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Comparative Toxicity of Strychnine to Eight Species of Ground Squirrels

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Comparative Toxicity of Strychnine to Eight Species of Ground Squirrels[1]

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Abstract.--The toxicity of 3 strychnine bait concentrations, 0.20%, 0.35%, and 0.50%, was evaluated on 8 species of ground squirrels (Spermophilus spp). Significant species-specific differences were evident in the relative toxicity of strychnine in our tests.

INTRODUCTION

The Environmental Protection Agency (EPA) completed a special review of strychnine as a rodenticide in September, 1983, and issued the PD-4 strychnine position document (EPA, 1983). The rationale for regulating strychnine-containing products was given and the studies required for the determination of the reregistration of strychnine bait formulations for ground squirrel control were listed.

The potential hazard of primary poisoning to nontarget mammals and to seed eating birds was of special concern to EPA. Thus, to reduce nontarget hazards, EPA proposed lowering strychnine concentrations from the currently registered 0.35% and 0.50% strychnine to 0.20%. Evaluation of the efficacy of the 0.20% strychnine bait through laboratory toxicity studies was essential to determine the need for testing under field conditions.

Data are presented in this report on laboratory studies on the comparative toxicity of 0.20%, 0.35%, and 0.50% strychnine on 8 species of ground squirrels (Matschke 1985a, b, c, Matschke et al. 1987d, e, f, g, h). Species tested were the Columbian ground squirrel (Spermophilus columbianus), Franklin's ground squirrel (Spermophilus franklini), golden-mantled ground squirrel (Spermophilus lateralis), Richardson's ground squirrel (Spermophilus richardsonii), rock squirrel (Spermophilus variegatus), thirteen-lined ground squirrel (Spermophilus tridecemlineatus), Townsend's ground squirrel (Spermophilus townsendii), and Uinta ground squirrel (Spermophilus armatus).

PROCEDURE

Ground squirrel procurement and care

Ground squirrels of both sexes were trapped in Montana (Columbian, golden-mantled, and Uinta), North Dakota (Franklin's), Colorado (Richardson's, rock, and thirteen-lined), Idaho (Townsend's), and South Dakota (thirteen-lined). Each ground squirrel was dusted with pyrethrum powder for flea control, and housed individually in steel cages (40.6 x 24.1 x 18.0 cm), except that rock squirrels were housed individually in stainless steel cages (61.0 x 45.5 x 23.0 cm). The animal room was maintained at about 21° C on a 12-h light-dark cycle (0600-1800 h light, 1800-0600 h dark). Squirrels were fed flaked barley, pelleted rodent laboratory chow (Ralston Purina Company[6]), and tap water (ad libitum).

Bait Formulation

Strychnine alkaloid (CAS No. 57-24-9) was purchased from Pocatello Supply Depot (PSD), Pocatello, Idaho, and assayed at 98% technical. Strychnine baits were formulated with steamed-crimped oats according to the procedures established by the (PSD) (Pocatello Supply Depot, n.d.). A sham-treated bait was formulated in the same manner with all the ingredients except strychnine. All strychnine concentrations were assayed by a procedure developed by the Denver Wildlife Research Center (unpublished) and reported as percent active ingredient. Only baits that assayed within ±10 percent of the

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[6] Reference to trade names does not imply U.S. Government endorsement.

desired concentrations, 0.20%, 0.35%, and 0.50%, were used for testing.

Testing Procedures

For each species, the number of ground squirrels tested per strychnine concentration and sex are given in Table 1.

On the day before testing, the ground squirrels were fasted beginning at 1600 h. The next morning (day 1), each ground squirrel in each strychnine treatment group received strychnine bait, and each ground squirrel in the control group received sham-treated bait. Bait was presented in aluminum dishes (8.2 cm diameter x 3.9 cm deep) fastened to the cages with metal springs. Spilled bait was caught in pans placed beneath the cages. Following presentation of test baits, the animal room was locked and entry denied until 1500 h or 1600 h the same day. Upon reentry, mortality was recorded, dishes and pans were removed from the cages of those ground squirrels that died, and remaining bait was weighed. At 0700 h the next morning (day 2), any additional mortality was recorded, dishes and pans were removed, and the remaining bait was weighed. Each survivor was given additional bait of the strychnine concentration initially received, and each control squirrel received additional sham-treated bait. Entry to the room was denied until 0700 h the next morning (day 3), when the day 2 procedure was repeated.

