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Cage Efficacy Trials with Cholecalciferol Plus Diphacinone and Cholecalciferol Plus Brodifacoum Baits Using Richardson's Ground Squirrels

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ABSTRACT: There are many species of ground squirrels in North America and some species can cause substantial damage to agriculture and other resources. Traps and rodenticides are the most commonly used methods to reduce populations and damage. We tested the efficacy of three new formulations of rodenticides containing two active ingredients: cholecalciferol and diphacinone or cholecalciferol and brodifacoum using wild-caught Richardson's ground squirrels. All three formulations had an efficacy of ≥60% and one cholecalciferol and brodifacoum formulation had an efficacy of 90%. Additionally, all formulations had lower concentrations of one or both active ingredients than commercial rodenticides that contain only one of the active ingredients. We also noted that squirrels that died generally consumed much more bait than those that survived. Most of these formulations had high efficacies in previous trials with voles and pocket gophers. We recommend that a field efficacy trial be conducted in a ground squirrel colony.

KEY WORDS: brodifacoum, cholecalciferol, diphacinone, Richardson's ground squirrel, rodent control, rodenticide, *Spermophilus richardsonii*

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INTRODUCTION

Ground squirrels can cause substantial damage to crops and rangeland forage, especially when densities are high (>10/ha; Banfield 1974, Askham 1994, Marsh 1994, Gebhardt et al. 2011, Baldwin et al. 2013). They also can undermine foundations, levees and road banks, damage irrigation systems, and prey upon eggs and young of ground-nesting birds. Soil mounds can be damaging to farm equipment. Ground squirrels are also reservoirs of plague in the western states. Populations and damage are reduced using rodenticide baits, burrow fumigants, and traps (Askham 1994, Marsh 1994). Exclusion is generally considered of limited effectiveness, especially over large areas (Marsh et al. 1990, Askham 1994, Witmer et al. 2008).

Primary control options for ground squirrels include trapping, burrow fumigation with aluminum phosphide, and baiting with rodenticides (Askham 1994, Marsh 1994). Two toxicants are used to control ground squirrels: zinc phosphide and anticoagulants. However, researchers are looking into new active ingredients and other formulations of current rodenticide baits. This is occurring because 1) manufacturers are removing some products from the commercial market for a variety of reasons, 2) some current rodenticide formulations have become much less effective, 3) non-target and humaneness concerns have increased, and 4) the USEPA rodenticide hazards mitigation measures have been implemented and resulted in fewer products available and many restrictions on uses.

Researchers in North America and New Zealand are investigating new "combination" rodenticides. These rodenticides have two active ingredients, combining an anticoagulant and an acute active ingredient (e.g., cholecalciferol). In New Zealand, Eason et al. (2010b) found that one having the two active ingredients, cholecalciferol and coumatetralyl, produced promising results with rats and mice. Interestingly, they were able to obtain high efficacy with lower concentrations of the active ingredients than the concentrations used when either active ingredient are used as the only toxicant in a commercial bait. Hence, there may be some synergistic effect. This is noteworthy because if lower concentrations can be used to effectively control rodent populations, there could be a lower risk of harm to non-target animals. More recently, researchers have shown that that a cholecalciferol plus diphacinone and a cholecalciferol plus brodifacoum pelleted bait were very effective with California voles (*Microtus* spp.; Witmer et al. 2014, Baldwin et al. 2016), pocket gophers (*Thomomys* spp.; Baldwin et al. 2017, Witmer et al. 2017), and house mice (*Mus musculus*; Witmer and Moulton 2014). The objective of this study was to test the efficacy of these baits using wild-caught Richardson's ground squirrels (*Spermophilus richardsonii*). This would complete the initial data sets for the U.S. Environmental Protection Agency (USEPA) to review when considering the registration of one or more combination rodenticide baits for use in the U.S. on major agriculture rodent pests.

