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ONE-GENERATION REPRODUCTION TOXICITY STUDY OF DITHANE M-45 (MANCOZEB) AND LEAD ACETATE

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The reproductive toxicity of lead acetate and of a fungicide formulation (Dithane M-45) containing 80% mancozeb was studied on rats. Lead acetate was applied in the feed in the following dose groups: control, 1,000, 5,000 and 10,000 mg/kg of diet. The three treatment groups received, in addition to the above doses of lead acetate, 4,500 mg/kg Dithane M-45 in the diet. The method was based on the OECD Guideline for Testing of Chemicals No. 415 (1981). Clinical symptoms and mortality were not found in the parent generation. The body weight of female animals decreased significantly before the pregnancy period. This tendency was also seen in males after the combination treatment. At the two high dose levels a remarkable body weight increase was seen in the female animals during the lactation period. As a result of treatment, decreased body weight of offspring was measured during the lactation period. No gross pathological changes were seen. Histological examination showed general tubulonephrosis in the experimental animals. It can be established that the administration of Dithane M-45 did not enhance the reproductive toxicity of lead acetate.

Key words: Lead acetate, mancozeb, reproduction, rat

There are many chemicals (agricultural, industrial, household agents, etc.) in the human environment which exert effects on humans as single compounds or in combination.

The presence of lead and its effects in the environment and in living organisms have been known for 8,000 years (Manuwald, 1989; Poór and Mituszova, 1989). Lead pollution originates from numerous sources and results in serious environmental and human health problems (Kákosy and Soós, 1995).

The lead burden is meaningful as a consequence of human activity. The leaded exhaust of the increasing number of vehicles cause environmental pollution, which is accompanied by a growing number of human diseases. Chemical

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substances occur in the environment in different concentrations and can cause poisoning individually or through combined exposure.

The literature contains several reports on the reproductive toxicity of lead: decreased number and size of pups, damage of offspring, neurological disorders and impaired spermatogenesis have been described (Stowe and Goyer, 1971; Assenato et al., 1986; Klaasen, 1996). Some data on the embryonic toxicity of mancozeb can also be found (Larsson et al., 1976; Fáy et al., 1989).

As agricultural farms often apply a mancozeb-containing fungicide (Dithane M-45) for plant protection, it seemed reasonable to study the interaction of that pesticide with lead derived from environmental pollution in a reproduction toxicity test.

Considering that a significant number of people are exposed to pesticides on a continuous or occasional basis in Hungary, the objective of this study was to assess the interaction of lead acetate and the above-cited fungicide in a one-generation reproduction toxicity study which was part of a 4-year-long research programme.

Materials and methods

Materials:	Lead (II)-acetate-3-hydrate (Reanal, Budapest) Dithane M-45 (80% mancozeb) (Rohm and Haas, US).
Animals:	Wistar rats, SPF (Human Company, Gödöllő), 5 weeks old, 15 males and 30 females per group.
Administration:	*Per os,* in the diet.
Doses:	Control (0) 0.0 mg/kg diet lead acetate[a] I 1,000 mg/kg diet lead acetate[a] II 5,000 mg/kg diet lead acetate[a] III 10,000 mg/kg diet lead acetate[a] and in treated groups I–III 4,500 mg/kg diet Dithane M-45[b] [a]Acute *per os* Dosis Toxica Minima (DTM) = 5,000 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 = > 5,000 mg/bwkg (The Pesticide Manual, 1994)
Food:	Standard rodent food (Bioplan Ltd., Budapest), *ad libitum*.
Water:	Tap water, *ad libitum*.
Environment:	Room temperature: 21 ± 2 °C, relative humidity 60–70%, lighting period: 12 hours light, 12 hours dark in a vivarium of controlled climate. Pregnant females were caged individually.

Body weight and food consumption measurement:
Weekly and on days 1, 4, 7, 14, 21 and 28 of lactation.

Mating procedure:	1 : 2 (male : females) matings were used.
Clinical observation:	Daily.
Litter size:	Without standardisation to the stage of weaning.
Gross necropsy:	At the time of sacrifice or death during the study (P adult animals and F_1 generation).
Histopathology:	In parental generation. Samples were taken from all the organs of 10 females and 5 males in all the groups. Staining: haemalum-eosin, examination: light microscopy.
Biometric evaluation:	Student's *t*-test (Finney, 1972).
Methodological basis:	OECD 415: Guideline for Testing of Chemicals (OECD, 1981).

Results

Clinical signs and deaths were not seen in the parental generation during the experiment. Significant decrease occurred in average body weight gain data in all treated groups of dams and in males receiving the two higher doses as compared to the control (Table 1), before pregnancy.

Table 1

Body weight gain data (g) before pregnancy in the one-generation reproduction toxicity study of mancozeb and lead acetate in rats

Dose groups	Females (F)	Males (M)
	Animals	
0	98.6 ± 15.9 (25)	212.7 ± 33.8 (15)
I	89.1 ± 13.0[a] (21)	190.2 ± 26.8 (15)
II	85.2 ± 13.0[c] (28)	157.7 ± 31.7[c] (15)
III	89.8 ± 13.0[a] (24)	165.4 ± 38.0[b] (15)

[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$; F = number of females; M = number of males

Combined administration of lead acetate and mancozeb induced the appearance of the following toxic effects: average maternal body weight gain decreased during pregnancy, and a slight decrease in the number of pups was seen in all the treated groups (Tables 2, 3 and 4).

