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INTERACTION OF DITHANE M-45 (MANCOZEB) AND LEAD ACETATE DURING A TERATOGENICITY TEST IN RATS

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The teratogenic effects of lead acetate (Trial 1) and the possible teratogenic effect of this compound administered in combination with a fungicide containing 80% mancozeb (Trial 2) were studied in rats. The test substances were administered by gavage on Days 6–15 of gestation. In Trial 1, five groups were treated with lead acetate administered at doses of 0.1, 0.5, 1.0, 10.0 and 1000.0 mg/kg body weight (bwkg), respectively. In Trial 2, lead acetate was applied at doses of 0.1, 10.0 and 1000.0 mg/bwkg, respectively. In the latter case the dose of the pesticide was 750 mg/bwkg in all treated groups. Lead acetate was not teratogenic after a single administration. Combined administration of lead acetate and mancozeb gave rise to the following toxic effects: average maternal weight decreased during pregnancy, the ratio of live fetuses decreased after the two lowest doses, and fetal mortality increased in the lowest and in the highest dose groups. The ratio of fetal resorption was higher in all the treated groups than in the control group. A significant decrease occurred in average fetal and placental weight in each treated group as compared to the control. Maternal toxicity was expressed in paralysis of the hindlimbs in the two lowest dose groups. Maternal mortality was between 16.7 and 23.3% at the three dose levels. Phocomelia and hernia cerebri occurred as characteristic fetal developmental anomalies in all the treated groups. It is concluded that the joint administration of lead acetate and a mancozeb-containing fungicide can cause maternal toxicity, embryotoxicity and characteristic teratogenic effects.

Key words: Lead acetate, mancozeb, teratology, rat

Increasing environmental pollution is accompanied by a growing number of human diseases. Chemical substances present in high concentrations can cause poisoning with severe clinical consequences and the harmful effects exerted by the long-term presence of low concentrations of pollutants or by combined exposures are especially difficult to measure.

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The use of lead, its environmental impact and poisonous effects have been known for 8000 years, for as long as the age of human civilisation (Manuwald, 1989; Poór and Mituszova, 1989). Lead pollution arising from different sources still poses serious environmental and health problems (Kákosy and Soós, 1995).

As both large- and small-scale farms often apply a mancozeb-containing fungicidal pesticide (Dithane M-45) for plant protection, it seemed reasonable to study the joint effects of lead and that pesticide in a teratological test. First a teratogenicity study of lead acetate was carried out, as publications available on that topic were of limited number and sometimes contradictory.

The possibility of a teratogenic effect of lead on both experimental an i-mals and humans is excluded by numerous publications (Wilson, 1973; WHO, 1977); however, lead is known to cross the placenta (Carpenter, 1974; Hapke, 1975; Barlow and Sullivan, 1982). Human exposure to lead increases the inc i-dence of developmental anomalies (Fergusson, 1991), and the teratogenic effect of lead has been established by other authors (Kákosy and Soós, 1995).

The ability of lead to pass through the placenta and cause birth defects at very high doses has been demonstrated in numerous animal experiments, but its teratogenic effect on humans was not known (WHO, 1977). Only a single publi-cation can be found about the results exerted by lead poisoning during pre g-nancy on children: nerve and muscle malfunction as well as retarded develo p-ment were described (WHO, 1977). The toxic effect of lead on the developing human fetus was reported by Klaasen (1996).

The dithiocarbamate fungicides maneb and zineb applied in a single oral dose of 1–4 g/kg and 2–8 g/kg, respectively, at days 11 or 13 of gestation caused fetal developmental anomalies (Petrova-Vergieva and Ivanova-Tchemishanska, 1973). The single oral administration of mancozeb on day 11 of gestation was teratogenic at the 1320 mg/kg but not at the 730 mg/kg dose (Larsson et al., 1976).

Based upon the available literature (Larsson et al., 1976), the applied dose of fungicide (750 mg/kg body weight (bwkg) Dithane M-45 = 600 mg/bwkg mancozeb) does not have teratogenic effects. The applied highest dose of lead acetate was 1000 mg/bwkg, which was equal to 20 percent of the acute Dosis Toxica Minima (DTM; Erdey-Grúz, 1963). In this study, a 10-day-long perma-nent administration period was used, in contrast with the above-cited publications where a single administration was used during pre gnancy.

