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Toxicity of Thiram (Tetramethylthiuram Disulfide) to Mink and European Ferrets

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Thiram (tetramethylthiuram disulfide) is a dithiocarbamate used extensively as a fungicide, seed disinfectant, bacteriostat, wood preservative, rubber accelerator, vulcanizer, and rodent repellent (Anon. 1976). It is one of the most important fungicides used in agriculture (Dive et al. 1984). The compound is generally prepared via the oxidation of sodium dimethyldithiocarbamate by hydrogen peroxide or iodine and is an oxidation product of the iron and zinc salts of dimethyldithiocarbamic acid (Fishbein 1976). Thiram is an irritant of mucous membranes and a skin sensitizer (Anon. 1976). When administered to rats, it reduces feed intake (Lowy et al. 1979; 1980) and causes neural dysfunction (Lee and Peters 1976; Lee et al. 1978). It is fetotoxic to mice (Fishbein 1976) and has been found to be teratogenic to mice and hamsters (Fishbein 1976; Woo 1983). When fed in combination with sodium nitrite, it produces an increased incidence of tumors (Lijinsky 1984). The acute oral LD_{50} for mammals ranges from 350 to 2,500 mg/kg (Fishbein 1976).

The widespread use of thiram has generated concern regarding its possible contamination of the environment and potential toxicity to mammalian species, including man. Few studies have investigated the toxicity of thiram to nontargeted wildlife species. This study was, therefore, conducted to investigate the toxicity of this compound to mink (Mustela vison) and European ferrets (Mustela putorius furo).

MATERIALS AND METHODS

Thiram toxicity evaluation consisted of a range-finding study, 28-day LC_{50} test, and 20 (ferret) or 24 (mink) week reproduction test for each species. Unless otherwise stated, the LC_{50} and reproduction tests were conducted in accordance with previously described procedures (Hornshaw 1984; Hornshaw et al. 1986) as summarized below.

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The tests were conducted in two similar rooms. The animals were housed individually in wire cages (76 cm L x 61 cm W x 46 cm H) suspended above the floor. Wooden nest boxes (31 cm L x 28 cm W x 27 cm H) attached to the outside of the cages were provided for the females during the reproduction test.

During the LC_{50} tests, the photoperiod was held constant at the natural light-dark regimen at the end of the acclimation period, while simulated natural light conditions were maintained during the reproduction tests. Temperature within the animal rooms approximated ambient temperature except during cold weather when the temperature was maintained above 0°C. Ventilation was provided by an exhaust fan and ceiling vents.

Standard dark mink and agouti-colored ferrets were randomly allocated to treatment groups (with the exception that littermates were not assigned to the same group). The 5 to 6 month old mink and 6 to 7 month old ferrets were acclimated to the test facilities for 18 and 21 days, respectively. The mink LC_{50} test started October 7, 1982 and the ferret LC_{50} test began December 16, 1982. In both the LC_{50} and reproduction tests, the thiram (analytical reagent; Pfaltz and Bauer, Stamford, CT) was suspended in water prior to mixing with appropriate quantities of the other components of the diet. The basal diet consisted of 24.2% mink cereal, 28.9% whole chicken, 18.1% ocean fish scrap, 9.7% beef tripe, 4.8% beef liver, 4.8% beef lungs, 4.8% beef trimmings, and 4.8% cooked eggs. "As fed" the diet contained 68.4% moisture, 14.3% protein, 7.9% fat, and 2.5% ash. Feed and water were provided ad libitum.

Based on an acute oral (gavage) range-finding trial in which up to 1000 mg/kg thiram was administered to mink without any adverse affects, dietary concentrations of 0, 476, 857, 1543, 2778, and 5000 ppm thiram were initially selected for the mink LC_{50} test. However, due to feed avoidance, the concentrations were changed to 0, 45, 82, 147, and 265 ppm. Ten animals (five males and five females) were assigned to each dietary treatment, except for the 45 and 82 ppm groups which consisted of two males and two females per treatment group because of the limited number of untreated, acclimated mink available after the treatment levels were adjusted.