At 0700 h the next morning (day 4), mortality was recorded, dishes and pans were removed, and remaining bait was weighed. Survivors were given flaked barley and rodent laboratory chow, and were observed twice daily for 14 days. Records for each animal were kept on daily strychnine or control bait consumption, total bait consumption, mg of strychnine consumed per kg of body weight[7] (day 1), and mortality.

Data Analyses

For each species, differences in consumption among the 3 strychnine bait concentrations and the sham-treated bait on day 1 and between the 2 sexes were analyzed by a 2-factor analysis of variance. Comparisons of mg/kg consumption of strychnine among the 8 species for the 3 strychnine concentrations also were analyzed as a 2-factor analysis of variance. If treatment or interaction effects were significant in the ANOVA, Duncan's Multiple Range Test was used to determine which individuals means were significantly different from others.

RESULTS

Mortality

The 0.20% concentration was 100% efficacious when consumed by 3 (Franklin's, Richardson's, and thirteen-lined) of the 8 species (Table 2), and the 0.35% concentration was 100% efficacious when consumed by 6 (Columbian, Franklin's, golden-mantled, thirteen-lined, Townsend's, and Uinta) of the 8 species. The 0.50% concentration was 100% efficacious when consumed by 6 (Columbian, golden-mantled, Richardson's, rock, thirteen-lined, and Uinta) of the 8 species, and 90% or more efficacious to all 8 species. Most mortality occurred during the first 24-h feeding period for all 3 bait concentrations, with 94-97% of all squirrels dying during this period.

Bait Consumption

Consumption by ground squirrels that consumed 0.20% strychnine bait and died ranged from 0.40 to 2.18 g on day 1 (Table 3); consumption

[7] All mg/kg calculations are based on the assayed concentration of the strychnine baits.

Table 1. Sex of ground squirrels tested per species, number of ground squirrels tested per strychnine bait concentration, and total number of ground squirrels tested per species.

Species	Sex M/F	Number of squirrels per strychnine bait concentration 0.20%	0.35%	0.50%	0.0%	Total tested
Columbian	40/40	20	20	20	20	80
Thirteen-lined	40/40	20	20	20	20	80
Richardson's	40/40	20	20	20	20	80
Franklin's	40/40	20	20	20	20	80
Golden-mantled	40/40	20	20	20	20	80
Uinta	40/40	20	20	20	20	80
Townsend's	31/45	19	19	19	19	76
Rock	35/45	20	20	20	20	80

Table 2. Percent of ground squirrel mortality observed for each strychnine bait concentration.

Species	Percent strychnine bait concentration							
	0.20		0.35		0.50		0.0	
	N[1]	% Mortality	N	% Mortality	N	% Mortality	N	% Mortality
Columbian	13	65	20	100	20	100	0	0
Thirteen-lined	20	100	20	100	20	100	0	0
Richardson's	20	100	19	95	20	100	0	0
Franklin's	20	100	20	100	18	90	0	0
Golden-mantled	17	85	20	100	20	100	0	0
Uinta	18	90	20	100	20	100	0	0
Townsend's	18	95	19	100	18	95	2	10
Rock	16	80	18	90	20	100	0	0

[1] N = Number of ground squirrels that died.

Table 3. Mean strychnine bait consumption and mg/kg intake of strychnine for ground squirrels that died.

