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Examination of the *In Vivo* Metabolism of Maneb and Zineb to Ethylenethiourea (ETU) in Mice

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The magnesium (maneb) and zinc (zineb) salts of ethylenebisdithiocarbamate constitute two of the most important fungicides currently used in agricultural practice. These compounds have a low order of acute toxicity. The major toxicological concern with these compounds is the breakdown product ethylenethiourea (ETU). ETU produces thyroid tumors in rats (ULLAND et al. 1972, GRAHAM et al. 1975) and liver tumors in mice (INNES et al. 1969). ETU is also teratogenic (KHERA 1973), and goitrogenic (SEIFTER and EHRICH 1948, GRAHAM and HANSEN 1972). A second major breakdown product is ethylenebisdiisothiocyanato sulfide (EBIS) (TRUHAUT et al. 1973; MARSHALL 1977, VONK and SIJPESTEIJN 1976, NEWSOME 1976). This compound is also referred to as ethylenethiuram monosulfide (ETM). No chronic studies have been carried out with EBIS. A sub-acute study (FREUNDENTHAL et al. 1977) has revealed a reversible paralysis of the hind legs and a measurable effect on thyroid function at 1000 ppm EBIS in the diet. Additional studies have shown that EBIS causes an inhibition of rat and mouse hepatic monooxygenase activity and a reduction in hepatic cytochrome P-450 concentrations when administered in vivo (YOSHIDA et al., in press). Also, EBIS inhibits hepatic delta-aminolevulinic acid synthetase activity when administered to mice (YOSHIDA and NEAL, in press). The purpose of the present study was to examine the metabolism of maneb and zineb to ETU and EBIS in the mouse in vivo.

METHODS

[^{14}C] Ethylenethiourea (ETU) was synthesized by the method of ALLEN et al (1955) using 1,2-[^{14}C] ethylenediamine. [^{14}C] Ethylenebisdiisothiocyanato sulfide (EBIS) was prepared as described by THORN and LUDWIG (1962) using [^{14}C] nabam. The radiochemical purity of the final products was >99% as determined by thin layer chromatography on silica gel using the solvent systems $CHCl_3$: n-butanol : methanol : water (100 : 5 : 2: to saturation) and $CHCl_3$: ethylacetate (1:1). [^{14}C] disodium ethylenebisdithiocarbamate (nabam) was prepared as described by SELLING et al. (1974). [^{14}C] magnesium ethylenebisdithiocarbamate (maneb) and [^{14}C] zinc ethylenebisdithiocarbamate (zineb) were synthesized from [^{14}C] nabam as described by SEIDLER et al. (1970). The [^{14}C] maneb and [^{14}C] zineb were extracted twice with methanol followed by two extractions with dry chloroform just prior to administration to the mice. No EBIS,

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ETU, EU or other products which migrated from the origin in the $CHCl_3$:n-butanol:methanol:water (100:5:2:to saturation) solvent system could be detected in the product resulting from these chloroform or methanol extractions.

Adult male ND/4(S)BR mice (Harlan, Ind. Inc., Indianapolis, Ind.) weighing approximately 25-30 g. were used in these studies. They were allowed access to food and water ad libitum throughout these studies. The mice were administered the labeled compounds p.o. either dissolved in 0.15 ml of water (ETU) or suspended in 0.15 ml olive oil (EBIS, maneb, zineb) and placed in an all glass metabolism apparatus which allowed for the separate collection of urine, feces and expired CO_2. The urine was collected over dry ice. The feces were collected every 24 hours and stored at -70° until assayed for radioactivity. Expired $[^{14}C]CO_2$ was trapped in freshly prepared 5N NaOH and an aliquot examined for radioactivity by scintillation counting.

The pooled 0-24 and 24-48 hour urines were analyzed for radioactive products of $[^{14}C]$ ETU, EBIS, maneb and zineb using thin-layer chromatography and the solvent systems $CHCl_3$:n-butanol:methanol:water (100:5:3:0.5) (CZEGLEDI-JANKO 1967) and $CHCl_3$:ethanol (1:1). In this procedure 2 mg each of unlabeled EBIS, ETU and ethyleneurea (EU) were dissolved in 1 ml of the urine containing the radiolabeled products and 50 µl of the mixture spotted on silica gel thin-layer plates (0.25 mm thickness). The plates were developed using the solvent systems described above. EBIS and ETU were detected on the developed chromatogram using iodine vapor. EU was detected using a 1 percent solution of 4-methylaminobenzaldehyde in ethanol (Ehrlich's reagent). The silica gel in the areas of the thin-layer plates corresponding to EBIS, ETU and EU were scraped from the plates and the radioactivity quantitated by scintillation counting.

