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# ZINC PHOSPHIDE: BLACK-TAILED PRAIRIE DOG—DOMESTIC FERRET SECONDARY POISONING STUDY

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# ZINC PHOSPHIDE: BLACK-TAILED PRAIRIE DOG—DOMESTIC FERRET SECONDARY POISONING STUDY

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**ABSTRACT:** A laboratory study was conducted in which tissues from zinc phosphide-killed black-tailed prairie dogs (*Cynomys ludovicianus*) were fed to domestic ferrets (*Mustelaputorius*). Prairie dogs were fed a 2.03% zinc phosphide bait and upon death, two tissue complexes were prepared: stomach, liver, and intestines, and the remaining carcass. Five male and five female ferrets were each fed one of the two treated tissue complexes. A similar number of ferrets were each fed one of the two control tissue complexes. No poisoning symptoms or emesis were observed and no ferret mortality occurred. Zinc phosphide residue was determined in 10 prairie dog carcasses; 99.9% was found in the stomachs and small intestines. Residues were also detected in the large intestine, caeca, kidneys, and gall bladder/liver, none were found in the lungs, heart, or muscle. The low amounts of zinc phosphide remaining in the carcasses, the absence of ferret mortality, poisoning symptoms or emesis, despite the emetic properties of zinc phosphide, confirm that the risk of secondary poisoning from zinc phosphide is unlikely.

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## INTRODUCTION

The Environmental Protection Agency established test standards for reregistration of zinc phosphide (ZP) as a rodenticide for controlling damage by field rodents. Two of these standards involved the determination of secondary poisoning hazards and ZP residues in the tissues of animals killed after feeding on ZP baits. A study protocol was designed to furnish required data for compliance with these two standards and included the following two objectives: determine the risk of secondary poisoning to domestic ferrets that consume tissues from prairie dogs killed after feeding on a 2.0% ZP bait, and determine the ZP and phosphine gas residues in tissues from prairie dogs that died after feeding on a 2.0% ZP bait. This report summarizes the results of the study.

## METHODS

### Secondary Poisoning Hazard Study

**Zinc phosphide and control bait preparation**—The purity of the technical zinc phosphide (CAS No. 1314-84-7) was assayed at 98.7% [Okuno et al. 1975 as modified by Denver Wildlife Research Center (DWRC) chemists (Revision #2)]. Therefore, the amount of technical ZP used to formulate the bait was adjusted accordingly to prepare a 2.0% bait. The 2.0% ZP bait was prepared on steamed crimped oats with Alcolec-S<sup>1</sup> as the adhesive. A control bait was prepared with the same ingredients except that ZP was omitted. After preparation, three samples from the 2.0% bait were analyzed by a gas chromatographic procedure developed at DWRC by Okuno et al. (1975). The bait assayed at 2.03% (SE = 0.09).

**Procurement of domestic ferrets and prairie dogs**—Eleven-week-old domestic ferrets were purchased from Marshall Research Animals, North Rose, New York, and shipped by air freight to the DWRC, Denver, Colorado. Black-tailed prairie dogs were livetrapped at Wind Cave National Park, Hot Springs, South Dakota, dusted with pyrethrum powder for ectoparasite control, and transported to the DWRC. Both species were sexed, weighed, identified by eartag number, and placed in individual stainless steel cages (60 x 32 x 45 cm) in different animal rooms. Ferrets were

maintained on Purina Cat Chow<sup>1</sup> and tap water *ad libitum*. Prairie dogs were maintained on a diet of flaked oats, pelleted rodent laboratory chow, horse hay cubes, and tap water *ad libitum*. Both species were quarantined and acclimated for a minimum of 14 days and inspected by a veterinarian. The two animal rooms were on a 12-h light-dark cycle (light 0600-1800 h, dark 1800-0600 h) and maintained at about 21° C.

### Preparing Prairie Dogs for Secondary Poisoning Study

**Prairie dog tissue used to precondition the ferrets**—The domestic ferrets were preconditioned to consume the stomach, liver, and intestinal tissue (SLI) of prairie dogs. The 88 prairie dogs selected were fasted beginning at 1600 h; the next morning at 0800 h they were given 40 g of steamed crimped oats. At 1600 h, the remaining bait was weighed to determine consumption. The animals were euthanized with carbon dioxide, and the stomach, liver, and intestinal tissue were excised from the carcass, and frozen.

