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ETHYLENETHIOUREA

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ETHYLENETHIOUREA

A Special Report on the Occurrence of Ethylenethiourea as a Terminal Residue Resulting from Agricultural Use of Ethylenebisdithiocarbamate Fungicides

Project Coordination: R. Engst (GDR)

Major Contributors:

R. Baron (USA)
D. G. Crosby (USA)
N. Drescher (FRG)
R. Engst (GDR)
R. Greenhalgh (CAN)
P. C. Kearney (USA)
F. Korte (FRG)
R. G. Nash (USA)
W. H. Newsome (CAN)
H. V. Morley (CAN)
W. Schnaak (GDR)
E. Turtle (UK)
K. Vogeler (FRG)
J. W. Vonk (NETH)

ABSTRACT - Ethylenethiourea (ETU) is a manufacturing, processing, and metabolic product of the ethylenebisdithiocarbamate (EBDC) fungicides. Toxicological studies indicate that ETU may produce goiterogenic, tumorogenic, and teratogenic effects in laboratory animals, raising the concern that residues may be found on agricultural commodities. Thin-layer chromatography, polarography, and radioisotopic methods are used for ETU analysis, though electron capture or flame photometric gas-liquid chromatography are the preferred methods because of their greater sensitivity, specificity, and accuracy. Derivatization of ETU is usually necessary prior to gas liquid chromatography. ETU is degraded by oxidative reactions in biological systems and by photolytic reactions, especially in the presence of photosensitizers. Degradation products of ETU detected in photochemical, soil, and plant systems include 2-imidazolidinone (EU), 2-imidazoline, 2,4-imidazolidinedione (hydantoin), and 3-(2-imidazolin-2-yl)-2-imidazolidinethione (Jaffe's base). Ethyleneurea, oxalic acid, glycine, and urea have been detected in animal urine. The amount of ETU residue detected in the environment depends on the amount applied with the EBDC fungicide, the rate of formation as a result of EBDC degradation, and the rate of degradation. Residues of ETU have been detected in crops receiving EBDC fungicides. Levels of ETU detected are well below 0.1 ppm and occur primarily as surface residues.

INTRODUCTION

Ethylenebisdithiocarbamates (EBDC) form the most important class of fungicides for controlling diseases of agricultural crops. The class includes nabam, maneb, mancozeb, metiram and zineb. The United States production of dithiocarbamates, which includes both ethylenebis- and dimethyldithiocarbamates, in 1971 was nearly 16 million kg (von Rumker et al. 1974); world production was estimated to be 5 to 8 times this amount.

During recent years much attention has been paid to the various findings that ethylenethiourea (ETU) may occur in plant samples following the use of these dithiocarbamate fungicides. The occurrence of ETU may result from the presence of ETU in the pesticide when applied or from its subsequent transformation. Similarly, propylenethiourea may occur in residues of the propylenebisdithiocarbamate fungicide propineb.

The amounts of ETU present in commercial formulations have been shown to vary from one sample to another, depending on the length of time from manufacture to use and the storage conditions, especially temperature and moisture. Bontoyan and Looker (1973) found an initial content of 0.02 - 2% ETU, increased during a 39 day storage experiment at 49°C (120° F) and a relative humidity of 80% up to a final content of 0.13 - 14.5% ETU. The degradation dynamics of formulations from several manufacturers was different. The products containing both manganese and zinc formed the least amount of ETU.

Ethylenethiourea is a well known and fairly stable compound. It is employed commercially or industrially as an accelerator in the production of synthetic rubber. When administered

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under various toxicological experimental conditions, ETU has been shown to have caused, or in some cases suspected to have caused various pathological effects. These, including goiterogenic, tumorogenic and teratological effects, have raised some questions regarding the possibly harmful effects as terminal residues of the relevant dithiocarbamate fungicides.

When considering this matter at the request of various governments, the Joint FAO/WHO Meeting of Experts on Pesticide Residues in 1974 drew attention to deficiencies in data which prevented an adequate evaluation of the situation concerning residues in food (FAO/WHO, 1975). So far as chemical questions are involved they drew special attention to inadequacies in the methods of analyses hitherto used by workers in this field and which usually did not differentiate between the dimethyl- and the ethylenebisdithiocarbamates; also to insufficient information on the amounts of ETU in residues occurring in marketed produce or in prepared, including cooked, foods at the point of consumption. After recognizing the importance of the fungicides in agricultural production but expressing concern over the situation, the FAO/WHO experts called for further work and information on these points. They also gave notice of their intention to re-evaluate the EBDC fungicides at their 1977 meeting.

Recognizing the above mentioned deficiencies and the importance of the subject, this report presents an outline of current knowledge. Possible environmental contamination beyond the occurrence of residues in foods has not been covered: any such consideration should take account of any other possible sources of ETU such as its use in the production of synthetic rubber.

1. ANALYTICAL METHODOLOGY

Various techniques have been derived for the determination of ETU and are discussed briefly. GLC methods predominate because of their greater sensitivity, specificity, and accuracy and are summarized in Table I.

1.1. Thin-layer Chromatography

A variety of adsorbants and developing solvents have been used to detect ETU in plants (Vonk and Kaars Sijpesteijn, 1970; Blazquez, 1973; Onley and Yip, 1971; Engst and Schnaak, 1974). A minimum limit of detection of 0.02 ppm has been reported (Onley and Yip, 1971) using alumina plates and Grotes reagent for visualization. Semiquantitative determinations are possible by comparison with ETU standards run simultaneously.