After parturition the body weight gain of females was powerful at the two higher dose levels (Table 2). The body weight data, the number of pups and the indices did not show a consistent dose-effect connection (Tables 3, 4 and 5). No

pathological changes were detected macroscopically. Histological findings consisted of inclusion-like forms, tubular epithelial cell necrosis, tubular epithelial cell regeneration with appearance of endomitotic cell nuclei, namely chronic tubulonephrosis. The severity of tubulonephrosis showed a mild dose-dependence, which was more expressed in dams.

Table 2

Body weight gain data (g) of dams during pregnancy and lactation in the one-generation reproduction toxicity study of mancozeb and lead acetate in rats

Dose groups	Pregnancy	Lactation (28 days)
0	70.3 ± 33.9	–11.8 ± 15.6
I	53.0 ± 39.3	–19.9 ± 17.0
II	–2.4 ± 2.7[c]	1.3 ± 18.4[a]
III	0.1 ± 2.6[c]	12.3 ± 10.1[c]

[a]P < 0.05; [c]P < 0.001

Table 3

Sex of pups and body weight data (g) of offspring in the one-generation reproduction toxicity study of mancozeb and lead acetate in rats

Dose groups	Sex* M/F	Days of lactation					
		1	4	7	14	21	28
0	122/138	7.35±0.87	11.36±1.67	16.49±2.76	29.57±5.80	43.14±7.71	70.82±12.34
I	81/111	6.92±0.74	10.34±1.15[a]	14.67±2.13[a]	26.88±4.60	40.92±7.14	62.79±11.56[a]
II	113/130	6.27±1.05[c]	9.40±1.85[c]	13.68±2.61[c]	25.72±4.93[a]	39.45±7.38	62.43±11.30[a]
III	118/106	6.30±0.78[c]	9.21±1.44[c]	13.38±2.34[c]	25.26±4.09[b]	38.59±6.77[a]	61.29 ± 9.26[b]

*On day 28 of lactation; [a]P < 0.05; [b]P < 0.01; [c]P < 0.001; M = Male; F = Female

Table 4

Number of live pups during the lactation period in the one-generation reproduction toxicity study of mancozeb and lead acetate in rats

Doses mg/kg diet	Lactation days				Loss %
	0	4	21	28	
0	250	249	241	241	3.6
I (1,000)	192	191	183	183	4.7
II (5,000)	244	241	238	238	2.5
III (10,000)	224	223	222	222	0.9

Table 5

Indices (%) in the one-generation reproduction toxicity study of mancozeb and lead acetate in rats

Indices (%)	Dose, mg/kg diet			
	0	I (1,000)	II (5,000)	III (10,000)
Copulation	86.7	73.3	96.7	83.3
Fertility	96.2	95.5	96.6	96.0
Gestation	100.0	100.0	100.0	100.0
Viability	99.6	99.5	98.8	99.6
Lactation	96.8	95.8	98.8	99.6
Weaning	96.8	95.8	98.8	99.6

Discussion

The toxic effects of lead on reproduction have been demonstrated in numerous publications on the basis of experimental observations. The gametotoxic effects of lead on rats in both sexes were described by Stowe and Goyer (1971). Some authors described the adverse effects of lead acetate on mouse embryonic development (Jacket et al., 1975; Jacket, 1977). In other cases developmental disorders of the corpora lutea, reduced serum progesterone level (Jacket et al., 1977) and some other alterations were detected. In mice experimentally treated with organic lead, an adverse effect on gestation was observed (Odenbro and Kihlström, 1977).

The neurotoxic effects of mancozeb after repeated administration are well known (WHO, 1988; Cs. László and Dura, 1989; Kékes-Szabó et al., 1990; Adamis-Borbély and Molnár, 1993).

Doses corresponding to 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-Tchemishanska, 1971).

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

In the one-generation reproduction toxicity study of lead acetate and the mancozeb-containing Dithane M-45 fungicide the animals survived the combined administration. The body weight gain decreased after the treatment. This phenomenon was similar in the one-generation reproduction study on female rats after lead acetate administration and after combined use of both chemicals in a 90-day subchronic test on rats (unpublished data). The body weight decrease was due, with great probability, to the durable lead burden.

Reduced number of pups after lead administration has been reported in the literature. The results of the present trial confirm this finding, indicating that lead exposure has such a consequence in rats (Dalldorf and Williams, 1945; Puhač et al., 1963).

The severity of confirmed tubulonephrosis seemed to be dose dependent on the basis of the light microscopic findings, which also indicates the toxic effects of lead administration.

Compared to data of the literature and to unpublished data of our one-generation reproduction toxicity study of lead acetate in rats, the macroscopic and microscopic findings of this experiment showed no increased toxicity after the simultaneous administration of lead acetate and the fungicide formulation. The mancozeb-containing Dithane M-45 did not enhance the reproductive toxicity of lead acetate.

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