**Prebaiting prairie dogs with untreated oats**—Before the prairie dogs were exposed to the ZP bait, they were prebaited with untreated oats. Each animal was prebaited with 40 g of untreated steamed crimped oats for 9-h; consumption was measured and those animals that refused the oats were eliminated from the study.

**Weight ranking and assignment of prairie dogs to day of treatment**—To allocate prairie dogs of similar body weight to each of the 3-days of treatment, we selected animals passing the pre-bait acceptance tests, ranked them by weight within each sex, and randomly assigned them from each weight and sex class to the control or treated group and to one of the 3-days of testing. Initially, 15 prairie dogs were assigned to the ZP group and 10 to the control group. However, observations of prairie dog mortality on Day 1 suggested additional prairie dogs would be required for Days 2 and 3, to meet the standard requirement of 10 dead animals. Therefore, an additional five prairie dogs (2 males, 3 females) that previously passed the prebait test were added to the treated bait group for Day 2. The control group remained at 10 animals. For Day 3, six addi-

<sup>1</sup> Reference to commercial entities or products does not imply endorsement by the U.S. government.

tional female prairie dogs that previously passed the prebait test were added to the treated bait group.

**Baiting of prairie dogs with zinc phosphide-treated and control baits**—On the day before testing, the first two groups of prairie dogs (treated and control) assigned to treatment Day 1 were fasted beginning at 1600 h. At 0500 h the next morning (treatment Day 1), each animal received 10 g of either 2.03% ZP bait or control bait. The animal room was closed until 1200 h (treatment Day 1) when the prairie dogs were checked for mortality. At 1500 h, the remaining bait was weighed to determine consumption. Each dead prairie dog was weighed and the mg of ZP ingested per kg of body weight was calculated. The 10 control animals were euthanized with carbon dioxide and weighed. The procedure for preparing the prairie dog tissues was as follows:

For the ZP tissue complex 1, the stomach, liver, and complete intestinal tract (SLI) were excised from 10 dead prairie dogs and weighed. For the ZP tissue complex 2, the remaining carcasses (RC) were weighed. For the control tissue complex 3, the stomach, liver, and complete intestinal tract (SLI) were excised from 10 dead control prairie dogs and weighed. For the control tissue complex 4, the remaining carcasses (RC) were weighed. The same procedures were repeated to prepare tissues for treatment Days 2 and 3.

#### Statistics

The prairie dog body weights measured pretreatment were analyzed to verify that differences did not exist between treatment groups, and to avoid bias due to weight differences. Consumption of treated bait and mg/kg intake of ZP also were analyzed. All three analyses were made using two-factor analysis of variance (ANOVAs), with Sex and Day of treatment as factors.

#### Preparing Domestic Ferrets for Secondary Poisoning Study

**Preconditioning of domestic ferrets to feed on prairie dog tissue**—Forty-four (22 males and 22 females) of the 52 ferrets were randomly selected to be preconditioned to feed on stomach, liver, and intestinal (SLI) tissue from control prairie dogs. SLI tissue was offered for two days and consumption was measured daily. On precondition Day 1 at 0600 h, the frozen SLI tissue from 44 prairie dogs was thawed and the 44 ferrets were fasted. At 1600 h each ferret was given preweighed SLI tissue from a single prairie dog. The animal room was closed until 1600 h the next day (Day 2), when the remaining SLI tissue was weighed. Each ferret then received a second preweighed SLI tissue at 1700 h. On Day 3 at 1600 h, the remaining SLI tissue was removed and weighed and the ferrets were returned to their regular ration.

**Assignment of tissue complexes to domestic ferrets**—The 20 male and 20 female ferrets that had the highest total consumption of the SLI tissue during the preconditioning test were ranked by weight within each sex. Representatives from each weight and sex class were randomly assigned to four groups, with each group containing 5 male and 5 female ferrets.

#### Secondary Poisoning Feeding Study

**Feeding zinc phosphide treated and control tissues to ferrets**—On the day of testing (treatment Day 1:0600 h), each ferret was weighed and food was removed. At 1700 h each ferret received its assigned preweighed tissue complex. The

number of the prairie dog carcass (RC) or SLI tissue given to each ferret was recorded. The animal room was closed until 1600 h (Day 2) when the unconsumed tissue was removed and consumption was calculated and recorded. The animals were observed for symptoms of poisoning and the cages were examined for vomitus.