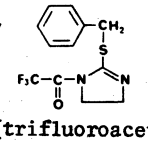
1.2. Polarography

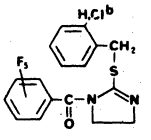
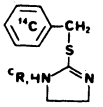
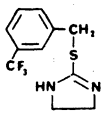
Engst and Schnaak (1974) have developed a procedure which utilized this technique. It involves cleanup on an alumina column followed by paper chromatography and determination of the nitroso derivative by polarography.

1.3. Radio-isotope

Nash (unpublished) employed ^{14}C-benzyl chloride to form S-benzylated ETU. The method has a sensitivity >1 ppm, but is considered impractical. A reverse isotope dilution method has been reported for ETU in the presence of its metabolites and is useful in the low milligrams range (Graham and Bornak, 1973).

TABLE 1. Methods for determining ETU in plant samples

Extraction Solvent	Extract Cleanup	Derivative Formation	Analysis Measurement	Detectability ppm	Reference
Plant Sap	Ethanol	None	Silica gel TLC[a]	10	Vonk & Kaars Sijpesteijn (1970)
Plant Sap	Ethanol	None	Paper electro-phoresis	Not Given	Vonk & Kaars Sijpesteijn (1971)
Ethanol + Chloroform	Cellulose Column	 2-(butylthio)= 2-imidazoline	GLC-thermionic 30% DC-200 5% SE-30 - GLC - Flame photometric	0.02	Onley & Yip (1971) Watts et al. (1974)
Methanol (1972) Ethanol (1975)	Chloroform/ HCL	 2-(benzylthio)-1-(trifluoroacetyl)-2-imidazoline	GLC-electron capture 2% butanediol succinate	0.005 0.01	Newsome (1972) Pecka et al. (1975)

Methanol	Al_2O_3 Column		GLC-Flame photometric 20% SE-30	0.01	Haines & Adler (1973)
	2-(butylthio)-2-imidazoline				
Dioxane + Water	None	None	Silica gel G TLC	1	Blazquez (1973)
Methanol/ Na-ascorbate	Al_2O_3 Column	None	GLC/FPD 3% Versamid 900	0.01	Otto et al. (1976)
Ethanol	Al_2O_3 + paper chromatography		Polarography	0.05-0.2	Engst & Schnaak (1974)
	1-nitroso-2-imidazolidinethione				
Methanol	Florisil Column		GLC-electron capture 3% OV-17, 3% OV-1, 3% XE-60	0.005	Nash (1974, 1975)
	2-(benzylthio)-1-(pentafluorobenzoyl)-2-imidazoline				
Methanol	Various		LSC - ^{14}C	1	Nash (Unpublished)
	2-(benzylthio)-2-imidazoline				
Water	None	None	Silica gel and cellulose TLC-^{14}C	Not Given	Vonk (1975)
Methanol	TLC	None	Silica gel TLC-^{14}C	Not Given	Hoagland & Frear (1976)
Ethanol	Ether/HCl partition		GLC/ECD 3% OV-275	0.01	King (1976)
	2-(m-trifluoromethylbenzythio)-2-imidazine				

[a]TLC - Thin-layer chromatography; GLC - Gas-liquid chromatography; LSC - Liquid-scintillation counting.
[b]H - 1974; CL - 1975.
[c]R - Trifloroacetic anhydride, pentafluorobenzoyl chloride, or others.

1.4. Gas-Liquid Chromatography

This technique involves the use of both electron capture (ECD) and flame photometric detectors (FPD). Smaller sample sizes are used in conjunction with electron capture detection but these methods require a derivatization step. The use of a selective GLC detector is most desirable because of the need to detect and confirm the residues. All methods employing GLC-flame photometric detection except that of Otto (1976) also require derivatization.

(I) Extraction

Methanol and ethanol have been preferred as extraction solvents for biological samples due to the high solubility of ETU in polar solvents. Mixed solvents such as methanol/chloroform (Onley and Yip; 1971) or methanol/acetone (Phillips, 1976) have also been employed. Improved recoveries with the latter solvent have been reported with the addition of trichloroacetic acid. Sodium ascorbate has also been found effective in ensuring good recoveries of ETU (Otto et al., 1976).

(II) Cleanup

The simplest procedures involve extraction of a derivative from aqueous acid and alkali (Newsome, 1972; Nash, 1974; King, 1976). Another approach has been to purify the initial extract by column chromatography prior to proceeding with derivatization (Onley and Yip, 1971; Haines and Adler, 1973) or determination steps (Otto et al., 1976). Where ETU is determined without derivatization (Otto et al., 1976), a solvent partitioning step is included to provide further cleanup.

(III) Derivatization
In all cases, derivatization involves first an alkylation of the thiocarbonyl group. The various derivatives which have been used are given in Table 1. Careful attention to reagent purity is essential to ensure quantitative results (Onley and Yip, 1971; Pecka et al., 1975; King, 1976). The benzyl chlorides react smoothly by refluxing in alcohol for 30 min, while alkylation with butyl bromide is carried out at room temperature in aqueous dimethylformamide containing NaOH and sodium borohydride. Solutions of ETU in aqueous dimethylformamide have been found extremely unstable and must be reacted immediately (Phillips, 1976). The n-butyl (Onley and Yip, 1971) and m-trifluoromethyl benzyl (King, 1976) derivatives are sufficiently volatile to be analyzed directly by GLC, whereas the benzyl derivatives must be concentrated and acylated prior to quantitation. Care must be exercised during the concentration step to prevent losses by evaporation (Pecka et al., 1975). Pentafluorobenzoyl chloride (Nash, 1974) and trifluoroacetic anhydride (Newsome, 1971) have been used as acylating reagents, the former requiring a column chromatographic step to remove excess reagent and by-products before GLC. Although the excess trifluoroacetic anhydride is easily removed by evaporation, the trifluoroacetate derivative is unstable in the presence of moisture and must be determined soon after removal of the excess reagent.