The teratogenic effect of lead acetate, which was described in some publ i-cations, was not confirmed in our studies, as developmental anomalies observed in the embryos occurred sporadically and the results did not show a dose-response effect.

The possible fetotoxic effect of the combined administration of lead acetate and the dithiocarbamate fungicide Dithane M-45 was investigated in this study.

Material and methods

Trial 1

Test material. Lead (II)-acetate-3-hydrate (Reanal, Budapest).

Experimental animals. 16–27 pregnant Wistar rats, SPF (Human Company, Gödöllő).

Administration. Per os, by gavage on days 6–15 of gestation, once a day (OECD, 1981); final volume: 10 ml/bwkg.

Doses. Control (vehicle; distilled water): 0.0 mg/bwkg; lead acetate: 0.1, 0.5, 1.0, 10.0 and 1000.0 mg/bwkg, respectively.

Trial 2

Test materials. Lead (II)-acetate-3-hydrate (Reanal, Budapest); Dithane M-45 (80% mancozeb) (Rohm and Haas, US).

Experimental animals. 20–21 pregnant Wistar rats, SPF (Human Company, Gödöllő).

Administration. Per os, by gavage on days 6–15 of gestation, once a day (OECD, 1981); final volume: 20 ml/bwkg.

Doses. Control (vehicle: distilled water): 0.0 mg/bwkg. Lead acetate[a]: 0.1, 10.0 and 1000.0 mg/bwkg. Dithane M-45[b]: 750 mg/bwkg in all the treated groups. [a] = Acute per os Dosis Toxica Minima (DTM) = 5000 mg/bwkg (Erdey-Grúz, 1963). [b] = Acute per os LD_{50} on rats = 10,700 mg/bwkg (Szabadi, 1998). Mancozeb acute per os LD_{50} on rats = > 5000 mg/bwkg (The Pesticide Manual, 1994).

Trials 1-2

Food. Standard rodent food (Bioplan Ltd., Budapest), *ad libitum*.

Water. Tap water, *ad libitum*.

Environment. Room temperature: 21 °C ± 2 °C; relative humidity 60–70%; Lighting period: 12 hours light, 12 hours dark in a vivarium of controlled climate.

Microscopic evaluation. Wilson's free-hand razor blade dissection and Dawson's bone staining (Dawson, 1926; Wilson and Warkany, 1965; Hayes, 1986).

Biometric evaluation. Student's *t*-test (Finney, 1972).

Results

Trial 1

The copulation and fertility indices showed no difference from the control data in any of the treated groups, except that the latter index decreased in the group treated with the 10.0 mg/bwkg dose.

A statistically significant ($p < 0.001$) reduction in maternal body weight occurred in the 1.0 mg/bwkg dose group, while in the other three dose groups no decrease was found with respect to the control.

Numerical data of the litters are summarised in Table 1. Compared to the control group, the ratio of live fetuses markedly decreased in the 1000.0 g/bwkg dose group. Total resorption increased remarkably in this group as compared to the control and the other three dose groups.

A significant decrease in placental weight occurred in the 0.1 mg/bwkg dose group as compared to the control. After the administration of lead acetate in a dose of 1000.0 mg/bwkg, the postimplantation loss and the ratio of dead f etuses showed a marked increase.

Soft tissue and skeletal anomalies occurred sporadically in the control and treated groups. Only a single rat showed decreased movement activity, faintness, anorexia and ruffled fur in each of the 0.1 and 10.0 mg/bwkg dose groups.

Two pregnant animals died in the 0.1 mg/bwkg dose group. At necropsy, septicaemia arising from abscess formation was established as the cause of death in one rat. Haemorrhagic gastritis and enteritis with loss of body weight were the necropsy findings in the other case.

Gastritis and enteritis together with the presence of a small haematoma on the right thoracic wall were found at necropsy in a pregnant animal that died in the 10.0 mg/bwkg dose group.

Trial 2

The copulation and fertility indices were not different from the control in any of the treated groups. A statistically significant reduction was found in m aternal body weight from day 10 to the end of gestation in all treated groups with respect to the control.

A significant decrease occurred in maternal body weight gain during the entire period of pregnancy in the treated groups (I, II) as compared to the control (Table 2).

