the bottom of the figure.

Plant Studies, Foliar Treatment. Both total ^{14}C-labeled residues and intact [^{14}C]ETU residues rapidly decreased from tomato and bean plants treated with either [^{14}C]maneb or [^{14}C]ETU (Tables I and II). One objective of this study was to find out if residual ETU increased as maneb disappeared. Table I shows little, if any, evidence that ETU is accumulating as the total ^{14}C-labeled residues on maneb treated crops disappear. The small amounts of ETU reported in Table I are attributed to problems with the analytical extraction method for isolating ETU when used in the presence of maneb residue. Pease and Holt (1977) have studied this problem and report that 0.5–1.0% conversion of maneb to ETU is commonly encountered with all isolation procedures.

In recovery studies with both tomato and bean foliage fortified with [^{14}C]ETU at levels of 0.01 to 0.44 ppm (Table III), we obtained by our procedure for intact ETU an average recovery of 83%, if the samples were extracted immediately after adding the [^{14}C]ETU to the plant tissue. The percent recovery decreased rapidly as the time between addition of [^{14}C]ETU and sample extraction increased. For example, when samples of frozen tomato plant, fortified with [^{14}C]ETU at 0.4 ppm, were extracted 1 h after spiking, only 18% of the added ^{14}C was extracted and only 8–10% of the added [^{14}C]ETU was recovered. It is not surprising, then, that in Table II, even on day 0, very little intact ETU was found, even in studies where ETU was applied directly. ETU is apparently so reactive that the rapid decreases from field-treated crops reported by most workers are understandable.

Table III. [^{14}C]ETU Recovery Studies on Tomato and Bean Foliage

Sample	Wt, g	Spike level, ppm	μg: Added	μg: Found	% recovery
Tomato plant	50	0.44	22	18.7	85
	50	0.44	22	19.1	87
	50	0.12	6.1	4.8	80
	50	0.02	1.2	0.96	80
					83
Bean plant	50	0.24	12.1	9.9	82
	50	0.12	6.1	5.1	84
	50	0.02	1.2	0.98	82
	50	0.01	0.6	0.52	86
					83

Table IV. TLC Analysis of Extracts from Tomato Plants Treated with [^{14}C]ETU

Compound	% extracted ^{14}C at days exposure: 1	7	10	14
ETU	2			
Ethyleneurea	21	16	25	40
Jaffe's base	75	83	73	56
Origin of TLC plate	2	1	2	4
Total ^{14}C residue (ppm)[a]	2.1	0.75	0.62	0.25

[a] Total ^{14}C residue based on combustion results (calculated as parts per million equivalent to ETU). See Table II.

For the [^{14}C]ETU studies reported in Table II, we somewhat arbitrarily chose the ETU treatment rate of 0.2 lb/acre. The usual maneb rate is 2–3 lb/acre. We felt 10× lower for ETU was a fair test because it represents a grossly exaggerated level over that which would be applied to crops at recommended maneb application rates.

The major degradation products of [^{14}C]ETU on tomato plants were ethyleneurea and Jaffe's base [1-(2-imidazolin-2-yl)-2-imidazolidinethione] as shown in Table IV. TLC separations of the methanolic extracts showed that 40 and 56% of the extracted ^{14}C cochromatographed with ethyleneurea (R_f 0.60) and Jaffe's base (R_f 0.43), respectively, for the 14-day sample. (Note: our extraction scheme removed 70–80% of the total ^{14}C from the plant tissues.) Infrared and mass spectral analyses of the radiolabeled materials isolated from the plant extracts confirmed that the major constituents in the two TLC fractions were ethyleneurea and Jaffe's base. Mass spectral evidence also suggested that a small amount of 2-[^{14}C]-imidazoline was present in the TLC fraction containing Jaffe's base.