Control and treated ferrets were given a second fresh preweighed tissue of the assigned tissue complex at 1700 h (Day 2). The animal room was closed again until 1600 h (Day 3) when the procedure was repeated. On Day 4 at 1600 h, the unconsumed tissue was removed and consumption was calculated and recorded. All ferrets were weighed and returned to their regular ration. During the posttreatment observation period, the ferrets were weighed on Days 1, 7, and 14.

#### Statistics

Total amounts and percentages of prairie dog tissue consumed by ferrets for both the SLI and RC tissues were analyzed by a three-factor, repeated-measures ANOVA. Ferret body weights were analyzed by a three-factor, repeated measures ANOVA, with Time as the repeated measure. For body weights, Time included the one pretreatment measurement and three posttreatment measurements on Days 1, 7, and 14.

#### Zinc Phosphide—Prairie Dog Residue Study

**Weight ranking and assignment of prairie dogs to treatments**—Ten male and 10 female prairie dogs were ranked by weight and divided into 5 weight classes within each sex. Each animal from each weight and sex group was randomly assigned to either the ZP or control treatment. Each treatment included 10 animals (5 males, 5 females).

**Prebaiting the prairie dogs with untreated oats**—On the day before testing, all the prairie dogs were fasted beginning at 1600 h. At 0500 h the next morning, each prairie dog was prebaited with 40 g of steamed crimped oats. At 1500 h the remaining bait was weighed to determine consumption and the animals were placed on their regular ration.

**Feeding treated and control zinc phosphide baits to prairie dogs**—Only one prairie dog could be tested daily because only the tissue from one animal could be analyzed each day. The daily selection alternated between treated and control animals. At 1600 h on the day before testing, one treated animal was randomly selected and fasted. At 0500 h the next morning (treatment Day 1) it received 10 g of 2.03% ZP bait. The animal room was closed until 1500 h when each prairie dog was checked for condition and symptoms of intoxication; checks were made hourly until 2000 h. If death did not occur by 2000 h, the animal was not used.

The contents of the stomach and small intestines were removed from each prairie dog and the addition of acid to these two tissues hydrolyzed the ZP into phosphine gas. The caecum, large intestine, samples of the hearts, gall bladder/liver, lungs, spleen, kidneys and muscle were taken from each animal. For these tissue groups the phosphine gas was measured without the addition of acid. The analytical method analyzes phosphine gas therefore the values reported in the results section were calculated as ZP. Upon death the remaining bait was weighed to determine consumption and the mg of ZP consumed per kg of body weight was calculated. Means and standard errors for the ZP present were calculated for each tissue type.

Control prairie dogs were tested in the same manner as

the treated prairie dogs with the following two exceptions. They received 10 g of control bait and at 1500 h they were euthanized with carbon dioxide.

## RESULTS

### Secondary Poisoning Hazard Study

**Prebaiting prairie dogs with untreated oats**—The 30 prairie dogs from which tissues were used for tissue complexes 1 and 2 and that had been assigned to Treatment Days 1, 2, and 3 each consumed an average of 27.39 g (SE = 0.92), 27.44 (SE = 1.29), and 23.03 g (SE = 3.27) of prebait respectively. The 30 control animals allotted to the same three days each consumed an average of 28.23 g (SE = 0.81), 24.59 g (SE = 3.00), and 27.92 g (SE = 0.95) of prebait, respectively.

### Baiting of Prairie Dogs with Zinc Phosphide Treated and Control Baits

**Zinc phosphide treated bait**—On Day 1, 10 (4 males, 6 females) prairie dogs died within 10 h after the bait was offered. They had consumed between 6.44 and 7.63 g ( $\bar{x}$  = 7.20 g, SE = 0.10) of ZP bait, equivalent to 131 to 155 mg ( $\bar{x}$  = 146.20 mg, SE = 2.12) of ZP. The average intake of ZP for both sexes was 227.12 mg/kg (SE = 23.34). On Day 2, 11 (5 males, 6 females) prairie dogs died within 10 h after the bait was offered. Ten (5 males, 5 females) of these animals were randomly selected to be processed for SLI and RC tissue. These 10 prairie dogs had consumed between 6.47 and 7.68 g ( $\bar{x}$  = 7.13 g, SE = 0.15) of ZP bait or between 131 and 156 mg ( $\bar{x}$  = 144.66 mg, SE = 3.00) of ZP. The average intake of ZP for both sexes was 212.84 mg/kg (SE = 12.72). On Day 3, 10 animals (5 males, 5 females) prairie dogs died within 10 h after the bait was offered. They had consumed between 5.40 and 7.80 g ( $\bar{x}$  = 6.78 g, SE = 0.23) of ZP bait or between 110 and 158 mg ( $\bar{x}$  = 137.55 mg, SE = 4.73) of ZP. The average intake of ZP for both sexes was 201.17 mg/kg (SE = 13.07).