Total implantation rate in dose group II and the ratio of total live fetuses in dose groups I and II showed a marked decrease with respect to the control. The r atio of total live fetuses in dose group III showed a similar tendency. It is remar kable that the number of dead fetuses in dose groups I and III and the ratio of fetal resorption in dose groups I and II were definitely higher than in the control (Table 3).

The preimplantation loss was higher in each treated group than in the control, but only at the middle dose level (II) were these differences significantly important (Table 4). A marked increase occurred in the postimplantation loss in all treated groups. A significant decrease in placental weight occurred only in dose groups I and II. Mean fetal weight showed a similar tendency. The ratio of overall fetal mortality increased remarkably in each treated group as compared to the control (Table 4).

Table 1

Summary of litter data obtained in the teratogenicity test of lead acetate in rats

Parameter	Dose (mg/kg body weight)					
	0.0	0.1	0.5	1.0	10.0	1000.0
Corpora lutea	274	286	256	361	171	255
Total implantation	246 (99.6%)	248 (86.7%)	217 (84.8%)	322 (89.2%)	143 (83.6%)	216 (84.7%)
Total live fetuses	239 (97.2%)	247 (99.6%)	216 (99.6%)	320 (99.4%)	139 (97.2%)	95 (44.0%)
Dead fetuses	0.0	1.0 (0.4%)	1.0 (0.4%)	0.0	3.0 (2.1%)	0.0
Total resorption	7.0 (2.8%)	0.0	0.0	2.0 (1.4%)	1.0 (0.7%)	121.0 (56.0%)
Postimplantation loss (%)	2.8	0.4	0.5	0.6	2.8	56.0
Overall fetal death (%)	12.8	13.6	15.6	11.4	18.7	62.8

Table 2

Summary of maternal body weight data obtained in the teratogenicity test of lead acetate and Dithane M-45 in rats (x ± SD)

Gestation period	Dose (mg/kg body weight)			
	0.0	I	II	III
Day 0	233.0 ± 18.9	231.3 ± 19.2	234.7 ± 14.7	236.0 ± 14.4
Day 6	248.9 ± 17.3	257.1 ± 16.6	260.2 ± 19.5	260.5 ± 17.5
Day 10	266.8 ± 23.4	243.9^{c} ± 13.8	248.5^{a} ± 22.4	245.6^{b} ± 15.3
Day 15	278.3 ± 21.5	220.4^{c} ± 15.9	231.3^{c} ± 27.2	256.7^{b} ± 18.2
Day 20	340.5 ± 27.3	195.3^{c} ± 28.3	218.6^{c} ± 33.3	310.4^{b} ± 27.8
Gain in weight (Days 0–20)	107.5 ± 19.0	-35.2^{c} ± 23.3	-14.6^{c} ± 30.8	-78.9^{b} ± 25.6

[a] = $p < 0.05$; [b] = $p < 0.01$; [c] = $p < 0.001$; I = 0.1 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; II = 10.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; III = 1000.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45

Table 3

Summary of litter data obtained in the teratogenicity test of lead acetate and Dithane M-45 in rats

Parameter	Dose (mg/kg body weight)			
	0.0	I	II	III
Corpora lutea	274	186	210	170
Total implantation	246 (89.8%)	159 (85.5%)	160 (76.0%)	146 (85.9%)
Total live fetuses	239 (97.2%)	67 (42.1%)	105 (65.6%)	124 (85.0%)
Dead fetuses	0	26 (16.3%)	0	11 (7.5%)
Total resorption	7 (2.8%)	66 (41.6%)	55 (34.4%)	11 (7.5%)

I = 0.1 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; II = 10.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; III = 1000.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45

Table 4

Summary of litter data obtained in the teratogenicity test of lead acetate and Dithane M-45 in rats

Parameter	Dose (mg/kg body weight)			
	0.0	I	II	III
Preimplantation loss (%)	10.2	14.5	23.8	14.1
Postimplantation loss (%)	2.8	57.9	34.4	15.1
Placental weight (g)	0.730 ± 0.056	0.510[c] ± 0.120	0.561[c] ± 0.125	0.676 ± 0.121
Fetal weight (g)	3.410 ± 0.619	1.702[c] ± 0.400	2.155[c] ± 0.741	3.495 ± 0.812
General fetal death (%)	12.8	64.0	50.0	27.1

[c] = $p < 0.001$; I = 0.1 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; II = 10.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; III = 1000.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45

The incidence of soft tissue and skeletal anomalies is summarised in T ables 5–6.