Species	Amount of bait given (g)	Mean strychnine bait consumed (g) - Day 1				Mean strychnine intake (mg/kg) - Day 1		
		0.20%	0.35%	0.50%	0.0%	0.20%	0.35%	0.50%
Columbian	5[1]	1.86±0.14[2]	1.23±0.25	1.37±0.20	-	7.82±1.62	7.82±1.62	13.46±1.74
Thirteen-lined	5[1]	0.46±0.08	0.26±0.07	0.35±0.08	-	5.48±0.85	6.35±1.54	14.30±3.35
Richardson's	10	0.44±0.09	0.35±0.07	0.19±0.06	-	3.67±0.62	3.99±0.08	3.57±1.08
Franklin's	10	0.71±0.10	0.61±0.09	0.35±0.06	-	3.18±0.39	5.47±0.77	4.92±0.80
Golden-mantled	6	0.98±0.14	0.83±0.09	0.50±0.06	-	8.58±1.24	13.67±1.52	11.66±1.40
Uinta	6	1.47±0.15	1.04±0.12	1.00±0.09	-	11.15±1.28	12.55±1.50	18.36±1.84
Townsend's	10	0.40±0.06	0.17±0.03	0.26±0.04	2.73±0.64	3.92±0.54	2.59±0.49	5.76±0.93
Rock	15	2.18±0.37	1.41±0.14	0.94±0.13	-	7.01±1.21	6.90±0.61	6.97±0.86

[1] Control given 10 g of sham-treated bait.
[2] Mean ±SE.

by survivors ranged from 0.00 to 5.58 g on day 1 (Table 4). Bait consumption by ground squirrels that consumed 0.35% strychnine bait and died ranged from 0.17 to 1.41 g on day 1 (Table 3); consumption by survivors ranged from 0.03 to 2.63 g on day 1 (Table 4). Consumption by ground squirrels that consumed the 0.50% strychnine bait and died ranged from 0.19 to 1.37 g on day 1 (Table 3); consumption by survivors was 0.0 g on day 1 (Table 4). On days 2 and 3, survivors on all 3 strychnine bait concentrations continued to consume bait Consumption by ground squirrels that consumed the 0.0% strychnine bait and survived ranged from 3.51 to 10.47 g on day 1 (Table 4).

Consumption by survivors of strychnine treated bait for the entire 3-day test averaged 10.56 g, 11.04 g, 13.34 g, and 19.56 g for the Columbian, golden-mantled, rock, and Uinta ground squirrels, respectively.

Two control animals (Townsend's) that consumed the 0.0% bait died during the 14 day posttreatment period, possibly because of environmental stress. Neither of the 2 treated Townsend's squirrels died during this time period. Sham-treated bait consumption by controls for the entire 3-day test averaged 26.48 g, 20.45 g, 11.58 g, 18.42 g, 32.28 g, 16.32 g, and 19.81 g for the Columbian, Franklin's, golden-mantled, Richardson's, rock, Townsend's, and Uinta ground squirrels, respectively. Because all 60 thirteen-lined ground squirrels on treated bait died on day 1, further testing of the 20 control animals ceased after the first 24-h feeding period.

Data Analyses

There was a significant difference among the treatment means for bait consumption (Table 5)

Table 4. Mean strychnine bait consumption and mg/kg intake of strychnine for ground squirrels that survived.

Species	Amount of bait given (g)	Mean strychnine bait consumed (g) - Day 1				Mean strychnine intake (mg/kg) - Day 1		
		0.20%	0.35%	0.50%	0.0%	0.20%	0.35%	0.50%
Columbian	5[1]	3.64±0.28	-	-	8.55±0.24	13.37±1.18	-	-
Thirteen-lined	5[1]	-	-	-	3.51±0.05	-	-	-
Richardson's	10	-	0.03±0.00	-	5.47±0.48	-	0.0±0.00	-
Franklin's	10	-	-	0.0±0.00	6.52±0.47	-	-	0.0±0.00
Golden-mantled	6	3.07±1.05	-	-	3.93±0.27	25.19±8.72	-	-
Uinta	6	5.58±0.40	-	-	6.45±0.14	30.41±0.16	-	-
Townsend's	10	0.00±0.00	-	0.0±0.00	5.98±0.43	0.00	-	0.0±0.00
Rock	10	2.96±0.77	2.63±1.77	-	10.47±0.34	8.58±2.10	10.86±6.34	-

[1] Control given 10 g of sham-treated bait.
[2] Mean ± SE.

Table 5. Treatment means for strychnine bait consumption on day 1 separated by Duncan's Multiple Range Test.