Plant Uptake Studies. Tomato plants grown outdoors in soil treated with [^{14}C]maneb at 2 lb/acre, surface applied, or in soil treated with [^{14}C]ETU at 1.1 lb/acre, surface applied, or in soil where [^{14}C]ETU was incorporated (0–4 in.) at 1.1 lb/acre, took up only trace ^{14}C-labeled residues (Table V). The total ^{14}C-labeled residues in the plants, based on combustion analysis but calculated as

Table V. Total ^{14}C Residues in Tomato Plants Grown in Treated Soil

Soil treatment	Tomato foliage and stems: 1 week, Total ^{14}C, ppm[a]	Tomato foliage and stems: 1 week, ppm of ETU	Tomato foliage and stems: 3 weeks, Total ^{14}C, ppm[a]	Tomato foliage and stems: 3 weeks, ppm of ETU	Tomato foliage and stems: 10 weeks, Total ^{14}C, ppm[a]	Tomatoes, 10 weeks, Total ^{14}C, ppm[a]
[^{14}C]Maneb; 2 lb/acre surface treatment	0.37	<0.01	0.05		0.03	<0.01
(duplicate field exposure)	0.29	<0.01	0.12	<0.01	0.03	<0.01
[^{14}C]ETU; 1.1 lb/acre surface treatment	0.14	<0.01	0.04	<0.01	0.01	<0.01
(duplicate field exposure)	0.05	<0.01	0.03		0.02	<0.01
[^{14}C]ETU; 1.1 lb/acre incorporated	0.35	<0.01	0.11	<0.01	<0.01	<0.01
(duplicate field exposure)	0.25	<0.01	0.09		0.01	<0.01

[a] Calculated as parts per million of ETU; ^{14}C analysis based on combustion data.

Table VI. Percent of Original ^{14}C Activity in [^{14}C]ETU Treated Field Soil Fractions (2 lb/Acre)

Soil depth, in.	Exposure time, weeks: 0	1	4	12	52
0-1	92.6	52.5	40.7	33.2	16.7
1-3	0.1	1.9	3.8	1.8	4.0
3-5	<0.1	0.2	0.3	0.6	1.0
5-8	<0.1	0.2	0.1	0.2	0.5
8-12	<0.1	<0.1	<0.1	0.2	0.1
	92.7	54.8	44.9	36.0	22.3
Total rainfall[a]	0	0.54	3.34	14.26	53.76

% of original ^{14}C activity in [^{14}C]maneb treated field soil fractions (2 lb/acre)

Soil depth, in.	Exposure time, weeks: 0	1	4	8	52
0-1	92.4	66.5	56.3	41.6	26.5
1-3	0.5	7.8	1.5	2.9	5.5
3-5	<0.1	2.4	0.3	1.3	0.2
5-8	<0.1	0.4	0.1	0.5	0.1
8-12	<0.1	0.2	0.1	<0.1	<0.1
	92.9	77.3	58.3	46.4	32.3
Total rainfall[a]	0.0	0.19	1.07	10.95	50.55

[a] Total rainfall (inches) from time of application to sampling.

Table VII. TLC Analysis of 8-h Exposure of Aqueous Solution of [^{14}C]ETU to UV Light

Compound	R_f	% total ^{14}C: No photoinitiator[a]	% total ^{14}C: 0.1 M acetone added[b]
Hydantoin	0.79	9.3	24.4
Ethyleneurea	0.60	13.9	7.3
Jaffe's base	0.45	10.9	16.5
Glycine	0.30	63.0	49.4
Origin of TLC plate	0.00	3.0	8.4

[a] ETU was completely degraded (>99%) after 6 h. [b] ETU was completely degraded (>99%) after 3 h.

ETU, ranged from only 0.1 to 0.4 ppm 1 week after treatment. These residues decreased to 0.01–0.03 ppm at maturity (10 weeks after treatment). The total ^{14}C-labeled residues represented only about 0.08% of the applied ^{14}C after 1 week and <0.01% after 10 weeks. No detectable [^{14}C]ETU (<0.01 ppm) was found in any sample. No ^{14}C residues at all were found in ripe tomatoes from any treatment (<0.01 ppm). These small total ^{14}C-labeled residues, taken up by tomato plants from field-treated soil, are considerably less than the levels reported taken up by potted soybeans in a greenhouse test (Nash, 1975, 1976), but our tests are closer to actual use conditions on a registered crop.