Dams in the control group showed normal behaviour. Clinical signs i ncluding decreased active movement, hypothermia, paralysis, loss of weight, bloody tear and urine (haematuria) were detected on five animals each in dose groups I and II. One animal showed ruffled fur, apathy, and diminished muscle tension on the forelimbs at dose level III.

Table 5

Fetal alterations observed in free-hand sections in the teratogenicity test of lead acetate and Dithane M-45 in rats

Type of alteration	Dose (mg/kg body weight)							
	0.0		I		II		III	
	No.	%	No.	%	No.	%	No.	%
Dark liver	5	4.1	–	–	2	3.3	4	5.6
Dilated stomach	4	3.3	2	5.4	2	3.3	5	6.9
Haemoperitoneum	4	3.3	–	–	–	–	1	1.4
Dilated intestines	5	4.1	1	2.7	2	3.3	2	2.8
Haemopleura	3	2.5	–	–	1	1.6	–	–
Enlarged kidney	2	1.7	–	–	1	1.6	1	1.4
Dilated thoracic aorta	1	0.8	–	–	–	–	–	–
Thymic haemorrhage	1	0.8	1	2.7	–	–	1	1.4
Dilated renal pelvis	1	0.8	–	–	1	1.6	1	1.4
Renal papilla short	1	0.8	–	–	–	–	–	–
Oesophageal dilatation	–	–	1	2.7	1	1.6	2	2.8
Small kidney	–	–	–	–	–	–	1	1.4

I = 0.1 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; II = 10.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; III = 1000.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45

No deaths occurred in the control group during the experiments. Five animals died in dose group I. Necropsy findings included the presence of blood-containing urine in the urinary bladder, hyperaemia of the small intestinal m ucosa, in some cases a gas-filled colon, and in one case hyperaemia of the gastric mucosa and presence of blood in the stomach content.

Seven female rats died in dose group II; in one case misgavage was detected. At necropsy, presence of 0.5–1.0 ml clear liquid in the thoracic cavity, mucosal hyperaemia of the pregnant uterine horn, thickening of the uterine horn wall, reddish spots on the cut surface of the lungs, and hyperaemia of the small intestinal mucosa were detected in three animals.

Table 6

Incidence of skeletal malformations in rat fetuses after administration of lead acetate and Dithane M-45

Type of anomalies	Dose (mg/kg body weight)							
	0.0		I		II		III	
	No.	%	No.	%	No.	%	No.	%
Ossified centre of sternebrae < 6	33	28.0	20	69.0	39	70.9	–	–
13^{th} rib present unilaterally	1	0.8	–	–	–	–	–	–
Incomplete ossification of ileum	1	0.8	–	–	–	–	–	–
Rib adhesion	1	0.8	–	–	–	–	–	–
Incomplete ossification of skull bones	–	–	8	27.6	–	–	–	–
Incomplete ossification of thoracic vertebral centres	–	–	3	10.3	13	23.6	–	–
Incomplete ossification of metacarpus	–	–	7	24.1	33	60.0	–	–
Incomplete ossification of metatarsus	–	–	8	27.6	33	60.0	–	–
General incomplete ossification	–	–	4	13.8	–	–	–	–
10^{th} rib rudimentary	–	–	–	–	2	3.6	–	–
13^{th} rib rudimentary	–	–	–	–	3	5.5	–	–
$7–10^{th}$ ribs rudimentary	–	–	–	–	2	3.6	–	–
Sternum centres fused	–	–	–	–	3	5.5	–	–
Absence of lumbar vertebral arch	–	–	–	–	9	16.4	–	–

I = 0.1 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; II = 10.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; III = 1000.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45

Seven animals died in dose group III. Necropsy findings included the presence of 0.5–1.0 ml clear liquid in the thoracic cavity and hyperaemia of the mucosa of pregnant uterine horn.

At necropsy, developmental anomalies were detected in fetuses of six dams, cerebral hernia was found in 29 cases and phocomelia in 28 cases in dose group I.

In dose group II, phocomelia was found in 73 fetuses and cerebral hernia in 56 fetuses of 10 dams. The numerical data of fetal necropsy findings and maternal deaths are shown in Table 7.