METHODS

Ground squirrels (henceforth, squirrels) for this study were Richardson's ground squirrels that were live-trapped in Montana and transported to NWRC, Fort Collins, CO, under another study protocol (QA-2243). Squirrels were kept in individual numbered, plastic shoebox cages in an animal room of the Invasive Species Research Building (ISRB). They were provided daily with rodent chow, hay, and a piece of fruit or vegetable; water was provided *ad libitum*. They each had a den tube, bedding, and a rawhide chew stick. A quarantine period was not required as they

had already been in captivity a considerable time under QA-2243. There were 4 treatment groups with 10 animals randomly assigned to each group for the two-choice cage efficacy trial. There was also a control group of 10 animals maintained on the maintenance diet. An effort was made to include both males and females in each group, but the ratio varied from 1:1; each group contained 4-5 females.

Four experimental rodenticides were tested, each with two active ingredients, but varying in concentrations:

1) Connovation, New Zealand, experimental rodenticide bait (0.005% diphacinone, 0.03% cholecalciferol)
2) Bell Laboratories, Wisconsin, experimental rodenticide bait (0.0025% brodifacoum, 0.015% cholecalciferol)
3) Bell Laboratories, Wisconsin, experimental rodenticide bait (0.0025% brodifacoum, 0.03% cholecalciferol)
4) Bell Laboratories, Wisconsin, experimental rodenticide bait (0.001% brodifacoum, 0.015% cholecalciferol)

The weight, sex, cage number, and treatment of each squirrel was recorded before the initiation of the trial. On Day 1 of the trial, about 50 g of the test rodenticide bait was placed in a small bowl and put in each cage as appropriate; the weight of the bait added was recorded. Squirrels continued to receive the maintenance diet as well. They continued to receive water *ad libitum* throughout the trial. Foods were replenished as needed, so that it was always available to the treatment squirrels during the next 10 days (i.e., throughout the rodenticide exposure period). The weight of the bait added was recorded. Uneaten rodenticide baits in the cages were gathered at the end of the 10-day exposure period and weighed. This allowed the determination of the total amount of rodenticide bait consumed during the trial by each squirrel. On Day 11, squirrels were placed in clean cages and were put back on their maintenance diet for another 14 days of observation (post-exposure period).

Squirrels were examined twice daily by the research staff and their condition and any mortalities were recorded. When an animal was observed by research or animal care staff to be experiencing more than momentary pain or distress, they contacted the Study Director or the Attending Veterinarian and had the animal examined and possibly euthanized. Dead squirrels were weighed and placed in individual, labeled zip-lock bags and refrigerated for later necropsy. When necropsied, they were examined for signs of anticoagulant poisoning as described by Stone et al. (1999). Additionally, during necropsy, animals were examined for signs of disease, endoparasites, or injury that may have contributed to their condition or demise. All surviving squirrels were euthanized and incinerated at the end of the study with isoflurane and CO_2.

RESULTS

Efficacy and Days-to-Death

The efficacy of the tested rodenticides varied from 60-90% (Table 1). The least effective rodenticide bait was the bait containing diphacinone. The most effective bait was the one containing 0.0025% brodifacoum, and 0.03% cholecalciferol. No control squirrels died during the trial.

The average days-to-death for treatment animals that died during the trial varied somewhat across treatment groups. It ranged from 6.0-10.3 days (Table 1).

Bait Consumption

The average bait consumption varied across treatment groups. It ranged from 8.3-25.0 g (Table 1). The most bait consumed was by the treatment group with the diphacinone bait, averaging 25.0 g. All three treatment groups with baits containing brodifacoum consumed similar amounts, averaging 8.3-12.0 g.

In each treatment group, the squirrels that died consumed substantially more bait than those that survived (Table 1). Squirrels that died consumed an average of 10.8-30.5 g of bait, whereas squirrels that survived consumed an average of 2.3-16.7 g of bait. Squirrels that died in the T2 treatment group (cholecalciferol plus brodifacoum) consumed significantly more bait ($F = 28.01$, $P = 0.0007$) than the squirrels that survived as did the squirrels in the T4 treatment group (cholecalciferol plus brodifacoum; $F = 6.51$, $P = 0.0341$). We could not do a statistical analysis for the T3 treatment group (cholecalciferol plus brodifacoum) because only 1 squirrel survived; hence, we did not have a mean and variance for the squirrel survival. In the T1 treatment group (cholecalciferol plus diphacinone) the squirrels that died consumed almost twice as much bait (30.6 g) on average as the squirrels that survived (16.8 g), but the difference was not significant ($F = 2.62$, $P = 0.1439$), perhaps because of the large variances in consumption amounts in those two groups of squirrels.

