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Teratogenicity of maneb and some of its mixture. I- maternal, Body weight and General parameter of pregnant mice.

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ABSTRACT

Technical (T) and formulated (F) maneb as well as two of its mixtures namely brestan-60: mancozeb: fentin and zinc acetates were tested on pregnant mice from the 9th - 15th day of gestation. Technical maneb and fentin acetate were more effective in reduction of maternal weight gain(MWG)than F.maneb and brestan-60. However fentin acetate was more effective than T.maneb in this respect.Also.mancozeb and zinc acetate were effective and significantly increased maternal body weight gain at all the tested doses. However the effect of these compounds was in the following order: fentin acetate > brestan 60 > T.maneb > F.maneb. On the other hand the tested compounds were also effective on the general maternal parameters (GMP) and the effects were in the following order:. brestan 60 > fentin acetate> F.maneb > T.maneb. It was obvious that brestan 60 and fentin acetate were more effective on MWG and GMP than maneb .The tested compounds showed appreciable effects on the tested liver alkaline phosphatase activity (LAP) and their effects were in the following order: mancozeb = zinc acetate > brestan 60 > F.maneb > fentin acetate > T.maneb. Thus mancozeb and zinc acetate were more effective on LAP than maneb and brestan-60.

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INTRODUCTION

The dithiocarbamate compounds form the most important group among fungicides and are used extensively as biocides in agriculture. Most of these are foliage fungicides while some are used for seed and soil treatments.For reasons and others this group of fungicides has attracted the attention of many investigators to study their toxicological effects. However,ethylene bis-dithiocarbamates were found in our food chain as residual amounts and they appear to be a hazard in our environment at the present time.Thus much attention is being given to their side effects e.g. teratogenic effects of ethylene bis-dithiocarbamates and related compounds as ethylene bis dithiocarbamate which was found to be a potent teratogen in rats(Khera 1973).

Teramoto et.al(1981)and Short et.al(1980) found that zinc ethylene bis-dithiocarbamate decreased body weight and food consumption in rats.Nakaur et al(1984) observed that zineb at 250 mg/kg decreased maternal body weight gain and food consumption on the rats were markedly suppressed,the same dose had no effects evidence teratogenicity in mice.Otherwise methyl mercury reduced maternal weight and increased offspring preweaning mortality at the higher dose(Vorhees,1985).

In addition maternal and fetal toxicity of dinocap was detected in rat, golden hamster and adult.The developmental(A/D)Toxicity ratio were compared with previously established A/D ratio of dinocap in the gestation days 1-20 (0,100,150 or 200 mg/kg/day).In rat maternal extrauterine weight gain was affected at 150 and 200 mg/kg/day. Relative liver weight was elevated at 100 mg/kg/day and above,fetal weight was lower at 150 and 200 mg/kg.In hamster maternal extrauterine weight was lower

at 12.5 mg/kg/day and above fetal weight was reduced. (Rogers et.al 1988).

Mirkova and Georgiev (1975) indicated that single teratogenic doses at 250,500 and 1000 mg/kg of basfumgin as well as non teratogenic doses 62.5 and 125 mg/kg administered prenatally to pregnant rat. decreased the liver alkaline phosphatase and acid phosphatase activities in fetuses.

A correlation was established between placental weights and maternal circulating placental alkaline phosphatase (Adeniyi and Olatun-bosun 1984).The purpose of the present study was to determine the acute toxicity and the effects of maneb and its mixture on the maternal body weight of mice and also to find out their effects on the activity of the liver alkaline phosphatase in vivo.

Materials and Methods.

I - **Compound tested .**
- Maneb formulated : (80%) and technical (99%).
- Brestan 60 : (62% maneb + 18% Fentin acetate).
- Fentin acetate : (Technical brestan)
- Zinc acetate :

II - **Animals used .**
The mated females were randomly assigned to experimental group (each group was five pregnant females). Doses levels were (10,20,40,80,100 mg/kg) of each tested compounds.

III- **Daily dose administration .**
The compounds were dissolved in corn oil and given by oral administration , five pregnents femals were used as control.

IV -**Maternal weight gain (MWG).**
Body weight was measured daily during different stages of gestation : stage I (preimplantation stage) from the Ist-5th day. StageII (embryonic stage)from 6th - 15th day of gestation and stage III (organogensis stage) from 16th - 19th day of gestation(Komeil and Abd-Allah,1991).