(IV) Determination
A variety of column packings and conditions have been used in the analysis of ETU and its derivatives (Table I). Detectors used have included thermionic (Onley and Yip, 1971), flame photometric (Haines and Adler, 1973; Otto et al., 1976) and electron capture (Newsome, 1971; Nash, 1974; King, 1976). Although quantitation by GLC/EC enables the use of smaller samples (5-10 g) for monitoring ETU residues at the 0.01 pm level, it requires confirmation of suspected residues by mass spectrometry, a second derivative, or by element selective detectors. Methods employing GLC/FPD with large samples (40-100 g) have the advantage of both quantitating and confirming ETU residues.

Of the methods mentioned for the determination of ETU, three have been subjected to collaborative evaluation. A modification of the Onley-Yip method involving extraction with methanol/aqueous NaCl, addition of Gas Chrom S, and cleanup on alumina (Onley - personal communication, 1976) was tested in potato, apple sauce, and spinach. The method of Otto et al., (1976) has been examined in apples, tomatoes, and grapes, and that of King (1976) in tomato juice.

The possibility of conversion of the parent compound to ETU during the analytical procedure has been examined for some of the methods. Thus, a maximum conversion of 1.7% was observed by Otto et al., (1976), 2% estimated by Nash (1974) and 0.17% determined for the Newsome procedure (Newsome, personal communication, 1976). No conversion was reported for the Haines-Adler method (1973) while a range of 7 - 16% was found using a modification of this procedure (Phillips, 1976).

2. CHEMISTRY OF ETHYLENETHIOUREA (ETU)

2.1. Physical characteristics
Some physical data of ETU are summarized as follows:

Empirical formula:	$C_3H_6N_2S$
Synonyms:	2-imidazolidinethione
Appearance:	white, crystalline
Molecular weight:	102.17
Odor:	odorless
Melting point:	203-204°C
Stability:	stable under normal condition
Solubility:	water 20,000 ppm at 30°C, 90,000 ppm at 60°C, 440,000 ppm at 90°C, ethanol: slightly soluble, chloroform: nearly insoluble.

2.2. Formation
EBDC's are unstable in the presence of moisture and oxygen as well as in biological systems. By splitting off carbon disulfide and hydrogen sulfide as well as by oxidative degradation reactions, the formation of a great number of secondary products are produced, among them, ETU. Reactions of EBDC in the formation of ETU are shown in Figure 1.

2.2.1 ETU is prepared by the reaction of ethylenediamine and carbon disulfide in aqueous alcohol.

2.2.2 Formation from EBDC.
The amount of ETU formed by degradation of EBDC depends on the stability of the parent compounds, the substrate and reaction conditions. Under neutral and alkaline conditions, temperature increases promote the formation of ETU. Thus, boiling residue amounts of EBDC's or 5,6-dihydro-3 H-imidazo[2,1-c]-1,2,4-dithiazole-3-thione (DIDT) yielded considerable

quantities of ETU (Watts et al., 1974; Phillips, 1976). Under acid conditions and high temperatures, EBDC's decomposed to carbon disulfide and ethylene diamine (EDA) reversing the synthesis.

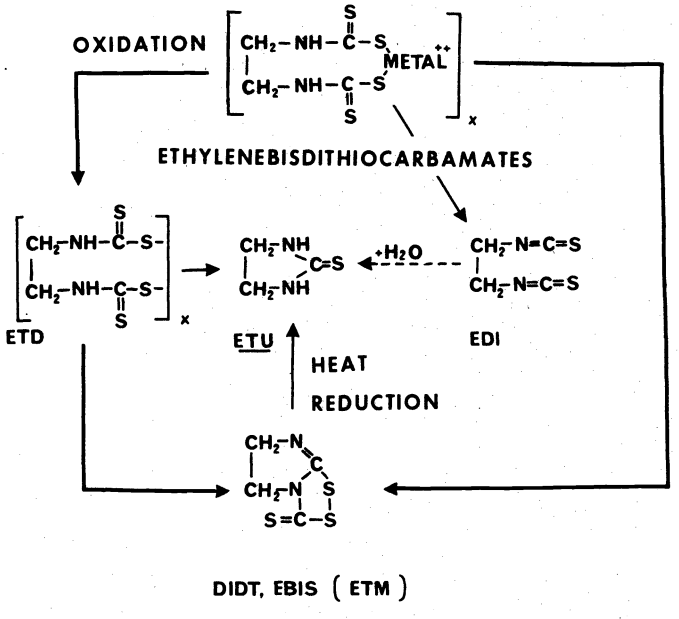


Fig. 1. Reactions leading to ETU formation. (Dashed line indicates proposed reaction.)

ion mechanisms leading to ETU formation are not completely understood, however, a number of hypothesis have been advanced. According to Marshall (1977) intermediary products of the thermal bis-dithiocarbamate degradation to ETU are β-amino ethylenedithiocarbamate and DIDT, but not ethylenediisothiocyanate (EDI). EDI was, however, postulated and detected several times as a secondary reaction product of the EBDC degradation at normal temperatures (Klopping and van der Kerk, 1951; Engst and Schnaak, 1970).

2.3. Transformation products of ETU

ETU is not a final product but is a relatively stable intermediate in the ultimate degradation of the EBDC fungicides. It is stable with respect to hydrolytic reactions but is easily oxidized to EU. Oxidation of ETU takes place primarily in biological systems and by photolytic reactions especially in the presence of photosensitizers, e.g. acetone, riboflavine and others (Cruickshank and Jarrow, 1973; Ross and Crosby, 1973).