RESULTS

Table 1 shows data from representative experiments examining the urinary and fecal excretion of radioactivity from $[^{14}C]$ ETU, EBIS, maneb and zineb administered in vivo to adult male mice. The total recovery of administered radioactivity in urine and feces was less in the mice receiving 0.05 mmole as compared to 0.25 mmole/kg ETU. Also, the total recovery of radioactivity from $[^{14}C]$ EBIS and zineb was less than with ETU and maneb. A smaller percentage of the radioactivity from maneb and zineb was excreted in the urine than was the case for ETU and EBIS. None of the administered $[^{14}C]$ ETU, EBIS, maneb or zineb was excreted as $[^{14}C]$ CO_2.

Table 2 shows the percentage of the radioactivity in the pooled 0-24 and 24-48 hr urines described in Table 1 which is present as ETU, ethyleneurea (EU), compounds more polar than EU (polar products) and less polar than ETU (other). Approximately half of the radioactivity in the urine following administration of $[^{14}C]$ ETU was present as unchanged ETU. This represented about 24 and 18%, respectively, of the orally administered dose of 0.25 and 0.05 mmol/kg $[^{14}C]$ ETU. Approximately 12% of radioactivity in the urine

TABLE 1

Excretion of radioactivity from [^{14}C] ETU, EBIS, Maneb or Zineb orally administered to adult male mice[a]

Compound (Dose, mmole/kg)	Recovery of Administered Radioactivity[b] (%)	Total Excretion of Radioactivity[c] 0-24 hr (%)	24-48 hr (%)	Excretion in 48 hours[c] Feces (%)	Urine (%)
ETU (0.25)	100.6	96.8	3.8	53.3	46.7
ETU (0.05)	71.7	92.3	6.6	51.8	48.1
EBIS (0.25)	54.0	96.9	3.1	26.0	74.0
EBIS (0.05)	53.5	95.5	4.5	60.3	39.7
Maneb (0.25)	90.6	83.9	16.1	91.0	9.0
Maneb (0.05)	80.4	68.5	31.5	92.7	7.3
Zineb (0.25)	65.1	96.5	3.5	90.4	9.6

[a] [^{14}C] ETU, dissolved in distilled water (0.15 ml), or [^{14}C] EBIS, Maneb or Zineb suspended in olive oil (0.15 ml), were administered by stomach tube to three adult male mice and the animals placed in a common metabolic chamber which allowed for the separate collection of expired CO_2, urine and feces. The animals remained in the chamber for 48 hrs. They were allowed excess to food and water ad libitum during this period.

[b] Calculated on the basis of the administered radioactivity.

[c] Calculated on the basis of total radioactivity recovered in the urine and feces.

TABLE 2

Identity of the radioactive compounds excreted in the urine of adult male mice administered [^{14}C] ETU, EBIS, Maneb or Zineb[a]

Compound (Dose, mmole/kg	% of Radioactivity in Urine Present as:			
	ETU	EU[b]	Polar Products[c]	Other[d]
ETU (0.25)	52.3	12.1	36.7	0.5
ETU (0.05)	50.5	11.5	37.4	0.5
EBIS (0.25)	10.8	10.7	76.6	1.9
EBIS 0.05)	0	0.3	99.7	0
Maneb (0.25)	15.8	4.0	77.8	2.4
Maneb (0.05)	7.8	6.8	82.4	3.0
Zineb (0.25)	15.2	1.0	81.0	2.7

[a] The pooled 0-24 hr and 24-48 hr urines from the experiments described in Table 1 were examined for products as described in METHODS.

[b] Ethyleneurea

[c] Compounds more polar than ethyleneurea (EU)

[d] Compounds less polar than ETU

following administration of either dosage of [^{14}C] ETU was present as EU with the remainder being unidentified polar products. EU is a non-enzymatic (RHODES 1977) and, perhaps, enzymatic product of ETU degradation. No unchanged EBIS could be detected in the urine of mice following administration of [^{14}C] EBIS. Only at the high dose of EBIS (0.25 mmol/kg) were significant amounts of ETU and EU seen in the urine. The majority of the radioactivity in the urine following administration of [^{14}C] EBIS was present as unidentified polar products. Approximately 16% of the radioactivity in the urine following the oral dose of 0.25 mmol/kg [^{14}C] maneb was present as ETU. This represents about 1.3% of the administered dose of maneb or 0.1 µmol ETU. Following the 0.05 mmol/kg dose of [^{14}C] maneb approximately 0.5% or 0.007 µmol of the radioactivity was present in the urine as ETU. Following the 0.25 mmol/kg dose of [^{14}C] zineb 1.0% or 0.07 µmol of the radioactivity was present in the urine as ETU. The majority of the radioactivity in the urine following the administration of the maneb and zineb was present as unidentified polar products. No EBIS was detected in the urine of the mice administered maneb or zineb.