Prior to the ferret LC_{50} test, a palatability trial was conducted in conjunction with range-finding in an effort to avoid the previously mentioned problem. Dietary concentrations of 0, 8, 20, 50, 125, and 312 ppm were chosen for the ferret LC_{50} test. Each dietary group consisted of 10 animals (five males and five females). In both the mink and ferret LC_{50} tests, feed consumption and body weights were measured weekly and observations for clinical signs of toxicity, and mortality were made daily. Organ weights were recorded at necropsy. Blood was collected (via heart puncture) for hematologic parameters from all survivors at termination.

Based on the LC_{50} test results, dietary concentrations of 0, 2.5, 10, and 40 ppm thiram were selected for mink and 0, 4, 16, and 64 ppm for ferrets, for the reproduction tests. Randomly selected animals (4 males and 12 non-parous females) were assigned to each concentration. Acclimation periods of 21 and 9 days were employed for the mink and ferret tests, respectively. Diets were fed starting eight weeks prior to the breeding season (March 1 for mink; April 15 for ferrets) and continued through weaning of offspring (kits) at six weeks of age. Feed consumption and body weights were measured at two week intervals until breeding began. The animals were mated within their respective groups. Kits were counted and weighed the day of birth and at three and six weeks post-partum. After weaning, the four adult males and four randomly selected adult females from each group were sampled for determination of hematologic parameters, killed (CO_2 gas), and necropsied. Brain, liver, spleen, kidney, heart, and lung weights were recorded.

Body weight changes, hematologic parameters, and reproductive parameters were analyzed by one-factor analysis of variance and significant differences ($P \leq .05$) determined by Dunnett's method for comparison with a control (Gill 1978). Organ weights were transformed and analyzed by two-factor ANOVA. Significant differences were tested by comparison with a control (Gill 1978). Whelping and kit survival data were analyzed by contingency table and significant differences tested by Bonferroni's Chi-square test (Gill 1978).

RESULTS AND DISCUSSION

Body weight changes and feed consumption are summarized in Table 1. For the mink LC_{50} test, dramatic signs of intoxication were seldom noted. Mink fed 82 ppm thiram showed reduced feed consumption, loss of body weight, and subsequent bloody stools, while animals in the 147 and 265 ppm groups avoided the diets after approximately two weeks, at which time these two dietary treatments were terminated. No mortality occurred in the treated mink, including those in the 147 and 265 ppm groups, nor were any gross lesions observed at terminal kill necropsy (7 December). No significant differences were found between organ weights of treated and control mink (data not shown). Hematocrit values for the mink fed the thiram-treated diets (45 and 82 ppm) were, however, significantly ($P < .01$) less than the controls.

In the ferret LC_{50} test, signs of intoxication were first observed on day 4, when two animals fed the 312 ppm diet were found to have bloody stools. Reduced feed consumption was noted. A transient reduction in feed consumption was also observed for some animals in the 50 and 125 ppm groups in the first week. Clinical signs of toxicity were only noted in the 312 ppm group. These signs included inanition, bloody stools, listlessness, incoordination, and occasional convulsions accompanied by intense vocalizations. All animals fed the 312 ppm diet died between days 11-16, while no deaths were noted in animals on any other

Table 1. Mean body weights, body weight changes, and feed consumption of mink and ferrets fed various concentrations of thiram for 28 days.

Parameter measured	Thiram concentration (ppm) 0 ♂	0 ♀	45 ♂	45 ♀	82 ♂	82 ♀	147 ♂	147 ♀	265 ♂	265 ♀
Mink										
Body weight (g)										
Initial	1753	962	1453	1200	1677	1053	1536	1135	1577	920
Final[a]	1867	974	1626	1249	1469	935	1213[b]	907[b]	1072[b]	637[b]
Change	114	12	173	49	-208	-118	-323	-228	-505	-238
N	5	5	2	2	2	2	5	5	4	4
Feed consumption by period (g/d)[d]										
Week 1	366	287	353	304	361	98	362	276	177	144
Week 2	309	238	321	244	121	148	117	72	63	54
Week 3	272	239	327	185	277	183	--[b]	--[b]	--[b]	--[b]
Week 4	272	195	177	164	209	34	--[b]	--[b]	--[b]	--[b]