2.4. Photooxidation

After ultraviolet irradiation of ETU on silica gel, Cruickshank and Jarrow (1973) found 9 secondary reaction products. 2-Imidazolidinone (ethyleneurea, EU), was identified as a main degradation product along with smaller amounts of 3-(2-imidazolin-2-yl)-2-imidazolidinethione (Jaffe's base).

Other secondary reaction products of the photooxidation of ETU are 2-imidazoline (Vonk, 1975) and glycine (Ross and Crosby, 1973) via the intermediate product 2,4-imidazolidinedione (hydantoin).

Photooxidation might be expected to be the major degradation reaction of ETU occurring as surface deposits on EBDC treated plants or in water. Excluding the catalytic effect of ultraviolet light and biological oxidation, ETU degradation either does not occur or does so slowly (Ross and Crosby, 1973).

2.5. Soil Metabolism

Kaufman and Fletcher (1973) observed a considerably slower ETU degradation in autoclaved soils than in nonsterile soils. Only ethylene urea was identified as a final product. In biologically active soils, ETU was oxidized to carbon dioxide and four other degradation products. Two were identified as hydantoin and Jaffe's base. Degradation of ETU to carbon dioxide in nonsterile soils was also reported by Lyman and Lacoste (1974).

The above results indicate that ETU is oxidized under both biological and nonbiological conditions to ethyleneurea. EU is considerably more stable than ETU and can be considered as a major metabolite. EU, however, is oxidized photochemically using a catalyst to give glycine and carbon dioxide (Ross and Crosby, 1973) and microbially in soil. In this context, Jaffe's base might be considered as an intermediate product in ETU degradation.

2.6. Plant Metabolism

After systemic uptake of ETU by plants, EU and 2-imidazoline were identified as metabolites (Vonk, 1975). Surface deposits of ETU which may occur as a result of EBDC-treatment form an additional unidentified substance as the main metabolite and ethylene diamine (Vogeler et al. 1976). This unknown metabolite was also observed by Vonk (1976) on zineb-treated lettuce plants. Propineb (methyl analog of zineb) as well as propylene thiourea also form an identical but unidentified major metabolite (Vogeler, 1976).

Nash (1976) reported the presence of 7-10 different degradation products in methanol extracts of soybeans after both soil or foliar treatment with EBDC as well as after treatment with ETU. In these cases EU was a degradation product.

2.7. Animal metabolism

The degradation products of ETU in animals are similar to those found in plants. Lyman (1971) detected EU, ethylene diamine, oxalic acid, glycine, and urea as major metabolites in cow urine. In addition, ^{14}C originating from ^{14}C-ETU was found in naturally occurring substances, i.e. in protein and lactose of milk. A summary of secondary reaction products in biological and non-biological systems is given in Figure 2.

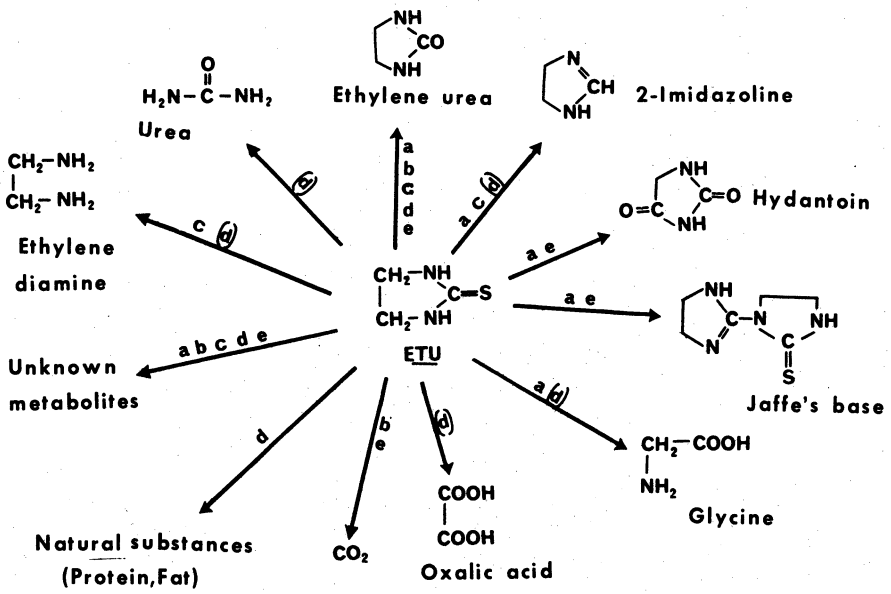


Fig. 2. Reaction products of ETU in biological and non-biological systems. (a= photodecomposition, b=chemical oxidation, c=plants, d=animals, and e= soil. Letters in parenthesis ( ) indicate proposed pathways.

3. DYNAMICS AND TRANSFORMATION OF ETU RESIDUES

The dynamic behavior of ETU residue in the environment is determined by three factors:

1. By the amount of ETU formed before the ethylenebisdithiocarbamate fungicides are applied.
2. By the rate of ETU formation as a result of dithiocarbamate degradation.
3. By the rate of ETU degradation.

Two different patterns of behavior of ETU residues have been observed; ETU residues, already present directly after application of EBDC fungicides, can either continuously decrease or as a result of EBDC degradation to ETU and further ETU metabolism, first increase and then decrease. Both patterns are described in literature, however, generalizations are not possible. The dynamics of residues are not only determined by the differing stability of the parent compounds, (Bontoyan and Looker, 1973), but also by manifold environmental conditions (temperature, moisture, air, catalysts, biological systems and so on). The stability of the parent compound is determined by the degree of polymerization, its salt characteristics and by impurities present in the technical formulation.

