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## Lack of Relay Toxicity in Ferret Hybrids Fed Carbaryl-Treated Prairie Dogs

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**ABSTRACT:** Carbaryl (1-naphthol methylcarbamate) is being considered for control of fleas on prairie dogs (*Cynomys* spp.) used in black-footed ferret (*Mustela nigripes*) recovery in the western United States. The potential for relay toxicity in ferrets was determined by feeding carbaryl treated prairie dogs to black-footed ferret × Siberian polecat (*M. eversmanni*) hybrids. Adult prairie dogs were treated topically with 2.5 g of commercial 5% carbaryl dust sold as flea powder. After 14 days prairie dogs were killed and fed to ferrets. Potential for relay toxicity was evaluated by analyzing ferret blood cholinesterase (ChE), prairie dog brain ChE, and hepatic carbamate concentration. There was no difference between pre- and post-exposure blood ChE activity, nor did treated prairie dog brain ChE differ significantly from controls. Post-exposure blood ChE did not exhibit reactivation after dilution in aqueous buffer. Hepatic carbaryl concentrations were less than detection limits (50 ppb). Based on these results, we conclude that short-term use of carbaryl for flea control on prairie dogs does not pose a hazard of relay toxicity in black-footed ferrets.

**Key words:** Black-footed ferret, carbaryl, *Mustela nigripes*, prairie dog, residue, toxicity.

Prairie dogs (*Cynomys* spp.) are the primary food source of wild black-footed ferrets (*Mustela nigripes*) in the western United States. Fleas associated with prairie dogs are vectors for the plague bacilli, *Yersinia pestis*, which causes sylvatic plague. Live trapped prairie dogs are utilized as a food source in the black-footed ferret recovery and reintroduction program. Thus, sylvatic plague is a potential health hazard to both black-footed ferrets and human caretakers (Williams et al., 1994) and a safe, effective method of flea control is desirable.

Carbaryl (1-naphthol methylcarbamate), a carbamate insecticide with low mammalian toxicity, is one potential flea control tool. Carbaryl is efficiently degraded in mammals (Kuhr and Dorough, 1976) and

therefore has a short biological half-life. It is widely used for ectoparasite control on domestic animals (Ware, 1994). Black-footed ferrets might be exposed to insecticide residues when carbaryl is used to control fleas on prairie dogs. The purpose of this experiment was to evaluate the potential for relay toxicity when carbaryl is used to control fleas on prairie dogs.

Twenty-five adult white-tailed prairie dogs (*Cynomys leucurus*) were live trapped in Phillips County, South Dakota (USA) and transported to the Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA) where they were weighed and housed in wire bottomed cages. Water and rat chow (Lab Cubes, Manna Pro, St. Louis, Missouri, USA) were provided *ad libitum*. Locally raised black-footed ferret × Siberian polecat (*M. eversmanni*) hybrids were housed in wire bottomed cages at the same facility and fed commercial ferret ration (Purina Ferret Chow, St. Louis, Missouri) or a mixture of dry cat food (Wayne KitKat Glo, Pet Products Plus, Inc., St. Charles, Missouri, USA) and pelleted mink food (Gro-fur Dark Pellets, Milk Specialists, Co., New Holstein, Wisconsin, USA). Hybrid ferrets were selected as the best available model of the black-footed ferret (Williams et al., 1996). Water was provided *ad libitum*.

A commercial carbaryl preparation sold as pet flea powder (Sevin® 5% dust, C. J. Martin Co., Nacodoches, Texas, USA) were applied topically at the rate of 2.5 g/animal to 20 prairie dogs and rubbed in to assure thorough contact with the skin. Carbaryl which fell off during treatment was collected and weighed resulting in >100 mg active ingredient delivered per animal. Five untreated prairie dogs served as controls. Animals were observed post-

exposure and during feeding and cage cleaning. After 14 days, prairie dogs were weighed and killed by CO<sub>2</sub> inhalation. Brain and a portion of liver were removed as quickly as possible (<90 sec after death) and quick frozen in liquid nitrogen until assayed. Thorax, including skin and viscera, and remaining liver were fed to the hybrid ferrets. Ferrets were observed several times within 2 hr of feeding for clinical signs of carbaryl intoxication. Uneaten portions of prairie dog were removed from ferret cages after approximately 12 hr.