Discussion

The toxic effects of lead on reproduction have been described in numerous publications on the basis of experimental observations and human clinical exper i-ence. The gametotoxic effects of lead on male and female rats were described by Stowe and Goyer (1971). In humans, suppression of spermatogenesis as a result of lead exposure was described by Assenato et al. (1986) and the toxic effects of lead on endocrine testicular function were reported by Rodamilans et al. (1988).

Table 7

Summary of maternal deaths and fetal necropsy findings in the teratogenicity test of lead acetate and Dithane M-45 in rats

Dose group	Maternal death		Fetal anomalies			
			Phocomelia		Cerebral hernia	
C	0/29	(0.0%)	0/239	(0.0%)	0/239	(0.0%)
I	5/30	(16.7%)	28/67	(41.8%)	29/67	(43.3%)
II	7/30[a]	(20.0%)	73/105	(69.5%)	56/105	(53.3%)
III	7/30	(23.3%)	35/124	(28.2%)	31/124	(25.0%)

[a] = Misgavage in one case; C = control; I = 0.1 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; II = 10.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45; III = 1000.0 mg/bwkg lead acetate + 750 mg/bwkg Dithane M-45

In mice experimentally treated with lead acetate, adverse effects on embryonic development (Jacket et al., 1975; Jacket, 1977), developmental disorders of the corpora lutea and reduced serum progesterone level (Jacket et al., 1977) were observed. Odenbro and Kihlström (1977) reported adverse effects of organic lead on gestation in mice.

In order to prevent the harmful effects of human exposure to lead, pregnant women and nursing mothers must not work in an environment where lead occurs at dangerous levels (Kertai, 1982).

Lead acetate did not cause developmental anomalies in rats in this teratogenicity study in rats. The developmental anomalies observed in embryos of the treated animals occurred sporadically. The results did not show a dose-response effect, and lead acetate was not found to be teratogenic in rats in the present teratogenicity test.

Repeated oral administration of mancozeb gives rise to neurotoxic effects. Mancozeb can produce such an effect at a high dose because of its slight absorption (WHO, 1988; Cs. László and Dura, 1989; Kékes-Szabó et al., 1990; Adamis-Borbély and Molnár, 1993). Paralysis of the hindlimbs was confirmed in this study.

The maternal deaths observed in the treated groups (I, II, III) can be attributed to the cumulative toxic effect of mancozeb (Ivanova-Tchemishanska, 1971; Fáy et al., 1989). Few papers have been published on the toxic effects of mancozeb on reproduction.

Administration of the 1/5, 1/10 or 1/20 part of the oral LD_{50} of mancozeb increased perinatal and postnatal mortality and the incidence of macroscopic developmental anomalies in rats (Ivanova-Tchemishanka, 1971).

The toxic effect exerted by Dithane M-45 at the 700 and 350 mg/bwkg dose on the reproduction of animals was established by Hungarian authors (Fáy et al., 1989). The single oral administration of 1320 mg/bwkg mancozeb on day 11 of gestation caused teratogenic effect in rats, but the 730 mg/bwkg dose did not give rise to such effects (Larsson et al., 1976).

The 750 mg/bwkg dose of Dithane M-45 (which is equal to 600 mg/bwkg mancozeb active ingredient) proved to be teratogenic in this experiment when given in combination with lead acetate on days 6–15 of gestation.

It is known that ethylene-bis-dithiocarbamate compounds (like mancozeb) less readily form complex compounds with metal, and thus their synergistic e f-fects are moderate (Cs. László and Dura, 1989).

No data could be found in the literature on the teratogenicity testing of mancozeb applied by the conventional experimental method (OECD, 1981, No. 414). The potential teratogenic effect of mancozeb was declared by the Env i-ronmental Protection Agency (EPA, 1992).

The results of this study indicate that combined exposure to mancozeb (Dithane M-45) and lead acetate can cause distinct teratogenic effects in rats.

The understanding of chemical interactions is an essential requirement when applying pharmaceuticals, but it is a meaningful toxicological area also in the case of other chemicals. In the practice, many xenobiotics act on the living organism simultaneously. These effects can be modelled in toxicological tests, in this case in a teratological study.

Combined exposure to lead and pesticides poses a problem in both human and veterinary hygiene. Evaluation and assessment of the risks and elaboration of preventive measures are impossible without the results of scientific studies that complement the practical exper ience.

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