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Effect of Zineb and Its Metabolite, Ethylenethiourea, on Hepatic Microsomal Systems in Rats and Mice

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Ethylene bisdithiocarbamate fungicides (EBDC) are widely used in agriculture for food crops. Zineb (Zinc ethylene bisdithiocarbamate) belongs to eight compounds of EBDC group registered in Italy for use on all cereals, various fruits and vegetables as well as for treatment of many seeds. The significance of Zineb as a residue of agricultural products is enhanced by reports on toxicity of its degradation products such as ethylenethiourea (ETU). ETU is present in commercial formulations of EBDC, increases during their environmental degradation and during heat processing of food containing EBDC residues. Marked differences between rats and mice in acute toxicity and in teratogenicity after a single dose of ETU have been observed (Ruddick and Khera 1975; Khera and Tryphonas 1977; Khera 1984). In all these studies the rat appeared to be more susceptible to its toxic effects than the mouse. The question arises whether different responses of the xenobiotic metabolizing systems are responsible for these species differences. A four-week administration of Zineb in the diet (Albrecht et al 1975, Pélissier et al 1981) as well as its single oral dose (Miladi et al 1981) caused a decrease in the activity of hepatic microsomal mixed function oxidases in rats. To our knowledge the effects of a single dose of orally administered Zineb on liver microsomal metabolizing systems in mice have not yet been investigated. In the present paper comparative data regarding such effects induced by Zineb and by ETU in rats and mice are reported.

MATERIALS AND METHODS

Commercial preparations of Zineb, i.e., those actually present as residues on agricultural products (purity 89,5%, containing 0.07% of ETU) were obtained from Farmoplant (Milano, Italy). ETU of analytical grade (98%) was purchased from Fluka (Buchs, Switzerland).

Adult male albino Swiss mice having body weights in the range 24-27 g, and Wistar male rats in the range 200-250 g, purchased from Charles River Italia (Calco, Como) were used. They were allowed free access to water and standard pelleted food. Zineb

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(suspended in arachis oil) and ETU (dissolved in water) were administered by gastric lavage in doses from 50 to 400 mg/kg, and of 100 and 200 mg/kg body weight, respectively. Control animals received the vehicle. The animals were killed 24 hr after treatment. In time-course experiments the animals received a single dose of Zineb (200 mg/kg body weight) 2, 18, 24 and 48 hr before sacrifice. Livers were immediately removed and 12.5% (w/v) homogenates prepared with 0.01 M phosphate buffer containing 1.15% KCl, pH 7.4, using a Potter-Elvehjem homogenizer with teflon pestle. The homogenates were centrifuged at 9,000 g for 20 min at 4°C and the supernatants used for enzymatic assays. Aniline hydroxylase was measured by formation of p-aminophenol, and aminopyrine-N-demethylase by production of formaldehyde according to the methods of Mazel (1971). In some experiments the supernatants were centrifuged at 100,000 g for 60 min to obtain the microsome fraction. Cytochrome P-450 was estimated by the carbon monoxide difference spectrum of dithionite reduced microsomes (Mazel 1971). The proteins were determined by the method of Lowry et al (1951) using bovine serum albumin as standard. Statistical analyses were performed by ANOVA and Duncan's multiple range test.

RESULTS AND DISCUSSION

Table 1. Effects of Zineb on hepatic mixed function oxidase system in rats and mice.

Dose mg/kg	Aniline hydroxylase nmol p-aminophenol formed/ min/g liver		Aminopyrine-N-demethylase nmol HCHO formed/ min/g liver	
	Rats	Mice	Rats	Mice
0	27 ± 2.6	50 ± 3.6	248 ± 11	359 ± 25
50	25 ± 1.8	50 ± 3.8	198 ± 11	336 ± 16
100	23 ± 2.2	57 ± 3.0	200 ± 18	248 ± 17 *
200	20 ± 1.9 *	57 ± 2.4	162 ± 10 *	260 ± 20 *
400	21 ± 2.0 *	60 ± 3.2 *	143 ± 18 *	211 ± 20 *

Animals received single oral doses of Zineb and were killed 24 hr after treatment. Values are expressed as mean ± SE from eight animals in each group. Microsomal proteins in control animals were of 21.8 ± 0.4 and 18.7 ± 1.1 mg/g of liver, respectively, for the rat and the mouse. Protein contents were not significantly affected by Zineb treatment.

* Significantly different from controls treated with the vehicle ($p<0.05$, Duncan's multiple range test).

Table 2. Time course of hepatic mixed function oxidase system and cytochrome P-450 content after Zineb in rats and mice.