**Control bait**—The 10 prairie dogs assigned to each of Days 1, 2, and 3 had each consumed an average of 8.14 g (SE = 0.11), 8.14 g (SE = 0.21), and 8.38 g (SE = 0.19) of control bait, respectively.

**Statistics on body weights, bait consumption levels, and mg/kg dosages**—There were no significant differences ( $p = 0.70$ ) in mean body weights of prairie dogs allotted to the three treatment days or their mg/kg intake of ZP. However, a significant difference ( $p = 0.033$ ) occurred in the mean consumption levels of bait across days. (These means were Day 1, 6.99 g; Day 2, 7.05 g; and Day 3, 6.51 g). Separating the mean bait consumption levels among days by Duncan's multiple range test showed that a significant difference ( $p = 0.05$ ) occurred only between Days 2 and 3 with animals consuming significantly less on Day 3.

For ZP bait consumption, a borderline ( $p = 0.062$ ) significant Day x Sex interaction was indicated. A Duncan's multiple range test on the interaction means showed that males consumed significantly less on Day 2 (mean = 6.20 g) than they did on Day 3 (mean = 7.32 g), whereas, the females showed the same level of consumption on both days. **Preconditioning of domestic ferrets to feed on prairie dogs**—All 44 ferrets tested consumed the SLI tissue. Over the 2-day feeding period, the 10 ferrets later assigned to each of the four groups consumed an average of SLI tissue that

ranged from 248.5 to 269.7 g. Of the total SLI tissue that was presented to the four groups (1, 2, 3, and 4), the ferrets ingested an average of 89.5% (SE = 4.08), 91.9% (SE = 2.60), 86.2% (SE = 5.30) and 83.4% (SE = 4.23), respectively.

### Secondary Poisoning Feeding Study

**Feeding zinc phosphide treated (SLI and RC) and control (SLI and RC) tissue to ferrets**—The ferrets consumed both the ZP and control (SLI and RC) tissue over the 3-day feeding period, consuming less of the treated tissue (SLI and RC) than the control tissue (SLI and RC) (Table 1). Ferrets consumed an average of 83.84% (SE = 3.4) of the total treated SLI tissue offered, a quantity less than the 96.56% (SE = 1.91) consumed by the ferrets feeding on the control SLI tissue (Table 2). Of the 30 individual SLI tissues offered to the control ferrets, they consumed the entire contents of 17. The treated ferrets consumed the entire contents of only 5 of the 30 individual SLI tissues issued. The carcass tissue offered exceeded what the ferrets would consume in one day. Ferrets consumed an average of 18.06% (SE = 1.69) of the total treated RC tissue presented, a quantity higher than the 14.48% (SE = 2.19) consumed by the ferrets feeding on the control tissue. At this level of bait consumption, no symptoms of poisoning or emesis were observed in any of the ferrets, and no mortality occurred.

Unfortunately, it was not possible to determine the level of ZP residue in the prairie dog tissues presented to the ferrets. However, the mg of ZP consumed by each prairie dog

Table 1. Domestic ferret consumption of zinc phosphide treated and control black-tailed prairie dog tissue, total consumption per day and total consumption over the 3-day feeding period (N = 10 ferrets per treated and control tissue).

	Tissue consumption (g)			
	Treated SLI	Control SLI	Treated RC	Control RC
Day 1	1047.39	1039.89	977.74	810.38
Day 2	1025.68	1079.44	1051.65	1025.25
Day 3	1010.46	1019.30	797.72	999.32
Total	3083.53	3138.63	2827.11	2834.95

Table 2. Mean percentage of zinc phosphide and control black-tailed prairie dog tissue consumed by domestic ferrets over a 3-day feeding period (N = 10 ferrets per each treated and control tissue).

	Mean percentage of tissue consumption			
	Treated SLI	Control SLI	Treated RC	Control RC
Day 1	87.91	97.44	19.8	12.37
Day 2	79.14	97.54	19.4	17.22
Day 3	84.46	94.71	15.5	13.88
Mean	83.84	96.56	18.07	14.49
SE	2.55	0.93	1.54	1.43

was calculated. The three prairie dog SLI tissues consumed by each ferret were then identified and the mean consumption of ZP by ferrets (mg) was determined as: 428.41 mg (SE = 4.57) for treated SLI tissue and 428.41 mg (SE = 7.21) for treated RC tissue.