Soil Studies. Total ^{14}C analysis of soil, treated with [^{14}C]ETU at 2 lb/acre by surface application, show that [^{14}C]ETU and its degradation products have an overall half-life in soil (Keyport silt loam) of less than 4 weeks under field conditions in Delaware (Table VI). Total ^{14}C residues in [^{14}C]maneb treated soil (2 lb/acre) at the same location have a half-life of 4 to 8 weeks. Specific analyses of the [^{14}C]ETU treated soils showed that intact ETU actually had a half-life of less than 1 week in this test. Table VI shows clearly that ^{14}C from neither maneb nor ETU leached much below the 5-in. soil depth.

Only about 58% of the ^{14}C remaining in the [^{14}C]ETU treated soil was extractable after a 1-week exposure to field conditions. TLC analysis showed that 79% of the extracted ^{14}C was ethyleneurea and the rest of the extracted radioactivity was polar material which remained at the origin of the TLC plate. Kaufman and Fletcher (1973) have reported all [^{14}C]ETU on soil treated at 2 ppm was converted to ethyleneurea in 2 days and 43% was degraded to $^{14}CO_2$ within 4 days after treatment.

Water/UV Studies. The TLC analyses of aqueous solutions of [^{14}C]ETU exposed to mercury-vapor UV light showed that ETU is rapidly converted to other compounds. The principal mode of photodegradation (Figure 1; Table VII) leads to glycine sulfate with ethyleneurea and hydantoin formed as intermediate degradation products. Small amounts of Jaffe's base [1-(2′-imidazolin-2′-yl)-2′-imidazolidinethione] were also found, 11–16% of total degradation products. The structures of the identified compounds, listed in Table VII, were confirmed by comparison of the mass spectra of the isolated materials with the mass spectra of the respective reference compounds. The infrared spectra of ethyleneurea and Jaffe's base were also equivalent to the infrared spectra of the corresponding reference compounds; the infrared spectra of the other degradation products were not obtained. Ross and Crosby (1973) previously reported ethyleneurea and glycine from ETU in water/UV exposures.

CONCLUSION

ETU is readily degradable. Our studies in water/UV, in soil, and in plants all show rapid degradation and no tendency to accumulate in the environment. Even in recovery studies in the laboratory, the degradability of ETU caused some analytical problems. Hoagland and Frear (1976) have reported similar handling difficulties (see p 130 of their report, first paragraph in the Results and Discussion section).

Direct tests starting with [^{14}C]maneb confirm very little, if any, [^{14}C]ETU on plants under outdoor conditions. A companion paper (Pease and Holt, 1977) gives extensive direct conventional residue data on several edible food crops from several different locations. Their data also show

little, if any, ETU on edible crops (<0.05 ppm).

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance of H. L. Klopping and K. Lin for the synthesis of the radiolabeled maneb and ETU, respectively, and of R. Sutton for treating and growing the crops for the foliar spray tests. The author is also indebted to R. W. Reiser and O. R. Hunt for obtaining mass spectrographic and infrared analyses and to G. A. Smith for his assistance.

LITERATURE CITED

Blazquez, C. H., *J. Agric. Food Chem.* **21**, 330 (1973).
Dekhuijzen, H. M., Vonk, J. W., Sijpesteijn, A. K., paper presented at the 2nd International Symposium on Pesticide Terminal Residues, Tel-Aviv, 1971; *Pestic. Terminal Residues, Invited Pap. Int. Symp., 1971*, 233 (1971).
Graham, S. L., Davis, K. J., Hansen, W. H., Graham, C. H., *Food Cosmet. Toxicol.* **13**, 493 (1975).
Graham, S. L., Hansen, W. H., *Bull. Environ. Contam. Toxicol.* **7**, 19 (1972).
Graham, S. L., Hansen, W. H., Davis, K. J., Perry, C. H., *J. Agric. Food Chem.* **21**, 324 (1973).
Hoagland, R. E., Frear, D. S., *J. Agric. Food Chem.* **24**, 129 (1976).
Kaufman, D. D., Fletcher, C. L., Abstracts of the 165th National Meeting of the American Chemical Society, Dallas, Tex., 1973.
Lyman, W. R., paper presented at the 2nd International Symposium on Pesticide Terminal Residues, Tel-Aviv, 1971; *Pestic. Terminal Residues, Invited Pap. Int. Symp., 1971*, 243 (1971).
Lyman, W. R., LaCoste, R. J., paper presented at the 3rd International Congress of Pesticide Chemistry, Helsinki, 1974; "Pesticides" Georg Thieme, Stuttgart, 1975, p 67.
Nash, R. G., *J. Assoc. Off. Anal. Chem.* **57**, 1015 (1974).
Nash, R. G., *J. Assoc. Off. Anal. Chem.* **58**, 566 (1975).
Nash, R. G., *J. Agric. Food Chem.* **24**, 596 (1976).
Newsome, W. H., Shields, J. B., Velleneuve, D. C., *J. Agric. Food Chem.* **23**, 756 (1975).
Pease, H. L., Holt, R. F., *J. Agric. Food Chem.*, preceding paper in this issue (1977).
Ross, R. D., Crosby, D. G., *J. Agric. Food Chem.* **21**, 335 (1973).
Yip, G., Onley, J. H., Howard, S. F., *J. Assoc. Off. Anal. Chem.* **54**, 1373 (1971).