	0 ♂	0 ♀	8 ♂	8 ♀	20 ♂	20 ♀	50 ♂	50 ♀	125 ♂	125 ♀	312 ♂	312 ♀
Ferret												
Body weight (g)												
Initial	1929	933	1900	796	2033	907	1907	861	1998	902	1952	949
Final[a]	2082	950	2074	803	2097	862	1957	834	1876*	807	1628**[c]	763[c]
Change	153	17	174	7	64*	-45	50*	-27	-122**	-95**	-324**[c]	-186**[c]
Feed consumption by period (g/d)[d]												
Acclimation	245	141	293	121	283	139	269	145	279	138	249	133
Week 1	274	155	248	144	230	116	177	106	165	106	158	124
Week 2	338	189	334	171	315	183	240	172	272	203	157	53
Week 3	314	158	275	146	316	158	227	160	251	148	---[c]	---[c]
Week 4	305	133	273	130	309	124	218	137	232	119	---[c]	---[c]

[a] Based on body weight at time of death, at time of removal from test, or at terminal kill.
[b] Mink taken off treatment after 15 days.
[c] All ferrets died between days 11-16.
[d] Feed consumption based on the average of two consecutive day's consumption.
* Significantly different from control ($P \leq .05$).
**Significantly different from control ($P \leq .01$).

dietary concentration. No gross lesions were noted at necropsy. The organ weight data showed increased splenic weights for females fed 125 ppm, and an increase in lung weight for males fed 312 ppm. Red blood cell counts and hemoglobin concentrations were significantly ($P < .01$) reduced from control values in the 50 and 125 ppm treated ferrets. Hematocrit values were also significantly ($P < .05$) less than control values in ferrets fed 50 ppm thiram.

Feed consumption and body weights are negatively affected in rats (Lee et al. 1978; Lowy et al. 1980), chickens (Waibel et al. 1957; Rasul and Howell 1974), and turkeys and geese (Waibel et al. 1957) during subacute exposure ranging from 150 to 400 ppm of thiram. In the mink and ferret LC_{50} tests, thiram adversely affected feed consumption and body weight gains at dietary concentrations of 82 ppm, or more, and 50 ppm, or more, respectively. Male ferrets that received 20 ppm (mg/kg) dietary

thiram showed significantly reduced body weights although feed consumption was not significantly depressed, which suggests that thiram may affect body weight in a manner not entirely due to feed intake.

It is difficult to draw conclusions from the mink LC_{50} data, or to make comparisons to the ferret data based on the fragmentary results. It is apparent that 82 ppm thiram added via a water vehicle was detrimental to mink, while the results from the 45 ppm group indicate that this concentration may be safe for mink (it must be remembered that only four animals were exposed in both of these groups).

Body weight changes and feed consumption during the reproduction tests are summarized in Table 2. A significant reduction in body weight from the control was noted for the male mink fed 40 ppm thiram, while female ferrets fed 64 ppm lost less weight than did controls during the 8-week pre-breeding exposure period. Since female ferrets, and, to a lesser extent, female mink, are known to reduce feed consumption and lose body weight as

Table 2. Mean body weights, body weight changes, and feed consumption of mink and ferrets (4 males and 12 females) fed various concentrations of thiram for 8 weeks prior to breeding in a reproduction test.

Parameter measured	Thiram concentration (ppm)							
	0		2.5		10.0		40.0	
	♂	♀	♂	♀	♂	♀	♂	♀
Mink								
Body weights (g)								
Initial	2095	1128	1790	1061	1832	1142	2080	1109
Final	2222	1090	1849	1034	1840	1101[a]	1940	1045
Change	127	- 38	59	- 27	8	- 41[a]	-140*	- 64
Feed consumption by period (g/d)[b]								
Acclimation	386	163	228	124	318	120	322	145
Weeks 1-2	362	173	315	191	370	175	348	158
Weeks 3-4	387	219	357	174	341	154	297	180
Weeks 5-6	322	177	308	153	360	177[a]	327	122
Weeks 7-8	292	162	380	167	343	173[a]	249	152
	0		4.0		16.0		64.0	
	♂	♀	♂	♀	♂	♀	♂	♀
Ferrets								
Body weights (g)								
Initial	1980	925	1819	878	1728	870	1780	910
Final	1882	787	1710	743	1658	777	1689	833
Change	- 98	-138	-109	-135	- 70	- 93	- 91	- 77*
Feed consumption by period (g/d)[b]								
Acclimation	433	138	372	144	404	151	393	142
Weeks 1-2	412	126	341	137	337	115	313	111
Weeks 3-4	344	127	285	130	282	107	278	114
Weeks 5-6	261	134	223	112	267	114	244	96
Weeks 7-8	225	90	230	112	267	95	249	83