DISCUSSION

In a previous study (SEIDLER 1970) [^{14}C] maneb (390 mg/kg) was administered to rats and the excretion of radioactivity in the urine and feces followed for five days. A total of 55 percent of the administered dose was excreted in that time with the majority (49.5 percent) being excreted within the first 24 hours. In contrast to the results of the studies reported here, the majority of the radioactivity was excreted in the urine (64 percent) as compared to feces (36 percent). In another study (LYMAN 1971), [^{14}C] dithane (20 mg per animal) was administered by stomach tube to rats daily for seven days. Urine and feces were collected and analyzed for recovery of radioactivity. In this experiment approximately 90 percent of the administered radioactivity was recovered in the urine and feces with the majority (70 percent) being recovered from the feces. [^{14}C] dithane was also administered orally in various doses to cows (LYMAN 1971). Depending on the dose, from 86 to 101 percent of the administered dose was recovered in the urine and feces. Again, the majority of the radioactivity was recovered from the feces.

The excretion of radioactivity in the urine of rats (NEWSOME 1974, KATO et al. 1976, RUDDICK et al. 1976) and guinea pigs (NEWSOME, 1974) receiving [^{14}C] ETU has also been reported. In rats, 61 percent (NEWSOME 1974), 80 percent (KATO et al. 1976) and 73 percent (RUDDICK et al. 1976) of the radioactivity from [^{14}C] ETU was excreted in the urine during the first 24 hours following oral administration. In the guinea pig approximately 47 percent was excreted in the urine during the first 24 hours (NEWSOME 1974). These latter results are similar to those reported here in mice.

In vivo studies in cows have shown that ETU is metabolized to EU and ethylenediamine (LYMAN 1971). *In vivo* studies in rats

have also suggested that ETU is converted to EU in that species (KATO et al. 1976, RUDDICK et al. 1976). These animal studies as well as chemical studies of the breakdown of ETU in soils, on plants and under aqueous conditions (RHODES 1977) indicate that EU is formed by the desulfuration of ETU. The results of the present study indicate that ETU is metabolized in the mouse to EU and products which are more polar than EU. Thus, in contrast to results reported in rats (RUDDICK 1976, IVERSON et al. 1977), ETU is rather extensively metabolized in mice. The extensive metabolism of ETU in mice as compared to rats is of interest in light of the fact that ETU causes hepatic tumors in mice (INNES et al. 1969) but not in rats (GRAHAM et al. 1975).

In concert with the results of VONK and SIJPESTEIJN (1976) using microorganisms and animal and plant enzyme systems, EBIS appears to be metabolized by the mouse to ETU. EBIS is also extensively degraded to "polar" compounds. It is not known whether these "polar" compounds result from a further breakdown of ETU formed from EBIS or represent an alternate pathway for degradation of EBIS. EBIS is quite stable in aqueous solution under non-reducing conditions. Under reducing conditions it is converted almost exclusively to ETU and CS_2 (VONK and SIJPESTEIJN 1976). Therefore, it is possible these "polar" products represent a further breakdown of ETU formed from EBIS. No EBIS itself was detected in the urine of the mice administered [^{14}C] EBIS. Metabolism of EBIS to a number of products, including ETU, has also been reported in rats (IVERSON et al. 1977).

[^{14}C] maneb is metabolized in rats to ETU, EBIS, ethylenediamine and other unidentified products (SEIDLER et al. 1970). The metabolism of [^{14}C] zineb to CS_2, sulfur, ETU and EBIS in rats has also been reported (TRUHAUT et al. 1973). Although only a small percentage of the orally-administered doses of [^{14}C] maneb and [^{14}C] zineb are excreted in the urine of mice (Table 1), 15 percent or more of the radioactivity in the urine is represented by ETU or a metabolite of ETU (EU). The major unknown is what percentage of the "polar" products in the urine of the mice receiving [^{14}C] maneb and zineb represent further breakdown products of ETU formed from maneb or zineb or are products formed by alternate pathways of breakdown of maneb and zineb which do not lead to the formation of ETU. Without knowledge of the structures of these "polar" products it is not possible to speculate in this regard. It is possible that the ETU and EU detected in the urine of the mice administered [^{14}C] maneb and [^{14}C] zineb may have been present as impurities in the administered maneb or zineb. However, this is not likely since these samples of maneb and zineb were extracted with dry chloroform and dry methanol prior to administration to the mice. Thin-layer chromatography of the extracted maneb and zineb indicated that no ETU or EU were present. Thus it appears from these studies that a small percentage of maneb and zineb orally administered to mice is converted to ETU. The ETU is, in part, further metabolized and the ETU and its metabolic products are excreted in the urine, and, perhaps, feces.

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