

Fate of Ethylenethiourea in Hawaiian Soil and Water

Carl J. Miles* and Daniel R. Doerge

Department of Agricultural Biochemistry, University of Hawaii at Manoa, 1800 East West Road, Honolulu, Hawaii 96822

Ethylenethiourea (ETU) degradation rates and products in soil and groundwater were determined by using standard and isotopically labeled ETU (4,5-¹⁴C₂, 1,3-¹⁵N₂, and 2-¹³C). ETU is rapidly degraded and detoxified to ethyleneurea (EU) and sulfate ion in the soils studied by chemical oxidation, possibly by hydroxyl radicals. EU is further degraded by soil microorganisms to ethylenediamine and CO₂. ETU degraded very slowly in groundwater, but the rate increased significantly with addition of basalt. ETU should not reach deep groundwater because of rapid breakdown in overlying soils.

INTRODUCTION

The ethylenebis(dithiocarbamate) (EBDC) fungicides, such as mancozeb and maneb, have proven to be invaluable for agriculture. Although the parent fungicides show low toxicity, a formulation byproduct and environmental metabolite, ethylenethiourea (ETU; 2-imidazolidinethione), is goitrogenic and carcinogenic in laboratory animals (National Research Council, 1987). Because of these effects and the exposure of humans to ETU residues in food, EBDC fungicides are under Special Review by the U.S. Environmental Protection Agency to determine if the EBDCs can continue to be used. The occurrence of ETU in groundwater (Frakes, 1988) underscores the importance of its residue determination and environmental fate.

Degradation rates of ETU in soil are dependent upon soil type, ETU concentration, sunlight, and storage history of the samples (Cruickshank and Jarrow, 1973; Ross and Crosby, 1973; Helling and Thompson, 1974; Lyman and Lacoste, 1975; IUPAC, 1977; Rhodes, 1977; Nash and Beall, 1980) with soil half-lives ranging from 1 day to 4 weeks. Carbon dioxide, ethyleneurea (EU; 2-imidazolidone), hydantoin (2,4-imidazolidinedione), Jaffe's base [1-(4,5-dihydro-1*H*-imidazol-2-yl)-2-imidazolidinedione], and possibly elemental sulfur have been identified as degradation products. ETU was converted to EU in both sterile and nonsterile soils, suggesting that both biological and chemical oxidations occur in soils. Carbon dioxide formation from ETU was found only in nonsterile soils (Lyman and Lacoste, 1974; IUPAC, 1977). ETU is relatively mobile in most soils except muck (high organic matter) (Helling and Thompson, 1974), and trace amounts (0.3 ppb) leached through a sandy loam soil treated with EBDC fungicides (Nash and Beall, 1980). Three of 24 wells in an agricultural area of Maine had detectable levels of ETU (3-5 ppb) (Frakes, 1988).

Although several environmental fate studies on ETU have been performed, the methods often were nonspecific and mechanisms were not defined. This study employs standard and isotopically labeled ETU to determine its fate in some Hawaiian soils and groundwater. A degradation scheme for ETU oxidation in these soils is presented, and a possible mechanism is discussed.

EXPERIMENTAL PROCEDURES

Isotopically labeled ETU (4,5-¹⁴C₂, 1,3-¹⁵N₂, and 2-¹³C) was prepared by appropriate reaction of labeled ethylenediamine or carbon disulfide and purity determined by thin-layer chromatography (TLC), liquid chromatography (LC), and nuclear

magnetic resonance (NMR) (Doerge et al., 1990). LC determination of ETU and EU was performed on a silica column (5 μ m, 0.8 \times 10 cm, NovaPak, Waters) with 100% water as mobile phase and UV detection at 200 nm. Ion chromatographic (IC) determination of sulfate was performed by anion exchange (0.4 \times 25 cm, AS4A, Dionex), bicarbonate/carbonate mobile phase, and suppressed conductivity detection. TLC determination of ¹⁴C metabolites was performed on silica with 5:1 chloroform/methanol followed by autoradiography of developed TLC plates. Proton (300 MHz), ¹³C (76 MHz), and ¹⁵N (30.4 MHz) NMR spectra were obtained on a General Electric QE 300 or a Nicolet NT 300 spectrometer. Spectra were determined in D₂O or a deuterated phosphate buffer (0.1 M; pH 7) with 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), dioxane, and [¹⁵N]acetamide as proton, ¹³C, and ¹⁵N references, respectively. ¹³CO₂ was determined by headspace GC with mass spectrometric detection (HP 5970A).