3.1. Water and Soil

Ethylenebisdithiocarbamates, either in suspension or dissolved in water, are relatively rapidly decomposed. According to Lyman and Lacoste (1974) the half-life of mancozeb is less

than 1 day as a suspension of 20 ppm in sterile water. ETU and EU are formed as degradation products. Values on the amounts of ETU formed under these conditions are not available. In contrast, under actual use conditions, mancozeb in a spray tank slurry (0.45 kg/380 l) was relatively stable. The ETU concentration increased only slightly within 4 hours, from 2.08 ppm to 3.62 ppm. As the degradation of EBDC fungicides is accelerated by oxidation (Hylin, 1973), the high stability of the parent compounds in concentrated suspensions might be due to the exhaustion of oxygen dissolved in water. In contrast, the rapid degradation of the EBDC fungicides in the environment may be due to the prevailing aerobic conditions.

Ross and Crosby (1973) have reported on ETU degradation in water. ETU is stable in deionized water in the absence of photosensitizers, but is rapidly oxidized in their presence. Several sensitizers were added at 10 ppm to a 25 ppm solution of ETU and exposed to sunlight. After 4 days with riboflavine as a sensitizer, the concentration of ETU was less than 5 per cent of that in the dark control. To minimize microbial degradation, the procedure was repeated after filtration and boiling the water samples, with the same results. Furthermore, ETU degradation was investigated in several boiled samples of agricultural drainage water to which, before irradiation, had been added 0.5 mg ETU per liter (Table 2).

TABLE 2. Photodecomposition of ETU in agricultural waters (Ross and Crosby, 1973)

Source	Irradiation	% ETU remaining
Irrigation ditch (sugar beet)	3 days, lamp	10-20
	3 days, dark	100
Paddy flooding ditch (rice)	24 days, sun	25-50
	24 days, dark	100
Paddy (rice)	24 days, sun	10-25
	24 days, dark	100

Numerous samples of natural water were collected from rivers, lakes, and agricultural areas and almost without exception, were found to degrade ETU to ethyleneurea in sunlight. The same samples degraded ETU in the dark but only after prior exposure to sunlight, indicating that rather stable photooxidants had been generated. The substances responsible for ETU oxidation were isolated and identified as tryptophane and tyrosine (Ross 1974); the pure amino acids also caused conversion of ETU to ethyleneurea in light, apparently by their ability to form hydroperoxides or other strong oxidants (Ross and Crosby 1976). As both the amino acids and photosensitizers such as acetone, riboflavine, and chlorophyll are known to occur worldwide in water and soil, and this photolysis also has been shown to take place rapidly on a silica surface (Cruickshank and Jarrow 1973), the degradation of ETU to harmless products in the field seem entirely plausible.

Various investigators have examined the persistence of EBDC's and ETU in soils. ETU sprayed on soil surfaces (Immokalee fine sand soil type) did not degrade as rapidly as mancozeb. ETU could still be detected at 68 ppm 13 days after application, but only a trace could be found after 27 days. Mancozeb was detected up to 6 days after application, however, no ETU was detected (Blazquez, 1973). According to Lyman and Lacoste (1974) the half-life of ETU (10 ppm) and mancozeb in Hagerstown silt loam soil was, at least 22 days and about 90 days, respectively. Normal microbial CO_2 production was not disturbed at these concentrations. Because these values were determined on the basis of $^{14}CO_2$-formation from ^{14}C-ETU, they might be too high. The formation of $^{14}CO2$ did not parallel the disappearance of ETU from the soil. According to Kaufman and Fletcher (1973), ETU is oxidized to EU, whereas whereas CO_2 was formed slowly. In Hagerstown silt loam 2 and 20 ppm ETU were entirely converted into ethylene urea within 2 days and 200 ppm ETU within 8 days. In contrast, four days after treatment of soil with 2, 20, and 200 ppm ETU, only 43.4, 8.9, and 0.9 per cent, respectively, was degraded to CO_2. A slow but constant conversion of ETU into EU was also found in autoclaved soil, whereas the formation of CO_2 was only observed in nonsterile soils (Kaufman and Fletcher, 1973: Lyman and Lacoste, 1974).

Ethyleneurea and propyleneurea, respectively, were found to be the main metabolites in soils, besides small amounts of CO_2, from ^{14}C-zineb and ^{14}C-propineb in laboratory tests (Vogeler, 1976). ETU and propylenethiourea (PTU) also form ethyleneurea and propyleneurea, respectively, and CO_2. The parent thioureas were not detectable within the duration of the experiment (21 days). EU was found to be an intermediate in the degradation of ETU to CO_2. These results show that the possibility of ETU accumulation in soils, as a result of application of EBDC's can be excluded under normal practical conditions.

3.2. Residues in Animals

Orally administered polymeric water-insoluble EBDC fungicides are poorly absorbed through the

gastrointestinal tract by animals. Seidler et al. (1970) found, after a single dose of labeled maneb to rats (360 mg per kg body weight), about 35 percent of the applied label in the urine of the test animals within 5 days. After administration of 100 mg of mancozeb per kg rat, Lyman (1971) found only about 15 percent of the applied dose in the urine of the test animals, but 71 percent (47 percent as unchanged mancozeb) in the feces. Similar results were obtained with cows (Lyman, 1971).

The EBDC incorporated into animals is rapidly metabolized, with ETU observed as a main metabolite. From the above mentioned studies it cannot be determined to what extent the resorption of bisdithiocarbamates from the gastrointestinal tract takes place either as unchanged EBDC (for instance after the conversion into the water-soluble alkali salt, along with complex binding of the metal, Mn or Zn) or as previously formed degradation products. Engst and Schnaak (1975) found an unknown compound, which released CS_2 after acid hydrolysis in slaughter products from pigs, given 500 ppm maneb with their feed.