Strychnine bait concentration %	Mean grams of bait consumed[2]						
	Columbian	Thirteen-Lined	Richardson's	Franklin's	Golden-mantled	Uinta	Rock
0.00	8.55 a[1]	3.51 a	3.51 a	5.47 a	6.52 a	3.93 a	10.47 a
0.20	2.45 b	0.46 b	0.45 b	0.71 b	1.29 b	1.88 b	2.33 b
0.35	1.23 c	0.26 b	0.34 b	0.52 b	0.83 bc	1.04 c	1.54 c
0.50	1.37 c	0.35 b	0.19 b	0.27 b	0.47 c	1.00 c	0.94 c

[1] Means with no letter in common are significantly different, at an experimental-wise error rate of 0.05.
[2] ANOVA was not performed on data from the Townsend's ground squirrel due to negative consumption values.

for each species. The response was not uniform for the 8 species (excluding Townsend's[8]), except that the ground squirrels consumed significantly more of the control bait than of the strychnine-treated baits. No significant differences were found among the 3 strychnine concentrations for the Franklin's, Richardson's, and thirteen-lined. However, significant differences in bait consumption occurred among the 0.20%, 0.35%, and 0.50% concentrations for the remaining 4 species. The Columbian, rock, and Uinta consumed significantly more of the 0.20% concentration than the 0.35% and 0.50% concentrations. The golden-mantled consumed significantly more of the 0.20% concentration than the 0.50% concentration; however, no significant difference in consumption occurred between the 0.20% and 0.35% concentrations, or between the 0.35% and 0.50% concentrations for this species.

Analysis of the mg/kg of strychnine intake data among the 8 ground squirrel species that died on day 1 revealed highly significant interspecific variability ($p = 0.0005$). The results from separating the interaction means with Duncan's multiple range test are presented in Table 6. These results, plus the plot of the interaction means in Fig. 1, clearly indicate that the pattern of mg/kg strychnine consumed across concentrations is different among the 8 species. For 2 species (Richardson's and rock), mg/kg of strychnine consumed is fairly constant for all 3 concentrations. For 4 species (Columbian, thirteen-lined, Townsend's, and

[8] ANOVA was not performed on data from the Townsend's ground squirrel due to negative consumption values.

Table 6. Mean[1] intake of strychnine (mg/kg) for concentration and species of animals that died on day 1.

Concentration (%)	Species	Mortality	Mean (mg/kg)	Letter
0.2	Columbian	12	7.10	d e f g
0.35	Columbian	20	7.82	d e f
0.5	Columbian	20	13.46	b
0.2	Franklin	20	3.19	g
0.35	Franklin	18	5.78	e f g
0.5	Franklin	14	5.27	e f g
0.2	Golden-mantled	17	8.58	c d e
0.35	Golden-mantled	20	13.67	b
0.5	Golden-mantled	19	11.66	b c
0.2	Richardson's	20	3.67	f g
0.35	Richardson's	19	3.99	f g
0.5	Richardson's	20	3.57	g
0.2	Rock	16	7.01	e f g
0.35	Rock	18	6.90	e f g
0.5	Rock	20	6.92	e f g
0.2	Townsend's	18	3.92	f g
0.35	Townsend's	17	2.75	g
0.5	Townsend's	17	5.76	e f g
0.2	Thirteen-lined	20	5.48	e f g
0.35	Thirteen-lined	16	6.35	e f g
0.5	Thirteen-lined	16	14.29	b
0.2	Uinta	18	11.26	b c d
0.35	Uinta	20	12.55	b
0.5	Uinta	20	18.36	a

[1] Means with a common Letter were not significantly different at the 0.05 level of significance using Duncan's Multiple Range Test.

Uinta), mg/kg of strychnine consumed increased with increasing concentration, while for the remaining 2 species (Franklin's and golden-mantled), the mg/kg strychnine consumed was highest at the 0.35% concentration.

DISCUSSION

Our laboratory tests indicate that 3 species (Franklin's, Richardson's, and thirteen-lined) may be effectively controlled at the 0.20% or lower strychnine concentration. Four species (Columbian, golden-mantled, Townsend's, and Uinta) may be effectively controlled at strychnine concentrations between 0.20% and 0.35%. One species (rock) may be effectively controlled at strychnine concentrations between 0.35% and 0.50%. Further research will be required on all species to determine the minimum strychnine concentration that will cause 100% mortality.