Clay materials were prepared or purchased from Wards Scientific (Rochester, NY), and details of clay treatment were described previously (Miles and Moye, 1988). Groundwater was obtained from the Palolo section of the Pearl Harbor, Honolulu basin aquifer. Crushed basalt (ca. 0.2-2-mm diameter) was obtained from a local source (Kapaa Quarry, Kailua) and rinsed with groundwater before use. Jaucus (Typic Ustipsamments carbonatic, isohyperthermic), Wahiawa (Tropeptic Eustrustox clayey, kaolinitic, isohyperthermic), and Kaiwiki (Typic Hydrandepth thixotropic, isothermic) soils were obtained from the Soil and Agronomy Department, University of Hawaii. Soils were passed through a 2-mm sieve and used without further treatment unless described. Aqueous soil extracts (2.5:1 aqueous/solids and 1 h of mixing) were filtered and acidified before metal analysis by using inductively coupled plasma (ICP) atomic emission.

For degradation studies, fortified and control water and soil samples (2.5:1 aqueous/solids ratio) were incubated in Teflon bottles at room temperature on a shaker in the absence of light. Some groundwater samples were treated by addition of 10 g of unsterilized crushed basalt, 0.1% sodium azide, or a combination of both. Separate soil samples were treated by Γ irradiation (1 Mrad), autoclaving (25 min at 150 $^{\circ}$ C), a combination of these two treatments, addition of 0.1% sodium azide, addition of 72 800 units of catalase/10 g of soil, addition of 0.01 M Tris buffer, addition of 0.01 M mannitol, addition of 1500 units of superoxide dismutase/10 g of soil, or purging soil/water with nitrogen for 15 min to lower dissolved oxygen. Some soils were also treated by additions of various combinations 10 mM solutions of ferric iron, EDTA, diethylenetriaminopentaacetic acid (DTPA), and Desferal (deferoxamine). Periodically, samples were centrifuged and filtered before analysis by LC, IC, TLC, or NMR. Degradation was assumed to follow first-order kinetics; thus, rates were determined from plots of ln [pesticide remaining] vs time. For NMR analysis, samples were concentrated by lyophilization.

Soil column studies were performed with the Wahiawa soil according to the method described by Lemley et al. (1988). A 2.5 \times 30 cm glass column with a thermostated jacket (15, 25, or 35 $^{\circ}$ C) was filled with water-saturated Wahiawa soil and eluted

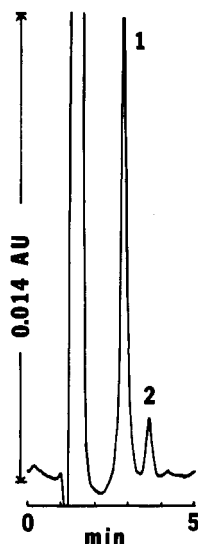


Figure 1. Chromatogram of Wahiawa soil/water extract on silica column with 100% water as mobile phase (2.0 mL/min) and UV detection (200 nm). (1) 5.91 mg/L ETU; (2) 1.48 mg/L EU.

for several days with 0.02 M CaCl_2 at 0.04 mL/min. After the 10-mL samples (in CaCl_2) were injected at the same flow rate, elution with CaCl_2 was reestablished and 4.73-mL (100 drops) fractions were collected. Fractions were analyzed by LC/UV for ETU and EU. Injection of $^3\text{H}_2\text{O}$ and scintillation counting was used to determine the soil column void volume. Solutions to the CXTFIT (Parker and van Genuchten, 1984) model used an apparent diffusion coefficient of $0.09 \text{ cm}^2 \text{ h}^{-1}$, a pore water velocity of 0.67 cm h^{-1} , a volumetric water content of $0.55 \text{ cm}^3 \text{ cm}^{-3}$, and a soil bulk density of 1.3 g cm^{-3} .