[a] One female died from causes not related to effects of the test substances.

[b] Feed consumption based on the average of two consecutive day's consumption.

* Significantly different from control ($P \leq .05$).

they approach estrus, it may be that the normal pre-estrus pattern of the ferrets had been disrupted, as only 7 of the 12 females were judged to be in estrus (vulvar swelling) after eight weeks on the 64 ppm diet. Thiram has been associated with disruptions of the estrous cycle in other species. Davydova (1973) noted an extension of the estrous cycle at the expense of the resting phase in rats exposed to 3.8 mg thiram/m^3 of air for 5 hr/day, 5 days/week for 4.5 months. A prolonged diestrous phase has also been reported in rats fed 400 ppm thiram for 14 days prior to mating (Short et al. 1976). Wedig et al. (1968) observed inhibition of ovulation in bobwhite quail at oral doses as low as 8.8 mg/kg/day.

No signs of intoxication or thiram related mortalities were noted in either species. No gross lesions were noted at necropsy for mink or ferrets, and no birth defects were observed.

Reproductive performance and kit growth and survival are summarized in Tables 3 and 4. The only significant reproductive effect noted in the mink was a decrease in average birth weight at 40 ppm, although the number of females that whelped on the 40 ppm diet was also quite low. The other reproductive indices were within the normal range for mink.

None of the ferret females fed 64 ppm thiram whelped and kit body weights at three weeks were significantly decreased at 16 ppm. Litter weights at birth, three, and six weeks were also significantly decreased in the 16 ppm group. In both the mink and ferrets, kit survival was inversely proportional to the dietary concentration. Decreases in the number of offspring produced per female, birth weights, and/or offspring viability have been reported in other species treated with thiram. Robens (1969) noted increased resorption of fetuses, low birth weights, and decreased viability of offspring from hamsters given 125 or 250 mg/kg body weight thiram from days 5-15 of gestation. Short et al. (1976) found a significant decrease in the number of implanted fetuses and number of pups produced by female rats fed 400 ppm thiram for 14 days prior to mating. These authors also reported reproduction failure in some female rats due to 100 percent resorption at oral doses of 164 and 200 mg/kg, decreased number of pups per female at 136, 164, and 200 mg/kg, and reduced birth weights of pups produced by female rats given 40, 90, and 136 mg/kg thiram during days 6-15 of gestation. In similar studies with mice, these authors failed to observe changes in litter sizes, birth weight, or incidence of resorption at oral doses of 100 or 300 mg/kg administered during days 6-14 of gestation. They also found no effect on viability or growth in pups from rats fed 300 ppm thiram from day 16 of gestation through day 21 post-partum, but did observe 100 percent pup mortality by day 21 post-partum at the dietary concentration of 1000 ppm.

Analyses of the hematologic data for both the mink and ferrets at the termination of the reproduction tests showed significant

Table 3. Reproductive performance of female mink and ferrets fed various concentrations of thiram.