Lyman (1971) reported the occurrence of dithiocarbamates in various organisms as well as of secondary reaction products of dithiocarbamates in fat after application of mancozeb to cows. The residues disappeared very quickly, approximately within 3 days, from all parts of the body, including the fatty tissue. ETU as a metabolite seems to be distributed rather uniformly in all animals tissues. After administration of radioactively labeled ETU, a significant accumulation of radioactivity of ETU was only observed in the thyroid gland (Lyman, 1971; Iverson and Newsome, 1976).

The elimination of ETU from the organism takes place by two principle routes:

1. excretion (urine, feces, milk)
2. metabolism

After application of 20 mg ETU per kg to rats and guinea-pigs, 50 percent of the unchanged ETU were excreted with the urine within 24 hours (Newsome, 1974). Lyman (1971) found in cows, given 1 ppm ^{14}C ETU with their feed, a significantly smaller quantity of unchanged ETU in the urine as well as in the milk of the test animals. However, high levels of ^{14}C were detectable in metabolites appearing partly as naturally occurring materials (i.e., glycine, urea, lactose), (Table 3).

TABLE 3. ^{14}C activity in the milk and urine of cows fed with 1 ppm of ^{14}C-ethylenethiourea (Lyman, 1971)

Material	Conc. (ppm)	Milk % of total ^{14}C	Conc. (ppm)	Urine % of total ^{14}C
Ethylenethiourea	0.011	31	0.12	7
Ethyleneurea	0.0025	8	0.27	18
Ethylenediamine	-	-	0.14	14
Glycine	-	-	-	6
Oxalic acid	-	-	-	12
Urea	-	-	-	11
Fat	-	3	-	-
Protein	-	18	-	-
Lactose	-	16	-	-
Totals (per cent)		76		68

From these results it can be concluded that a rapid elimination of EBDC's and ETU from the animal organism is to be expected.

3.3. Residues in Crops

Many workers have shown that ETU residues occur in crops. Thus Sato and Tomizawa (1960) identified ETU in extracts from zineb treated cucumbers. Engst et al. (1968) reported that field tomatoes, shortly after treatment with zineb, had levels of 0.05 ppm of ETU which disappeared within a few days. Vonk (1976) also noted the presence of ETU residues immediately after treatment of lettuce with ^{14}C-zineb. The ^{14}C-ETU residues were found only on the surface of the lettuce. The variety of products formed from the ^{14}C-zineb over a 21 day interval is given in Figure 3.

Experiments with propineb on apples and grapes carried out by Vogeler et al. (1976) showed analogous degradation pathways. Propylenethiourea was detected shortly after treatment and was rapidly transformed to an unknown metabolite together with small amounts of propyleneurea, and other unidentified reaction products.

Unlike the parent EBDC's, ETU is taken up by the plant root systems, translocated and metabolized (Vonk and Kaars Sijpesteijn, 1970; Vonk, 1975; Nash, 1976; Hoagland and Frear, 1976).

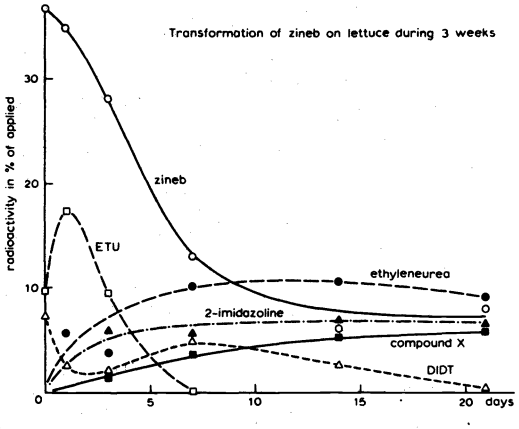


Fig. 3. Degradation of [^{14}C]-zineb into identified metabolites on lettuce plants as a function of time.

From the preceding work it may be concluded that since EBDC's are contact fungicides, ETU residues occur primarily at plant surfaces. These surface residues would then be expected to be degraded photochemically (Cruickshank and Jarrow, 1973; Ross and Crosby, 1973; Hoagland and Frear, 1976).

Residue levels of parent EBDC's, ETU, EDI and ETM (DIDT) on field tomatoes treated with 4 different EBDC formulations were reported by Newsome (1976). All formulations were applied 7 times at the maximum recommended rates at 7 day intervals. The results obtained are given in Tables 4 - 6 and demonstrate that levels of ETU (Table 6) are well below 0.1 ppm despite relatively high levels of parent EBDC's, especially in the case of the manganese-containing products. Similar levels of ETU have been reported from supervised field trials with apples (Wood, 1976), and on spinach, carrots and tomatoes (Phillips, 1976).

TABLE 4. Residues[1] of ethylenebisdithiocarbamate on tomatoes at various intervals after application by spraying (Newsome, 1976)

Elapsed Time (days)	Mancozeb	Manzate D	Polyram 80-W	Zineb 75W
0	8.96 ± 0.50	13.0 ± 1.89	3.22 ± 0.13	4.78 ± 0.13
1	10.9 ± 0.56	9.40 ± 0.92	3.45 ± 0.28	3.68 ± 0.26
2	9.23 ± 0.60	8.31 ± 0.87	3.58 ± 0.32	4.00 ± 0.17
3	9.15 ± 0.40	6.26 ± 0.58	2.54 ± 0.10	5.24 ± 0.12
6	4.08 ± 0.32	3.08 ± 0.28	1.43 ± 0.11	1.50 ± 0.06
10	3.92 ± 0.13	3.82 ± 0.17	1.30 ± 0.09	1.83 ± 0.15
14	3.29 ± 0.17	3.20 ± 0.13	0.778 ± 0.04	1.27 ± 0.06
Control	0.141 ± 0.014			

[1]Values (in ppm) are the means ± S. E. of 6 samples and are expressed as zineb.