DISCUSSION

Rodents cause substantial damage to crops in California and rodenticides have been major tools for reducing that damage (Witmer and Eisemann 2007, Baldwin et al. 2013,. However, numerous issues have arisen that may impact rodenticide use in the future. Some rodenticides are no longer effective because of resistance, over-use, or palatability issues (Salmon and Lawrence 2006, Witmer et al. 2013). Some commercial products are removed from the market because of increased costs of ingredients or because of new rodenticide mitigation measures implemented by the USEPA (Hornbaker and Baldwin 2010). There are also increasing concerns about impacts to non-target animals from both acute rodenticides (mainly primary hazards) and anticoagulant rodenticides (mainly secondary hazards; e.g., McMillin 2012, Crowell et al. 2013, Stansley et al. 2014). Finally, there is an increasing concern about the humaneness of some rodenticides (mainly with anticoagulants; e.g., Lapidge et al. 2009). Hence, various researchers in the U.S. and elsewhere are investigating alternative formulations and/

Table 1. Rodenticide treatments, percent efficacy, average days-to-death, and average bait consumption by all squirrels in the group, and by those dying versus those surviving in each treatment group.

Treatment	% Efficacy	Ave. Days-to-Death (S.D.) for Non-survivors	Ave. Bait Consumption g (S.D.), All Squirrels	Ave. Bait Consumption g (S.D.), Non-survivors	Ave. Bait Consumption g (S.D.), Survivors
T1 0.03% cholecalciferol + 0.005% diphacinone, pellet	60%	7.3 (3.5)	25.0 (14.3)	30.5 (13.5)	16.7 (12.7)
T2 0.015% cholecalciferol + 0.0025% brodifacoum, pellet	70%	6.0 (1.5)	8.3 (4.7)	10.8 (2.7)	2.3 (0.4)
T3 0.03% cholecalciferol + 0.0025% brodifacoum, pellet	90%	8.3 (3.6)	11.9 (6.1)	12.6 (5.9)	5.0 (0.0)
T4 0.015% cholecalciferol + 0.001% brodifacoum, pellet	70%	10.3 (3.7)	12.0 (5.2)	14.1 (4.5)	7.0 (2.3)

or active ingredients (Eason et al. 2010a, Eason et al. 2010b, Blackie et al. 2013, Morgan et al. 2013, Witmer et al. 2013, Witmer and Moulton 2014).

In this study, we looked at some new rodenticide formulations that contained two active ingredients. Currently, there are no such products registered in the U.S. However, recent studies with voles, pocket gophers, and house mice have shown that these formulations can be very effective (see citations in the Introduction). One advantage of the two active ingredient rodenticide is that lower concentrations of one or both active ingredients may be possible without reduced efficacy. In fact, the most efficacious rodenticide in this study contained 50% less brodifacoum than commercial brodifacoum baits and 60% less cholecalciferol than commercial cholecalciferol baits. This could result in lower costs of production, less toxicant being put into the environment, and less secondary hazards to non-target animals because of lower toxicant residue levels in poisoned rodents.

In this study, we showed the potential of two active ingredient rodenticides to control ground squirrel populations. While the efficacy was only very high (90%) with one of the four formulations and met the USEPA standard of 90% efficacy in a cage trial, the other formulations could perhaps be improved to increase palatability and/or effectiveness. We note this because the animals that survived tended to consume considerably less of the bait. It is also possible that the lower efficacy and consumption levels of some of the baits were related to the squirrels nearing the time of year (late summer) when they would go into hibernation in the prairies of northcentral U.S. and southcentral Canada (Banfield 1974). We recommend that a field efficacy study of the combination bait be conducted in agricultural fields infested with ground squirrels.

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