Thiram concentration (ppm)	♀s bred/ total	♀s whelped/ ♀s bred	Gestation[a] (days)	Live kits/ ♀ whelped	Total kits/ ♀ whelped
Mink					
0	12/12	10/12	48.9 ± 1.60[b]	3.9 ± 0.77	4.1 ± 0.65
2.5	9/12	6[c]/ 9	48.5 ± 2.07	5.0 ± 1.08	5.3 ± 0.84
10.0	11/11	11/11	47.9 ± 1.53	4.8 ± 0.73	5.3 ± 0.62
40.0	12/12	7/12	47.1 ± 1.92	3.7 ± 0.92	4.7 ± 0.77
Ferrets					
0	12/12	12[c]/12	41.3 ± 0.24	10.6 ± 0.80	11.2 ± 0.88
4.0	12/12	9/12	41.3 ± 0.28	11.3 ± 0.89	11.7 ± 1.02
16.0	12/12	8/12	41.5 ± 0.30	8.2 ± 0.94	9.2 ± 1.08
64.0	7/12	0.7	---	0	0

[a] Gestation calculated from date of last mating.

[b] Mean ± SEM.

[c] One female died from problems associated with parturition.

Table 4. Mean mink and ferret kit body and litter weights and kit survival for dams fed various concentrations of thiram.

Thiram concentration (ppm)	Kit body weight (g)			Litter weight (g)			% survival	
	Birth	3 weeks	6 weeks	Birth	3 weeks	6 weeks	3 weeks	6 weeks
Mink								
0	9.7 ± 0.29[a]	96.5 ± 3.02	254 ± 10.2	37.9 ± 6.75	410 ± 72.6	1126 ± 202.5	85.0	85.0[b]
2.5	9.8 ± 0.36	93.7 ± 3.84	260 ± 12.7	48.9 ± 9.54	393 ± 91.8	1041 ± 239.6	84.0	80.0
10.0	8.9 ± 0.25	114.2 ± 2.62	318** ± 9.1	43.1 ± 6.43	445 ± 64.9	1240 ± 169.4	73.6	73.6
40.0	7.9** ± 0.35	87.4 ± 4.40	258 ± 15.8	29.4 ± 8.07	350 ± 102.6	1118 ± 309.3	88.9[c]	72.2[c]
Ferret								
0	8.9 ± 0.14	86.3 ± 1.88	320 ± 5.4	94.2 ± 5.89	887 ± 46.2	3232 ± 204.0	95.8	94.1
4.0	8.8 ± 0.15	82.2 ± 2.06	293** ± 6.0	99.2 ± 6.51	868 ± 51.1	2996 ± 225.5	93.1	89.2
16.0	8.7 ± 0.18	76.7** ± 2.52	300 ± 7.8	69.7* ± 6.90	604** ± 54.2	1990** ± 239.2	96.9	81.5
64.0	---	---	---	---	---	---	----	----

[a] Mean ± SEM.

[b] Does not include litter killed by dam.

[c] Does not include litter lost from death of dam due to causes unrelated to effects of test substance.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

decreases in RBC count ($P \leq .01$), hemoglobin concentration ($P \leq .01$), and hematocrit percent ($P \leq .05$ for mink and $P \leq .01$ for ferrets) at the highest dietary concentration of thiram. The organ weight data revealed a significant ($P \leq .01$) increase in splenic weights at the highest thiram concentration in both tests, as well as at the 16 ppm thiram concentration ($P \leq .05$) in the ferret test.

Increased splenic weights were noted by Lee and Peters (1976) in rats fed 400 and 1000 ppm thiram for 8-9 weeks, but not in rats fed 100 ppm of thiram for the same length of time. These authors also reported increases in liver, kidney, and brain weights at 1000 ppm. Similar results were observed only in the ferrets fed the highest concentrations of thiram in the LC_{50} test, when these organ weights were expressed as a percentage of body weight.

In the LC_{50} tests in this study, a dietary no-effect level was not found for mink, since a decrease in hematocrit was noted at the lowest concentration (45 ppm) fed. The no-effect level for the ferrets was 8 ppm. The dietary no-effect level of thiram for rats has been reported to be 38 ppm (Lowy et al. 1979; 1980). The relative sensitivity of mink and ferrets to thiram in comparison to rats is further illustrated by the results of a chronic feeding study with rats, reported by Lee and Peters (1976), in which some animals fed 1000 ppm dietary thiram survived the 80-week exposure period, while in our 28-day LC_{50} tests, mink fed 147 ppm thiram, or more, were removed from the test (to prevent the animals from starving) and all the ferrets fed 312 ppm died by the 16th day of the test.

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