V -**General maternal parameters (GMP).**
Included females having hydrocephlus. early and late resorption. uterine growth retardation and aborted females.

VI -**Liver alkaline phosphatase (LAP).**
Alkaline phosphatase was determined in liver homogenat according to(Bessey et al 1946 and Komeil et al 1991).

VII -**Statistical analysis.**
All the data obtained were subjected to the statistical analysis according to(Steel and Torrie 1981).

RESULTS AND DISCUSSION

The data indicated that all tested compounds are of moderate acute toxicity than the technical one. This might be attributed to the masking effect of the solid carrier Also.the brestan 60 behaved similarly to maneb in this respect. The tested levels for teratogenisis were in the range between one tenth (oil) to one hundredth (oil) of the LD50 for rat levels (800; 1020. 780. 1600 and 1000 mg/kg) for the tested fungicides.

Effects of daily doses administration on (MWG) are presented in (Table I). There are significant differences among the three gestational stages.i.e.stages II and III showed significant increases in body weight gain compared with stage I with all the tested

Table (1) : Effect of tested compounds on maternal weight gain at different stages of gestation.

FUNGICIDES	MATERNAL WEIGHT GAIN (gm)		
	Stage 1	Stage 2	Stage 3
Control . A*	0.90±0.52^{a}	4.61±1.80^{bcd}	7.93±0.50^{bc}
Control . B**	0.91±0.40^{a}	3.90±1.20^{cd}	7.13±0.57^{cd}
Technical Maneb	1.42±0.21^{a}	3.51±0.60^{abc}	5.58±1.17
Formulated Maneb	1.03±0.17^{a}	4.79±0.37^{d}	7.31±1.3^{bc}
Technical Brestan	1.35±0.27^{a}	2.62±0.45^{cd}	3.85±1.^{a}
Formulated Brestane 60	1.06±0.24^{a}	3.57±0.54^{a}	4.69±1.76^{d}
Mancozeb	1.42±0.10^{a}	6.51±0.23^{ab}	10±2.6±0.31^{a}
Zinc Acetate	1.78±0.11^{a}	5.56±0.26	8.90±0.26^{ab}

Values having the same litters are not significant different from each other.

L.S D. for (fungicides stages) = 1.88
P,05

* corn oil only.

** corn oil + acetone.

Table (3) Teratogenic effect of tested fungicides administration on pregnant mice GMP

Fungicides	Number of females mated	Number of pregnant at necropsy	Number of females aborted		Number of females having uterine growth retardation	Number of females having hydrocephlus	Number of females showing resorption	
			Early	Late			Early	Late
Corn oil	5	5	0	0	0	0	0	0
Corn oil+acetone	5	5	0	0	0	0	1	0
Technical maneb	25	25	1	2	0	2	5	6
Formulated maneb	25	25	0	0	5	0	13*	6
Technical brestan	25	25	5	0	5	6	6	8*
Formulated brestan 60	25	25	4	5	9*	0	12*	2
Mancozeb	25	25	0	0	0	0	0	0
Zinc acetate	25	25	0	0	0	0	0	0

Values marked with asterisks are significant different from the control values.
· Chi- squar test (* $p < 0.05$)

Table (3) Effect of tested fungicides on liver alkaline phosphatase activity in vivo. (LAP)

FUNGICIDES	DOSES mg/kg	SPECIFIC ACTIVITY (u moles (P.N.P.)/ mg protein/min.		ACTIVITY (% of control value)
Technical maneb	Cont. B	17.73	b	
	10	34.40	c	194.02
	20	18.00	b	101.52
	40	17.50	b	98.70
	80	17.35	b	97.86
	100	15.59	a	87.93
Formulated maneb	Cont. B	17.73	c	
	10	24.10	c	135.93
	20	22.22	d	125.32
	40	15.40	b	86.86
	80	14.97	b	84.43
	100	13.67	a	77.10
Technical brestan	Cont. B	17.73	abc	
	10	23.43	d	132.15
	20	20.65	cd	116.47
	40	13.63	a	76.88
	80	18.43	bc	103.96
	100	15.55	ab	87.70
Formulated brestan 80	Cont. B	17.73	b	
	10	19.37	c	109.25
	20	18.57	b	104.74
	40	15.30	b	86.29
	80	13.00	a	73.32
Zinc acetate	Cont. B	17.73	f	
	10	10.80	e	60.91
	20	7.07	b	39.88
	40	4.33	b	24.42
	80	3.63	a	20.47
	100	5.73	b	32.32
Mancozeb	Cont.B	17.73	e	
	10	4.97	d	28.03
	20	2.43	b	13.71
	40	2.83	b	15.96
	80	1.80	a	5.64
	100	3.90	c	21.20