For sorption studies, 100 mg of soil or clay was combined with 1.0 mL of a $0.1 \mu\text{g/mL}$ solution of $[4,5\text{-}^{13}\text{C}_2]\text{ETU}$ (2 mCi/mmol) in a centrifuge tube and mixed for 2 h (kinetic experiments showed sorption was complete in 2 h). Samples were centrifuged, 0.1 mL of the supernatant was combined with 15 mL of Aquasol (New England Nuclear), and radioactivity was measured by scintillation counting. The sorption coefficient, K_D , was determined by the ratio of ETU in the solid phase (micrograms of ETU per gram of solid) to ETU in the solution phase (micrograms of ETU per gram of solution). The amount of ETU sorbed to the solid was determined by difference between the initial concentration and the solution concentration measured at the end of the experiment.

RESULTS AND DISCUSSION

Experiments required an analytical method capable of rapidly measuring parts per million concentrations of both ETU and EU in groundwater and soil/water mixtures. LC using a silica column with 100% water as mobile phase was the only method found that adequately separated ETU from EU in a short time. Good separation of the analytes from soil/water interferences was also observed (Figure 1). Capacity factors for ETU and EU were 1.81 and 2.25, respectively. Retention of the analytes on silica is probably by a mixture of processes including adsorption and electrostatic interactions. Others have reported good separation of organic compounds, especially amines, on silica with reversed-phase eluants (Bidlemeier et al., 1982; Adamovics, 1984; Law, 1987; Miles and Moye, 1987; Schmid and Wolf, 1987).

The absorption spectrum of EU ($\lambda_{\text{max}} = 190 \text{ nm}$) required detection at low wavelengths. Since the absorbance of EU and soil/water interferences increased with decreasing wavelength, 200 nm was chosen for LC detection as a compromise. At this wavelength detection limits for ETU and EU were 4.0 and 4.8 ng, respectively. With 20- μL injections, sub-ppm (0.20 and 0.24 ppm) detection limits were realized.

Table I. Degradation Rates ($\times 10^{-3} \text{ Day}^{-1}$) of ETU in Hawaiian Groundwater

| | control | pH | 0.1% NaN_3 | pH |
|----------------------|---------------|-----|---------------------|-----|
| groundwater | 0.6 ± 0.4 | 7.2 | 0.5 ± 0.3 | 7.9 |
| groundwater + basalt | 8.2 ± 0.8 | 7.5 | 4.4 ± 0.6 | 8.3 |

Table II. Chemical and Physical Characteristics of the Three Experimental Soils

| | sand, % | silt, % | clay, % | OC, ^a % | pH ^b |
|---------|---------|---------|-----------------|--------------------|-----------------|
| Jaucus | 95 | 5 | <1 | <1 | 6.2 |
| Wahiawa | 19 | 38 | 44 ^c | 1.5 | 6.2 |
| Kaiwiki | 12 | 33 | 55 ^d | 6.2 | 6.0 |

^a Organic carbon. ^b In 0.01 M CaCl_2 . ^c About 1:1 kaolinite and amorphous iron/aluminum oxides. ^d Mostly amorphous minerals with small amounts of quartz, anatase, goethite, hematite, maghemite, and ilmenite.

Table III. Sorption Coefficients (K_D) for ETU on Soils, Clay Materials, and Basalt

| sorbant | sorption coeff | sorbant | sorption coeff |
|-------------------------|----------------|-----------------|----------------|
| Jaucus soil | 0.03 | montmorillonite | 0.01 |
| Wahiawa soil | 0.09 | bentonite | 0.11 |
| Kaiwiki soil | 0.12 | kaolinite | 0.02 |
| Fe_2O_3 | 0.02 | basalt | 0.03 |
| illite | 0.01 | | |

ETU degraded very slowly in the groundwater tested, and addition of basalt to simulate the groundwater environment increased the degradation rate 10–20-fold (Table I). Enhanced degradation could be an effect of microbes introduced with the basalt or a surface effect as observed with soils (see below). EU was observed as a major product, indicating that oxidation is the primary degradation mechanism in the groundwater/basalt mixtures. Degradation was approximately 2 times slower in groundwater/basalt mixtures treated with azide, suggesting that either microbial metabolism is a significant pathway or azide inhibits degradation by iron chelation (see below).