TABLE 5. Residues[1] of ethylenethiuram monosulfide and ethylenebis(isothiocyanate) on tomatoes treated with ethylenebisdithiocarbamates in the field (Newsome, 1976).

Elapsed Time (days)	Mancozeb	Manzate D	Polyram 80-W	Zineb 75W
	Ethylenethiuram Monosulfide Found (ppm)			
0	0.019 ± 0.009	0.066 ± 0.018	0.008 ± 0.002	0.009 ± 0.002
1	0.032 ± 0.003	0.041 ± 0.005	0.020 ± 0.004	0.008 ± 0.001
2	0.035 ± 0.001	0.025 ± 0.001	0.012 ± 0.002	0.014 ± 0.004
3	0.017 ± 0.001	0.027 ± 0.001	0.007 ± 0.002	0.003 ± 0.001
6	0.019 ± 0.001	0.020 ± 0.001	na	na
Control	0.004 ± 0.002			

TABLE 5. Continued

	Ethylenebis(isothiocyanate) Found (ppm)			
0	0.009 ± 0.002	0.011 ± 0.001	0.007 ± 0.0	0.002 ± 0
1	0.016 ± 0.002	0.016 ± 0.003	0.006 ± 0.001	0.003 ± 0.001
2	0.013 ± 0.001	0.015 ± 0.001	0.002 ± 0.001	0
3	0.014 ± 0.002	0.013 ± 0.001	0.005 ± 0.001	0
6	0.003 ± 0.001	0.016 ± 0.001	na	na
Control	0.002 ± 0			

[1]Values are the means ± S.E. of 6 samples
na - not analyzed

TABLE 6. Effect of cooking on ethylenethiourea residues in tomatoes sprayed with ethylenebis(dithiocarbamates) (Newsome, 1976)

Time After Spraying (days)	Mancozeb M-45	Manzate D	Polyram 80-W	Zineb 75W
		Uncooked		
0	0.036 ± 0.004	0.033 ± 0.004	0.025 ± 0.005	0.033 ± 0.005
1	0.065 ± 0.015	0.018 ± 0.002	0.085 ± 0.015	0.024 ± 0.003
3	0.036 ± 0.009	0.026 ± 0.003	0.006 ± 0.003	0.007 ± 0.001
6	0.017 ± 0.004	0.015 ± 0.001	0.039 ± 0.004	0.006 ± 0.003
14	0.011 ± 0.001	0.008 ± 0.001	0.009 ± 0.002	0.011 ± 0.006
Control	0.001 ± 0.001			
		Boiled 10 min.		
0	1.14 ± 0.080	1.11 ± 0.138	0.217 ± 0.026	0.772 ± 0.144
1	1.33 ± 0.134	1.42 ± 0.162	0.754 ± 0.162	0.950 ± 0.117
3	1.08 ± 0.140	0.945 ± 0.173	0.584 ± 0.052	0.535 ± 0.080
6	1.03 ± 0.127	0.462 ± 0.051	0.174 ± 0.016	0.206 ± 0.005
14	0.935 ± 0.142	0.519 ± 0.049	0.184 ± 0.015	0.110 ± 0.007
Control	0.020 ± 0.002			

A study of the ETU levels in the Canadian food supply in 1972 was described by Pecka et al. (1976). Of the 167 samples collected only 33% of them contained detectable levels of ETU (>0.01 ppm), most of these being at the level of 0.02 ppm or less. The highest residues were found in canned spinach (0.047 ppm) and orange peel (0.083 ppm).

4. PROCESSING AND COOKING EFFECTS ON EBDC'S

Relatively little information is available in the literature regarding the formation of ETU during the processing of foods. Nevertheless the facile formation of ETU from EBDC's is now well established.

Pfeifer and Flora (1976) showed that ETU was formed by the thermal decomposition of zineb. Work by Farrow and Ralls (1970) established the disappearance of zineb, ziram and maneb residues during the normal canning operations for spinach and apricots. Following on from this work many reports appeared in the literature establishing the ready conversion of EBDC's to ETU. Thus, Blazquez (1973) showed that heated extracts containing maneb, zineb or mancozeb residues resulted in formation of ETU. Similar results were obtained by Newsome and Laver (1973) and Watts et al. (1974). Recent work by Newsome (1976) has shown that heating homogenates of field treated tomatoes resulted in a 38-48% (molar basis) conversion of EBDC's to ETU (Table 6).

Work with greenhouse tomatoes by Von Stryk (1976) with recommended rates of mancozeb application resulted in mancozeb residues ranging from 3.2-4.7 ppm. A marked reduction to 0.4-0.8 ppm was observed following mechanical brushing. Tomatoes fortified with 2 ppm of mancozeb and fried resulted in a 45% conversion to ETU (0.33 ppm-molar basis).

Work by Wood (1976) has established the levels of ETU formed from residues of mancozeb and Polyram 80 W present on apples processed for the manufacture of apple juice, sauce and pomace. The results are given in Table 7 and show that over 3 times as much ETU is present in pomace (0.17 ppm) as is present in juice (0.05 ppm). What is surprising is the high level of

unchanged mancozeb in the pomace (14.9 ppm) despite heat treatment for 15 hours at 150°C.

