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# Manganese Ethylenebisdithiocarbamate (Maneb) and Ethylenethiourea (ETU) Residues in Different Parts of Tomato Plant and Soil

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*ABSTRACT*

*Tomato plants, grown in greenhouse and field conditions, were sprayed with $^{14}C$-Maneb. $^{14}C$-Maneb and $^{14}C$-ETU were extracted from the plant by Soxhlet extraction, separated by thin-layer chromatography and quantified by liquid scintillation counting. Rapid decomposition of Maneb was observed during the first week. 0·03–0·06 ppm ETU were formed from about 4 mg Maneb applied per plant during this period. The highest content of Maneb was found on the leaves of the plant and the lowest in the fruit. The highest content of $^{14}C$-ETU was detected in the stem and the lowest in the fruit.*

## INTRODUCTION

Ethylenebisdithiocarbamates (EBDC) are widely used fungicides for the control of a variety of fungus diseases in plants. Ethylenethiourea (ETU), one of the metabolites of EBDC fungicides, has been reported to be goitrogenic (O'Neil & Marshall, 1984), carcinogenic, teratogenic and mutagenic in experimental animals (Teramoto *et al.*, 1975; Larsson *et al.*, 1976). Antithyroid properties of ETU have also been reported (Rose *et al.*, 1980).

Reports on amounts of ETU residues have been variable; high ETU levels in plants have been detected in tropical and subtropical countries where EBDCs were used (Ross & Crosby, 1973). Trace amounts of ETU have been reported on EBDC-sprayed crops (Yip *et al.*, 1971; Newsome, 1976; Nash,



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1976; Nitz, 1984). The reports on the persistence of ETU in plants and soil are controversial (Blazquez, 1973; Ankumah & Marshall, 1984).

Manganese ethylenebisdithiocarbamate (Maneb) which is used in this work, is an unstable chemical. The oxygen in the air and humidity increase its instability (Bontoyan & Looker, 1973). The purpose of this study was to measure and compare the ETU levels formed from Maneb in different parts of tomato plants grown in greenhouse and field conditions. The amount of Maneb and ETU in soil has also been determined.

## MATERIALS AND METHODS

### Radiolabelled materials

Maneb-ethylene-$^{14}$C (10·59 mCi/mmol) was obtained from Isinta, Budapest. ETU-ethylene-$^{14}$C (20 mCi/mmol) was received from the International Atomic Energy Agency, Vienna. ETU-free Maneb and $^{14}$C-Maneb were prepared according to the first and second FAO/IAEA meeting protocol (FAO/IAEA, 1987). These compounds were checked for ETU by TLC at one month intervals.

### Application to the plant

Technical Maneb (Dikotan M-22 80% Maneb) was obtained from a local supplier. 5 mg technical Maneb + 10 $\mu$Ci $^{14}$C-Maneb was sprayed with an atomizer onto each plant in a plastic tent. Three applications were made in one week intervals. The plants were harvested before and after applications and kept at $-20$°C until analyzed.

### ETU analysis

ETU was determined radiochemically on TLC plates by procedures similar to Yip *et al.* (1971) and Onley (1977). Ten grams of each plant were blended in 100 ml methanol at high speed for 2 min. Maneb and ETU were extracted into methanol by Soxhlet extraction, carried out for 20 h. After the extraction, the extract was evaporated to 5 ml in a vacuum at 35°C. The solution was filtered through a filter paper and 0·1 ml was taken for the total $^{14}$C-counting in a Packard Liquid Scintillation Counter (Model 3380).

An aliquot of each extract (0·1 ml) was applied to TLC plates (250 $\mu$m silicagel, 60-F-254). $^{14}$C-ETU standard, cold ETU standard and $^{14}$C-Maneb standard technical Maneb were run on the plates at the same time. The solvent system used was butanol–ethanol–water (120:33:57 v/v). One-

centimetre of the chromatograms were scraped off and added to a scintillation vial containing 10 ml Dioxin-based scintillation cocktail. Visualization of ETU was achieved using nitroprusside reagent.

Undecomposed Maneb was determined by $CS_2$ analysis (Keppel, 1969).

## RESULTS

TLC analysis showed ETU standard with $R_f$ 0·58–0·66 (Table 1). ETU was not detected in ETU-free prepared Maneb and $^{14}$C-Maneb. Cold ETU standard was visualized by nitroprusside reagent. In plant samples, 14 days after treatment with Maneb, ETU could be visualized. An unidentified metabolite of Maneb with a lower $R_f$ (0·38) has also been detected. Under these TLC conditions Maneb remained at the origin.