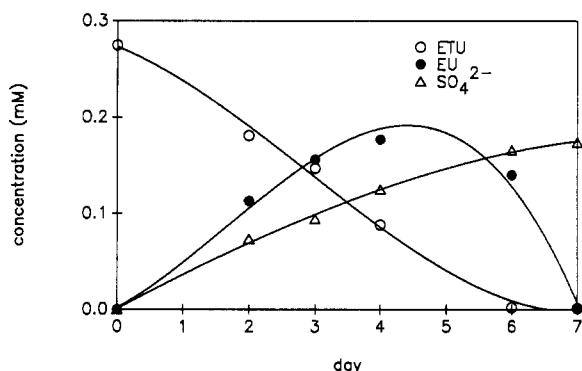
ETU sorption and degradation were tested in three Hawaiian soils with various physical and chemical compositions (Table II). Soils were flooded to simulate conditions in the soil saturated zone. ETU was only weakly sorbed to the three test soils and several selected clay materials (see Table III). Within the three soils, ETU sorption increased with increasing organic carbon content, although other factors may be responsible for the effect observed. Also, relatively high sorption was found with bentonite clay. Polar compounds, such as ETU, may bind to clay materials by mechanisms other than adsorption.

Injection of ETU on soil columns (Wahiawa silty clay) continuously eluted with 0.02 M CaCl_2 allowed observation of degradation and sorption (Lemley et al., 1988). Degradation and sorption were evaluated by a computer program, CXTFIT, that solves a convection/dispersion equation assuming linear adsorption and first-order decay (Parker and van Genuchten, 1984). A half-life of 1.4 days from the batch experiments compared well with the half-life of 1.3 days determined by the soil column and by CXTFIT. Also, a sorption coefficient of ≤ 0.1 calculated by CXTFIT is comparable to 0.09 determined by batch sorption experiments.

ETU degraded rapidly in all three soils (Table IV). Degradation experiments at three temperatures (15, 25, and 35 °C) showed that reaction rate approximately doubled for every 10 °C increase ($E_{\text{act}} = 15.7 \text{ kcal/mol}$). Soils were sterilized by Γ irradiation, azide treatment, and

Table IV. Degradation Rates (Day⁻¹) of ETU in Three Hawaiian Soils

| treatment | Wahiawa | Jaucus | Kaiwika |
|--|---------------|---------------|---------------|
| control | 0.484 ± 0.255 | 0.309 ± 0.062 | 0.201 ± 0.054 |
| 0.1% NaN ₃ | 0.086 ± 0.018 | 0.038 ± 0.006 | 0.007 ± 0.004 |
| Γ irradi | 0.354 ± 0.037 | 0.139 | 0.265 |
| Γ irradi + autoclaved | 0.100 ± 0.009 | | |
| autoclaved | 0.077 ± 0.006 | | |
| catalase (72 800 units/10 g) | 0.532 ± 0.228 | | |
| Tris buffer (0.01 M) | 0.217 ± 0.028 | | |
| mannitol (0.01 M) | 0.087 ± 0.011 | | |
| superoxide dismutase (1500 units/10 g) | 0.697 ± 0.141 | | |
| low oxygen | 0.161 | | |

**Figure 2.** Degradation of ETU and product formation in Wahiawa soil.

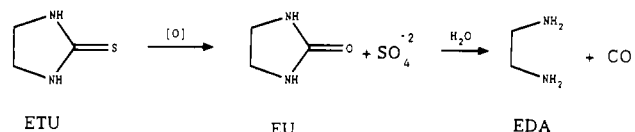
autoclaving to differentiate between biotic and abiotic degradation. Although autoclaving significantly slowed ETU degradation rates in Wahiawa soil, Γ irradiation (1 Mrad) had little effect. Autoclaving can affect soil surfaces that are involved in the degradation mechanism discussed below. Azide slowed the degradation rate by either retarding microbial growth or binding to iron as in the mechanism discussed below. These observations suggest an enzymatic or chemical degradation mechanism involving acellular oxidizing species.