Interval hr	Aniline hydroxylase nmol p-aminophenol formed/ min/g liver		Aminopyrine-N-demethylase nmol HCHO formed/ min/g liver		Cytochrome P-450 nmol/mg prot	
	Rats	Mice	Rats	Mice	Rats	Mice
0	27 ± 2.6	50 ± 3.6	248 ± 11	359 ± 25	1.31 ± 0.09	0.74 ± 0.03
2	28 ± 1.3	48 ± 2.1	266 ± 12	349 ± 26	1.27 ± 0.07	0.77 ± 0.05
18	29 ± 2.9	51 ± 3.9	180 ± 18*	254 ± 25*	1.30 ± 0.05	0.72 ± 0.06
24	20 ± 1.9*	57 ± 2.4	162 ± 10*	260 ± 20*	1.25 ± 0.10	0.71 ± 0.04
48	29 ± 1.8	52 ± 3.0	250 ± 25	328 ± 30	1.28 ± 0.09	0.73 ± 0.03

Animals received single dose of Zineb (200 mg/kg) and were killed after interval indicated. Values are expressed as mean ± SE from eight animals in each group.
*Significantly different from controls treated with the vehicle ($p<0.05$ Duncan's multiple range test).

As shown in Table 1 oral administration of single doses of Zineb 24 hr after treatment caused a depression of aniline hydroxylase in rats and its slight increase in mice. These effects reached the level of statistical significance at doses of 200-400 mg/kg body weight. Moreover, Zineb caused a depression of aminopyrine-N-demethylase, which at a dose of 200 mg/kg body weight was of about 30-35% both in rats and mice. Although the extent of decrease was not linearly dose-dependent, in rats reached almost 43% at the maximal dose and appeared more pronounced than that of aniline hydroxylase. A time course experiment (Table 2), using a dose of 200 mg/kg body weight indicated that the effects were absent at 2 hr, reached maximum at 24 hr, and disappeared 48 hr after treatment. The levels of cytochrome P-450 at all intervals were not modified by Zineb in rats nor in mice.

Table 3. Effects of ETU on hepatic mixed function oxidase system in rats and mice.

Dose mg/kg	Aniline hydroxylase nmol p-aminophenol formed/ min/g liver		Aminopyrine-N-demethylase nmol HCHO formed/ min/g liver	
	Rats	Mice	Rats	Mice
0	25 ± 1.8	55 ± 3.0	253 ± 8	331 ± 32
100	27 ± 2.5	89 ± 7.5*	176 ± 10*	316 ± 35
200	26 ± 1.9	120 ± 18.0*	135 ± 15*	296 ± 20

Animals received single oral doses of ETU and were killed 24 hr after treatment. Values are expressed as mean ± SE from eight animals in each group.
*Significantly different from controls treated with the vehicle ($p < 0.05$, Duncan's multiple range test)

A partial transformation of Zineb into ETU has been demonstrated both in rats (Camoni et al 1984) and in mice (Jordan and Neal 1979). Therefore, it appeared of interest to assess whether or not the described effects of Zineb on xenobiotic metabolizing systems depended on this metabolite. Table 3 shows that ETU treatment in rats caused a dose-dependent decrease of aminopyrine-N- demethylase (at 200 mg/kg of 47%) and did not modify this activity in mice. On the other hand ETU did not affect aniline hydroxylase activity in rats and caused a more than 2-fold increase in mice. Such an increase has been recently shown in this laboratory to depend on de novo protein biosynthesis with β-naphtoflavone like mechanisms of induction (Meneguz and Michalek, 1986). These effects of ETU on the two enzymatic activities (except the lack of depression of aniline hydroxylase in rats) appear similar to those

described for the first time by Lewerenz and Plass (1984) after a 3-day oral ETU treatment of the two species. Therefore the data support the recent suggestion of these authors that qualitatively different responses of hepatic microsomal enzymes may be at least partially responsible for the differences in acute toxicity and teratogenicity demonstrated in rats and mice.

In conclusion, the present study indicates that single oral administration of Zineb , in spite of its poor absorption (Blackwell-Smith 1953, Jordan and Neal 1979) is sufficient to induce transient dose-dependent modifications of xenobiotic metabolizing systems in both rats and mice. Depression of aminopyrine N-demethylase in rats, and the stimulatory effect on aniline hydroxylase in mice are also observed after ETU treatment. Therefore, it appears likely that these effects of Zineb may be related to its transformation into this metabolite. On the other hand the depression of aniline hydroxylase in rats and of aminopyrine N-demethylase in mice are observed after Zineb, but not after ETU, treatment. Therefore these effects of Zineb may depend on the fungicide itself or on its transformation into other metabolites such as ethylenebisdiisocyanato sulfide (EBIS) known to reduce aniline hydroxylase in rats (Yoshida et al 1978). It is of interest that Borin et al recently (1985) showed in experiments in vitro that not only ETU and EBIS but also Zineb itself at high concentrations (2.5×10^{-4} - 2.5×10^{-3} M) had a significant inhibitory effect on aniline and aminopyrine metabolism in rat microsomes.

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