Reducing the concentration of strychnine in the bait is one way to reduce the amount of toxic substance presented. Another way would be to reduce the quantity of bait applied per burrow entrance. The current directions for applying strychnine bait call for the placement of 1 tablespoon (12 g) per burrow entrance. Twelve g of bait may be excessive in view of the quantity of bait consumed by the 8 ground squirrel species. Two of the 8 species (Thirteen-lined and Townsend's) consumed no more than 1.72 g, suggesting that 2 g (1/2 teaspoon) of bait per burrow entrance may be sufficient. Four of the 8 species (Franklin's, golden-mantled, Richardson's and Uinta) consumed no more than 3.5 g, suggesting that 4 g (1 teaspoon) of bait per burrow entrance may be sufficient. For the Columbian and rock squirrel, reductions to 6 g of bait per burrow entrance may be possible, as the highest intake for a ground squirrel that died was 6.42 g.

Because survivors of 4 species (Columbian, golden-mantled, rock, and Uinta) continued to consume treated bait throughout the 3-day test period, we conclude that bait aversion did not occur under laboratory conditions. For 2 of these species (golden-mantled and Uinta), treated bait consumption by survivors equaled that of controls over the 3-day test period. While for the other 2 species (Columbian and rock), treated bait consumption by survivors was almost half the consumption by control animals.

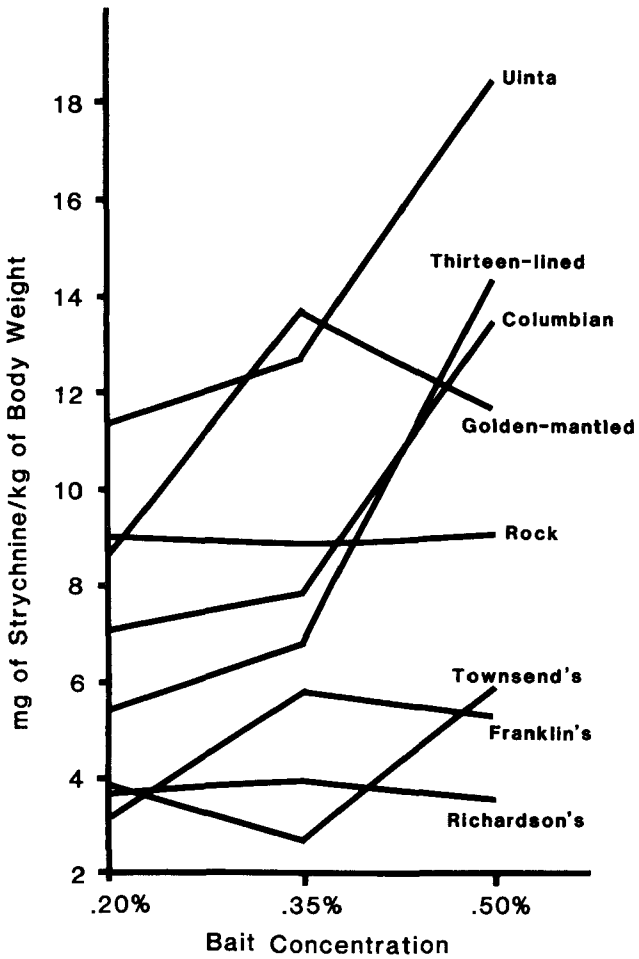

Figure 1.--Mean intake of strychnine (mg/kg) by concentration interaction for the animals that died on day 1.

The variable toxicity of strychnine to different species of ground squirrels is a function of physiological variability. The observed mg/kg of strychnine intake varied significantly among the species, and within a species, certain individuals appeared to be resistant to strychnine toxicity. Furthermore, the mg/kg intake of strychnine was constant for some species regardless of concentration, while for others, a sharp increase or decrease in mg/kg of strychnine consumed was observed at higher concentrations. Because of these differences among species, bait concentrations should be established in the laboratory before being used in the field.

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