In soil/water mixtures the degradation of ETU is accompanied by formation of sulfate and EU (Figure 2). EU is consumed in subsequent reactions. EU was stable for several weeks in irradiated and/or autoclaved samples, suggesting that EU degradation was microbially mediated. Incubation of soil with [2-¹³C]ETU produced [2-¹³C]EU as confirmed by ¹³C NMR. Except for minor soil peaks ($\delta = 60$) also observed in the control, ETU and EU ($\delta = 181$ and 167, respectively) were easily observed. Head-space GC/MS analysis of the same soil samples showed the formation of ¹³CO₂. Degradation of [1,3-¹⁵N₂]ETU ($\delta = 312$) to ethylenediamine (EDA; $\delta = 361$) in soil was observed by ¹⁵N NMR, and spectra were free of soil interferences. Experiments with [4,5-¹⁴C₂]ETU and TLC/autoradiography also confirmed formation of EU and other polar products like EDA.

Enhanced degradation of ETU was observed in the presence of both soil solids and ferric iron (see Table V). When ferric chloride was added to Wahiawa soil, the rate of ETU degradation increased ca. 200-fold above that observed with soil or ferric ion alone. Soil solids were essential as aqueous extracts of Wahiawa soil supplemented with ferric chloride did not give enhanced degradation. Treatment of Wahiawa soil with the chelators EDTA, DETPA, and Desferal increased the ETU degradation rate along with the soluble iron concentration as determined by ICP analysis. ICP analysis also showed increases in copper and manganese ions, but addition of these ions was

Table V. Degradation Rates of ETU in Wahiawa Soil and Solution

| treatment | degradation rate $\times 10^{-2}$, min ⁻¹ |
|---|---|
| Wahiawa soil | 0.034 |
| Wahiawa soil + 10 mM Fe ³⁺ | 8.0 ± 0.9 |
| Wahiawa soil + 10 mM Fe ³⁺ + 0.1 mM mannitol | 3.3 |
| Wahiawa soil + 10 mM Fe ³⁺ + 0.1 mM azide | 0.7 |
| Wahiawa soil + 10 mM DETPA | 5.3 |
| Wahiawa soil + 10 mM EDTA | 3.4 |
| Wahiawa soil + 10 mM Desferal | 2.3 |
| Wahiawa soil extract (no solids) | ca. 0.002 |
| Wahiawa soil extract + 10 mM Fe ²⁺ | 0.070 |
| deionized water + 10 mM Fe ³⁺ | 0.033 |

**Figure 3.** Degradation scheme for ETU in soil.

less effective in enhancing ETU degradation in Wahiawa soil than added ferric ion.

The inhibition of ETU degradation in soils by azide and mannitol ion suggests a hydroxyl radical mechanism for ETU oxidation. Iron-catalyzed hydroxyl radical formation requires at least one free ferric iron coordination site or a readily dissociable ligand like water (Graf et al., 1984). Azide can bind to this site and block hydroxyl radical formation (Graf et al., 1984). Since superoxide dismutase and catalase had minimal effects on ETU oxidation, it is proposed that the reactions producing hydroxyl radicals occur on soil surfaces and not in free solution. Molecular oxygen is required for ETU oxidation since nitrogen purging of Wahiawa soil/water greatly decreased the rate of degradation (Table IV). A mechanism of ETU oxidation by Wahiawa soil consistent with the experimental observations requires the chelation of ferric iron by soil surface components, reduction of the complex to the ferrous state by unidentified electron donors, and subsequent reaction with molecular oxygen to generate hydroxyl radicals, the ultimate oxidant.

This investigation shows that ETU is rapidly degraded and detoxified to EU and sulfate in the soils studied by a chemical oxidation mechanism, possibly by ferric ion mediated soil surface generated hydroxyl radicals. EU is further degraded by microbes to EDA and CO₂ (see Figure 3). ETU degraded very slowly in groundwater, but the rate increased significantly with addition of basalt. Although ETU is only weakly sorbed by soils and clays, it should not reach deep groundwater because of rapid breakdown in overlying soils. These results suggest a low potential for groundwater contamination by ETU.

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