### Statistics

Differences in total amounts and percentages of prairie dog tissue consumed by domestic ferrets were calculated as follows:

For the total SLI consumption measure, the Day x Sex effect was significant ( $p = 0.05$ ). These SLI results could be influenced by males possibly receiving less SLI tissue than they could consume. If this was a true Day x Sex interaction, then it could be explained by female consumption rising after 2 days with male consumption decreasing after 2 days.

For the mean percentage of SLI consumed measure, significant differences occurred for both the Treatment and Sex factors ( $p = 0.0025$ ). Overall, a lesser proportion of the treated SLI tissue offered ( $\bar{x} = 83.3\%$ ) was consumed than was the proportion of control SLI tissue offered ( $\bar{x} = 96.6\%$ ). Males consumed a greater proportion of SLI tissue offered ( $\bar{x} = 94.4\%$ ) than did the females ( $\bar{x} = 86.0\%$ ).

For the total RC consumption measure, the factor of Sex was highly significant ( $P = 0.0006$ ). This was the only significant effect observed. Females consumed less tissue overall, averaging 74.8 g compared to 114.3 g consumed by males.

For the percentage of RC consumed measure, the only significant difference was Sex ( $p = 0.0110$ ). Males consumed a greater proportion of RC tissue offered ( $\bar{x} = 19.7\%$ ) than did the females ( $\bar{x} = 13.2\%$ ).

### Body Weights of Ferrets

**Treated SLI Tissue (Group 1)**—All 10 ferrets lost weight during the 3-day testing period. The 5 males and 5 females weighed an average of 804.2 g (SE = 40.1) on the first day of treatment; whereas on Day 1 posttreatment, they weighed an average of 747.2 g (SE = 35.2), an average loss of 7.1%. When weighed on Days 7 and 14 posttreatment, all 10 ferrets had gained weight, weighing an average of 892.9 g (SE = 56.3), and 960.9 g (SE = 59.4), respectively.

**Treated RC tissue (Group 2)**—Nine ferrets gained weight, and one ferret lost weight during the 3-day testing period. The 5 males and 5 females weighed an average of 804.6 g (SE = 40.5) on the day before treatment; whereas on Day 1 posttreatment, they weighed an average of 837.1 g (SE = 45.8), an average gain of 3.9%. When weighed again on Day 7, all ferrets had gained weight. By Day 14 posttreatment, 9 ferrets had gained weight, and one had lost weight since Day 7. On Days 7 and 14 posttreatment, ferret body weights averaged 944.6 g (SE = 56.6), and 1000.9 g (SE = 61.9), respectively.

**Control SLI tissue consumption (Group 3)**—Six ferrets gained weight, and four ferrets lost weight during the 3-day testing period. The 5 males and 5 females weighed an average of 803.2 g (SE = 41.2) on the first day of treatment; whereas on Day 1 posttreatment, they weighed an average of 804.9 g (SE = 37.6), an average gain of 0.2%. When weighed on Day 7 posttreatment, 9 ferrets had gained weight, and one had lost weight. On Day 14, all 10 ferrets had gained weight. On Days 7 and 14 posttreatment, their body weights averaged 882.0 g (SE = 56.0), and 951.3 g (SE = 61.9), respectively.

**Treated RC tissue consumption (Group 4)**—Seven ferrets

gained weight, and three ferrets lost weight during the 3-day testing period. The 5 males and 5 females weighed an average of 808.8 g (SE = 37.6) on the first day of treatment; whereas on Day 1 posttreatment, they weighed an average of 817.2 g (SE = 46.9), an average gain of 1.0%. When weighed on Days 7 and 14 posttreatment, all 10 ferrets had gained weight, weighing an average of 923.6 g (SE = 50.2), and 979.0 g (SE = 55.9), respectively.

**Statistics**—Pre- and posttreatment ferret body weights were analyzed by a three-factor (pre- vs. posttreatment x Sex x Time in days), repeated measures ANOVA. Significant effects were found for Sex ( $p = 0.0001$ ), Time ( $p = 0.0001$ ), and the Time x Sex interaction ( $p = 0.0001$ ). The interaction contained the two main effects that were both significant; therefore, only the Time x Sex interaction means were examined using multiple comparisons.