L.S.D. for treatment means of (Technical maneb)=0.51
0.05

L.S.D. for treatment means of (formulated maneb)=0.93
0.05

L.S.D. for treatment means of(technical brestan 60)=3.5
0.05

L.S.D. for treatment means of(formulated brestan 60)=1.5

L.S.D. fortreatment means of(mancozeb) =0.63
0.05

L.S.D. for treatment means of(zinc acetate) =0.32

Cont. B = Corn oil +acetone

compounds.These findings are in contrast with those of Petrova - vergieva and Ivanova-chemishanska(1973)they found that maneb did not reduce maternal body weight of rats at 500 mg/kg but it did so to 50 at 4000 mg/kg. Maternal weight gain that was caused by the tested compounds could be arranged in the following order:T. brestan > brestan 60 > T. maneb > F.maneb.On the other hand mancozeb as well as zinc acetate increased significantly the maternal body weight gain compared with control.This finding speculated that effect of fentin acetate may be attributed to the presence of the triphenyltin acetate in the brestan 60.Also the additive materials in the formulatd maneb or brestan 60 may decrease the effects of the formulated compound on the maternal weight gain.

The effect of the tested compounds on (GMP) is summarized in(Table 2) and which can be arranged as follows:brestan 60 > fentin acetate > F. maneb > T.maneb.

In addition the effect of tested compounds on LAP activty can be arranged as follows:mancozeb = zinc acetate > brestan 60 > F. maneb > fentin acetate > T. maneb. as shown in (Table 3).

These results proved that maneb and formulated breston 60 decreased the enzyme activity, but this decrease was lower than that caused by mancozeb and zinc acetate. However these two compounds did not cause any teratogenic effect.These findings are in agreement with Mirkova and Georgiev (1975) who reported that basfumgin as a fungicide decreased the alkaline phosphatase activity. and also with Mirkova (1976) who reported that some fungicides decreased the activity of placental alkaline phosphatase.

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تاثير جزيئ المانيب وبعض خلائطه على الاجنه
١- وزن الامهات والياساتها العامه
لمعرفة التاثير على الفئران الصغيره

استخدم المانيب بصورتين النقيه و التجاريه وخلائطه الاخرى فى صور البرستان ٦٠ و المانكوزيب وكذلك خلات الفنتين و الزنك وتم اختبارها على حيوانات التجارب فى اليوم ٩ - ١٥ من الحمل. و أوضحت النتائج ان المانيب النقى وخلات الفنتين لهما تاثيرا واضحا فى خفض اوزان الامهات مقارنه بالمانيب التجارى و البرستان ٦٠ علما بان تاثير خلات الفنتين كان اكثر من المانيب التجارى. ومن ناحيه اخرى اوضح المانكوزيب وخلات الزنك تاثيرا فى زيادة الوزن الكلى للجسم. ويمكن ترتيب تاثير المركبات المستخدمه كما يلى:-
برستان ٦٠ > خلات الفنتين > المانيب التجارى > المانيب النقى.
ومن ناحيه اخرى اوضح كل من برستان ٦٠ وخلات الفنتين تاثير على وزن الامهات الحوامل والياساتها العامه مقارنته بجزيئ المانيب نفسه.
وعلى ذلك كان للمركبات تاثيرا متفاوتا على نقاط التزيم الفوسفاتيز فى الكبد و الذى يمكن ترتيبه كما يلى:-
المانكوزيب = خلات الزنك > البرستان ٦٠ > المانيب التجارى > خلات الفنتين. > المانيب النقى.
ومن ناحيه اخرى وجد ان المانكوزيب وخلات الزنك لهما تاثيرا كبيرا على نقاط الاتزيم مقارنته بجزيئ المانيب و البرستان ٦٠.
وعلى ذلك يمكن القول ان المانيب التجارى اقل المركبات المختبره تاثيرا على الكيمياويات التى تعملها البحث مما يجعله مامونا فى الاستخدام الحقلى ضد الفطريات.