$CS_2$ analysis of undecomposed Maneb in tomato plant leaves in greenhouse and natural conditions is given in Table 2. Table 3 shows the

**TABLE 1**
Thin-Layer Chromatography Method for the Determination of Ethylenethiourea

| *Compound* | *TLC conditions* | *$R_f$ in chromatographic system* | *Nitroprusside visualization reagent* |
|---|---|---|---|
| Ethylenethiourea | Silica gel; butanol:ethanol:water 120:33:57 | 0·58–0·66 | blue spot |
| Maneb (ETU free) | 120:33:57 | not detected | no spot |
| Plant sample | 120:33:57 | 0·63 | blue spot |

**TABLE 2**
$CS_2$ Analysis of Undecomposed Maneb + $^{14}$C-Maneb in Tomato Leaves Grown in Greenhouse and Natural Condition[a]

| *Days after the first application* | *Greenhouse (ppm Maneb)* | *Natural (ppm Maneb)* |
|---|---|---|
| 0 | 101 | 100 |
| 7 | 63 | 60 |
| 14 | 197 | 200 |
| 35 | 136 | 116 |
| Total | 497 | 476 |

[a] Control plant harvested immediately after application (0). Three applications at 1-week intervals.

**TABLE 3**
Total Maneb, $^{14}$C-Maneb and $^{14}$C-ETU Analysis of Different Parts of Tomato Plant and Soil in 36-Day Samples Grown in Natural Conditions

| *Part of the tomato plant* | *Total Maneb (ppm)* | *$^{14}$C-Maneb μCi/g plant (wet weight)* | *$^{14}$C-ETU (ppm)* |
|---|---|---|---|
| Leaves | 352 | 1·354 | 0·022 8 |
| Stem | 140 | 2·061 | 0·114 0 |
| Fruits | 0·117 | 0·573 | 0·010 0 |
| Roots | 0·156 | 0·512 | 0·020 0 |
| Soil | 0·396 | 1·142 | 0·007 7 |
| Total | 492 | 5·642 | 0·174 5 |

distribution of Maneb and ETU on different parts of tomato plant. Thirty-six-day samples grown in natural conditions were taken for this analysis. The highest contents of Maneb were detected on the leaves of the plant and the lowest in the fruit. The highest ETU content was in the stem and lowest in the fruit.

## DISCUSSION

There are several reports on the occurrence of ETU residues from EBDC fungicides (Vonk & Sijpesteijn, 1970; Blazquez, 1973; Ross & Crosby, 1973; Onley, 1977). In view of the extensive use of this fungicide on plant foods and that ETU exhibits higher toxicity than the active parent fungicide, we wanted to clarify the residue data further on tomato plants in greenhouse and natural conditions. The procedure for the extraction of ETU and Maneb from the plant was convenient. Both recoveries of ETU and Maneb were above 90%. Thin-layer analysis shows the ETU standard with $R_f$ 0·58–0·66 (Table 1). ETU $R_f$ has been reported to vary within the range 0·48–0·63 (Vonk & Sijpesteijn, 1970). In this region the ETU free standard Maneb and $^{14}$C-Maneb did not give significant amounts of radioactivity but, in the same region, second week samples gave a blue spot (ETU). However, there seems to be another metabolite of Maneb in the sample with a lower $R_f$ (0·38).

The radiochemical residue analysis method used in this study was similar to that used by other workers working with EBDC residue analysis (Onley, 1977; Rhodes, 1977; Ankumah & Marshall, 1984). The only point of dissimilarity was the rapid drying of the plates with cold air and immediate developing in order to prevent loss of $^{14}$C-ETU.

In the present study we have shown that Maneb was partly decomposed to

ETU and some other metabolite. In greenhouse plants the decomposition of Maneb was fast during the first week (38%). However, in natural conditions the decomposition of Maneb is faster during the third week. These values are obtained from the $CS_2$ analysis of Maneb after the intial applied dose of 4 mg per each plant, at one week intervals. No other weekly application of Maneb had been performed. It should be remembered that new leaves, formed during the three weeks of the experiment, could not originally be sprayed, a fact that provides a complication in interpretation. Our first week result was not in agreement with Newsome *et al.* (1975). Newsome found 35% decomposition of Maneb in 14 days after the original application since he observed a fast decomposition of Maneb on tomato plant during the first week in field conditions; exterior factors such as moisture, temperature, atmospheric humidity and organic acids excreted by the plant influence the decomposition of Maneb (Engst & Schnaak, 1974).

The analyses of Maneb and ETU on different parts of tomato plant show uneven distribution. The highest content of Maneb was found on the leaves of the plant and the lowest in the fruit after 36 days from the application of Maneb. This was in agreement with results obtained by Newsome *et al.* (1975).

Some of the ETU formed is sorbed into the plant from the superficial deposit and is transported within the plant; for this reason we determined ETU in different parts of the plant (Table 3). The highest content of $^{14}$C-ETU was detected in the stem and the lowest in the fruit. The low concentration of ETU in the leaves agrees well with its reported ability to be translocated from stem to fruit (Vonk & Sijpesteijn, 1970). The amount of ETU found in leaves and fruit (0·01–0·22 ppm) was acceptably low, being within the range determined by the Pesticide Analysis Advisory Committee, Dithiocarbamate Panel.

No significant quantities of Maneb or $^{14}$C-ETU were detected in soil.

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