Results of application of Duncan's multiple range test to the Time x Sex interaction means are shown in Table 3. All female means were significantly less than all male means. This suggested that the Sex effect was a true significant effect in addition to that associated with the Time x Sex interaction (mean weights: males 994.2 g, females 751.1 g). Within each sex, Days 7 and 14 differed from each other and from pretreatment and posttreatment Day 1. Pretreatment and Day 1 posttreatment body weights did not differ from each other. Because the locations of the differences among days are the same for each sex, the day effect is also significant. There was no difference between pretreatment and Day 1 posttreatment, but Days 7 and 14 differed from each other and from pretreatment and Day 1 posttreatment.

### Zinc Phosphide—Prairie Dog Residue Study

**Feeding treated and control zinc phosphide baits to prairie dogs**—All 10 prairie dogs feeding on the 2.03% ZP bait had residues in their carcasses. These animals had ingested a total of 1,379 mg of ZP but only 123.184 mg or 8.93% of the quantity ingested was recovered from the carcasses. The residue in the gastrointestinal tract was most highly concentrated in the stomachs 117.85 mg, followed by the small intestines 4.59 mg. The large intestines, the caeca, kidneys, gall bladder/liver and spleens contained minute amounts. No ZP resi-

Table 3. For the Time x Sex interaction, Duncan's multiple range test separated the mean body weights for the domestic ferrets pre- and posttreatment.

Days	Sex	Mean body weight (g)	Letter <sup>a</sup>
pretreatment	F	709.1	a
pretreatment	M	901.2	b
1	F	694.6	a
1	M	908.5	b
7	F	776.4	c
7	M	1045.3	d
14	F	824.2	e
14	M	1121.9	f

<sup>a</sup>Means with no letter in common are significantly different.

due was found in the lung, heart, or muscle tissues.

The first two control prairie dogs analyzed for ZP residue were positive. One animal contained 0.230  $\mu\text{g}$  of ZP in the small intestine; the second animal contained 0.576  $\mu\text{g}$ , also in the small intestine. No ZP was present in any other tissue of these two animals. The control bait analyzed positive for ZP, with a residue of 4.0  $\mu\text{g}/\text{g}$ .

We determined that, despite repeated cleaning of the bait mixer, ZP contaminated the next four control baits formulated. Replacement of the rubber gaskets solved this problem, and the fifth control bait formulated had no detectable ZP residue. The limit of detection for ZP was 0.086  $\mu\text{g}/\text{g}$  for this control bait. The samples for the remaining eight control prairie dogs tested showed no detectable ZP residues.

## DISCUSSION

No mortality, emesis, or visible symptoms of intoxication were observed among ferrets exposed to tissues obtained from prairie dogs killed with ZP, indicating little or no detectable risk of secondary poisoning from these gross symptom measures. We attributed this conclusion to the following factors.

The residue data showed that about 90% of the ZP ingested by the prairie dogs was not present in the carcass or not detected. However, this 90% value should be regarded as a minimal value. Had acid been added to the other tissues sampled, in addition to the stomach and small intestines, any ZP present should have converted to phosphine gas. Consequently, when sampled a higher phosphine gas concentration would have been detected.

Approximately 99% of the ZP residue was found in the gastrointestinal tract and none was in the muscle tissue. This result suggested that only those ferrets that fed on the SLI

tissue could be at risk. Even in these cases the amount of ZP contained in the tissue was apparently too small to induce emesis in ferrets consuming the treated SLI tissue (Group 1). The difference in tissue consumption between ferrets in the treated-RC tissue group and those in the control-RC tissue group was not significant ( $p = 0.95$ ), indicating no avoidance of the RC tissue by the treated group.

Ferrets that fed on treated-SLI tissue did show indications of avoidance by reducing food consumption. Although no significant difference ( $p = 0.79$ ) was observed in the total amount of tissue consumed by the SLI-treated and SLI-control groups, a significant difference ( $p = 0.0025$ ) in the percentage of tissue that was issued and consumed did occur between the treated and control SLI groups. The amount of SLI tissue was limited. Had more been available, such that all ferrets would have more opportunity to feed *ad libitum*, a difference in the total amount of tissue consumed might have been more readily detectable between the control and treated group.

The final contributing factor was the limited intake of the 2.03% ZP bait by prairie dogs. They consumed an average of about 7 g of bait; none consumed more than 8 g. The maximum ZP intake of the 2.03% bait did not exceed 150 mg. The 150 mg was sufficient to kill the prairie dogs. Any ZP remaining in the prairie dog tissues was insufficient to produce any visible symptoms of intoxication in ferrets.

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