Received for review September 13, 1976. Accepted December 13, 1976.

Persistence and Biodegradation of Carbofuran in Flooded Soils

K. Venkateswarlu, T. K. Siddarame Gowda, and N. Sethunathan*

The persistence of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) in four soils was studied in the laboratory with special reference to flooded conditions. After thin-layer chromatographic separation of residues, carbofuran in the soil samples was converted to its phenol by alkaline hydrolysis and then assayed colorimetrically following diazotization. More rapid degradation of carbofuran occurred in soils under flooded conditions than under nonflooded conditions. Carbofuran degraded rapidly between 20 and 40 days after flooding in most soils including an acid sulfate saline soil, Pokkali, capable of attaining near neutral pH upon flooding; but the insecticide persisted in another acid sulfate saline soil, Kari, perhaps due to its exceedingly low pH of 4.2, even after several weeks of flooding. Heat treatment of soils prior to incubation increased the persistence of carbofuran under flooded conditions. Moreover, a bacterium, isolated from flooded soil by an enrichment technique, decomposed carbofuran in a mineral salts medium. These studies indicate that microorganisms are involved in the degradation of carbofuran in flooded soils.

Brown planthoppers (*Nilaparvata lugens* Stal) cause direct damage to the rice crop by sucking the plant sap leading to a symptom known as "hopperburn" (PANS, 1970). In addition, it acts as a vector of grassy stunt virus disease. Recently, buildup of brown planthoppers particularly in areas where intensive cropping of rice is practiced has caused concern in India (Kulshreshtha et al., 1974) and in several southeast Asian countries.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) appeared to be the most effective insecticide in controlling rice brown planthoppers when broadcast in the flooded rice field soils as granules or when incorporated to the root zone in paper or gelatin capsules (IRRI, 1975). Although carbofuran has received attention in recent years because of its broad spectrum insecticidal and nematicidal properties, its fate in flooded soil ecosystem is little understood. This paper reports the relative persistence of carbofuran in Indian rice soils under flooded and nonflooded conditions and the role of biodegradation in its loss from flooded soils. The soils included two unique acid sulfate saline soils from coastal areas of Kerala, South India where carbofuran is used extensively to control the brown planthoppers in rice.

Laboratory of Soil Microbiology, Central Rice Research Institute, Cuttack-753006, Orissa, India.

MATERIALS AND METHODS

Persistence of Carbofuran in Different Soils under Flooded Conditions. The persistence of carbofuran was studied in four rice soils under flooded conditions. The soils included two unique acid sulfate saline soils from Kerala locally known as Kari and Pokkali. Some pertinent properties of the soils are listed in Table I. The soils were air-dried and ground to pass through 2-mm sieves.

Twenty grams of each soil contained in test tubes (25 × 200 mm) were treated with 1 mg of technical grade carbofuran (99.5% purity, FMC Corporation, Middleport, N.Y.) in 0.1 mL of methanol. After 24 h to allow evaporation of methanol, the soils were flooded with 25 mL of distilled water and incubated for 40 days. Carbofuran residues in two replicate soil samples were estimated at 20-day intervals.