Hybrid ferrets were anesthetized with ketamine hydrochloride (32 mg/kg) and diazepam (0.16 mg/kg) in (Williams et al., 1996). One ml of blood was collected from the jugular vein of each ferret into lithium-heparin vacutainers and quick frozen in liquid nitrogen. Blood for cholinesterase activity determination was collected 30 days and 24 hr prior to and 12 hr and 72 hr after feeding carbaryl-treated prairie dogs.

Prairie dog brain CHe and ferret blood were analyzed according to the Harlin modification of the Ellman method (Harlin and Ross, 1990). Two brains were inadvertently allowed to thaw. Cholinesterase activity in these two brains did not differ appreciably from the other brains, but the data were not included in analyses. Treated prairie dog livers were analyzed for carbamates by the method of Holstege et al. (1993).

Carbamate inhibition of cholinesterase is characterized by spontaneous reactivation when diluted in aqueous buffers (Nostrandt et al., 1993). A baseline for CHe reactivation was established by spiking pooled ferret blood with 10 µl of 1,000 ppm aqueous carbaryl. Blood was diluted in phosphate buffer (pH = 7.0) incubated at room temperature for 5, 10, 20, 40, 60, or 80 min and assayed for CHe as previously described (Harlin and Ross, 1990). Blood CHe increased to greater than 80% of unspiked baseline activity with 40 min incubation. Reactivation in post-exposure ferret blood was determined similarly.

Quantitative data (ferret blood CHe, prairie dog brain CHe) were analyzed by the SAS General Linear Models procedure (release 6.1, SAS Institute, Cary, North Carolina, USA). Differences between pre- and post-exposure means (blood) and treatment group means (brain) were considered statistically significant at  $P < 0.05$ .

Neither ferrets nor treated prairie dogs showed signs of illness during the experiment. All prairie dogs gained weight with the exception of one control which lost approximately 10% of body weight. Blood CHe activity of ferrets did not differ significantly between pre- and post-exposure values. Blood CHe (mean ± standard deviation) was  $2.44 \pm 0.46$ ,  $2.68 \pm 0.51$ ,  $2.45 \pm 0.41$  and  $2.41 \pm 0.41$  µmole/ml/min at 30 days or 24 hr pre-exposure, or 12 hr or 3 days post-exposure, respectively. Incubating blood samples drawn 12 hr post-exposure for 40 min resulted in a mean of  $3.5 \pm 0.9\%$  decline in activity which is within normal analytical variation for the Harlin method in our laboratory. Prairie dog brain CHe activity did not differ significantly ( $P > 0.05$ ) between treatment ( $13.7$  µmole/g/min) and control ( $12.5$  µmole/g/min) groups. Carbaryl liver values were less than the detection limit (50 ppb).

We interpret these data to indicate that there is little risk of relay toxicity to ferrets with short-term use of carbaryl followed by an appropriate withdrawal period in prairie dogs. The fact that there was no CHe carbamoylation in ferret blood suggests that any residual carbaryl from the anti-flea treatment in the prairie dogs had been cleared. This is further supported by the lack of detectable residues in prairie dog livers and by the fact that prairie dog brain CHe was not depressed 14 days post-exposure.

Although the question of carbaryl food residues should be evaluated with a chronic study, these data suggest that a single use of carbaryl to control fleas on prairie dogs, followed by a 14 day withdrawal,

does not result in carbaryl tissue residues and does not pose a hazard of relay intoxication when previously-treated prairie dogs are fed to ferrets.

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