TABLE 7. Residues (ppm) of EBDC's/ETU on apples and apple products (Wood, 1976)

Apples	Mancozeb	ETU	Metiran (80 W)	ETU
Before last application	4.2	0.05	0.90	0.03
After " "	12.8	0.05	12.50	0.17
2 days after	11.6	0.06	13.20	0.14
7 " "	11.1	0.05	7.00	0.07
14 " "	6.6	0.06	3.30	0.07
28 " "	3.1	0.03	1.20	0.03
42 " "	1.7	0.01	0.50	0.01
Apple juice	ND	0.05	ND	0.05
Apple pomace	14.90	0.17	3.30	0.15
Apple sauce	0.09	0.05	0.09	0.04

Four precover sprays of 2 lb/100 gls followed by 5 cover sprays of 1.5 lb/100 gls
Apples ground and pressed at 3000 psig. Racked, filtered, and heated to 93°C for 2 min and canned
Residue from juice extraction dried at 150°C for 15 hours
Apples peeled, cored into 2% saline solution, diced, drained. Heated to slow boil (100°C) and held for 5 min. Canned and heated in boiling water for 15 min.

A study sponsored by the U.S. Environmental Protection Agency (Phillips, 1976) to examine the effects of food processing on EBDC residues has confirmed and extended the results previously described. Washing the raw agricultural produce removed from 33 to 87% of the EBDC residue and the majority of the ETU residue prior to processing. An interesting result was that while almost instantaneous conversion of mancozeb to ETU took place in boiling water, the field weathered residue of the same product appeared to be more resistent to degradation to ETU. A summary of the results for raw and processed material is given in Table 8. These results were obtained using the recommended spray schedule. Additional unreported data using four times the recommended rate were also carried out with essentially the same results except for higher residue levels.

TABLE 8. Summary of EBDC - ETU residues (ppm) before and after processing

	Eastern USA		Western USA	
Tomato	EBDC	ETU	EBDC	ETU
Unwashed	0.3	--	2.1	0.01
Washed	0.2	--	0.6	0.01
Canned	--	0.03	0.5	0.11
Carrots				
Unwashed	0.6	--	0.1	0.01
Washed	0.3	--	0.1	0.01
Diced	0.1	--	0.1	--
Frozen	--	--	--	--
Canned	--	0.03	0.1	--
Spinach				
Unwashed	2.4	--	61.9	0.34
Washed	1.5	--	9.7	0.02
Frozen	0.1	0.04	0.6	0.50
Canned	--	0.18	0.1	0.71

Mancozeb was applied at the rate of 0.7 ai/0.5 ha in all cases. Spray schedules were as follows: spinach, 1 treatment with 10 day pre-harvest interval; carrot-eastern, 6 treatments at 7-10 day intervals, pre-harvest interval 7 days. Tomato-eastern, 4 treatments at 7-10 day intervals, pre-harvest interval 16 days. Tomato-western, 3 treatments at seven day intervals, pre-harvest interval 5 days.

A survey of foods from the Ottawa and Montreal areas of Canada was carried out using 50 processed and 50 fresh samples cooked before analysis by heating for 10 minutes in water under reflux. In general, the study established the relatively low levels of ETU present in cooked foods with the notable exception of spinach. The results are summarized in Table 9.

TABLE 9. ETU content of cooked foods sampled from Ottawa and Montreal

Commodity		Positive samples	Mean of positive samples (ppm)	Range of positive samples (ppm)
Grapes	Fresh[1]	1/10	0.012	-
	Processed	0/10	-	-
Apples	Fresh[1]	2/10	0.019	0.015 - 0.023
	Processed	3/10	0.015	0.011 - 0.018
Tomatoes	Fresh[1]	6/10	0.032	0.014 - 0.036
	Processed	7/10	0.032	0.012 - 0.060
Beans	Fresh[1,2]	5/10	0.042	0.021 - 0.104
	Processed	0/10	-	-
Spinach	Fresh[1]	8/10	1.62	0.014 - 5.84
	Processed	5/10	0.391	0.223 - 0.672

[1]Values after cooking fresh samples.
[2]Three frozen samples were substituted for fresh, one of which was positive.

CONCLUSIONS

1. ETU occurs as a primary reaction product of the EBDC fungicides.

2. ETU is present in commercial formulations in varying amounts (0.02 - 2%). The amount increases on storage under warm and humid conditions.

3. Environmental degradation (metabolism in plants, soils, animals and water) of the EBDC fungicides also leads to ETU formation.

4. There is no evidence for the persistence or bioaccumulation of ETU residues in plants, soils or water, although ETU is detected as a metabolite and accumulates in the thyroids of animals.

5. Currently, there are several adequate methods for the determination of ETU residues, however, there are no simple procedures that are universially applicable.

6. Monitoring data confirm the frequent presence of EBDC residues in or on raw agricultural crops treated using good agricultural practices. Generally, residues of EBDC do not exceed nationally recommended tolerances 1 - 7 ppm.

7. A substantial portion of the EBDC residue may be removed from the raw agricultural crop following a simple washing procedure. These procedures remove approximately from 30 - 90% of the residue.

8. Monitoring data confirmed the presence of ETU residues in or on certain raw agricultural crops. Generally, these residues were less than 0.1 ppm, most approaching the lower limits of analytical detection (0.01 ppm).

9. ETU is found in most heat processed foods, where EBDC residues were found prior to processing. Heat processing has been shown to convert from 16 to 23% (weight basis) of the EBDC residues to ETU. Consequently, the concentration of ETU may be higher in processed foods than in the raw agricultural products.

10. Current chemical data do not preclude the continued use of EBDC fungicides in good agricultural practices.

RECOMMENDATIONS

1. That the acceptable average daily intake of ETU and EBDC should be calculated immediately.

2. Analytical methodology for ETU and ETU forming compounds should be simplified to expediate monitoring programs. Simple, rapid and specific methods are required. An international collaboration study should be initiated.

3. Toxicological significance of residues reported in monitoring data should be determined by competent international bodies.

4. Further studies of the degradation and metabolism of EBDC and ETU in crops and animals should be conducted.

5. Further data on the conversion of EBDC residues to ETU in various food processing procedures should be developed. Studies to minimize the formation of ETU during food processing should be initiated.

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