# FIELD EFFICACY OF DIPHACINONE GRAIN BAITS USED TO CONTROL CALIFORNIA GROUND SQUIRREL

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ABSTRACT: Diphacinone treated oat groats were effective in reducing populations of California ground squirrels (*Spermophilus beecheyi*) by more than 84%. Two concentrations of active ingredient (0.005% and 0.01%) were compared, as well as two application methods: spot baiting and bait stations. Squirrel activity on test plots was assessed before and after bait applications using visual counts and active burrow counts. There was good correspondence between results of the two activity indices. There was no significant improvement in efficacy provided by the higher concentration of diphacinone. Bait consumption was much lower on bait station plots. Squirrel carcasses were found on treated areas at a rate of approximately one carcass per acre. Tissue residue analysis determined that residue loads were nearly identical regardless of the concentration of bait consumed or method of baiting.

KEY WORDS: vertebrate pest control, *Spermophilus beecheyi*, California ground squirrel, rodenticides, diphacinone, efficacy

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## INTRODUCTION

The California ground squirrel (*Spermophilus beecheyi*) is responsible for millions of dollars of damage annually to agriculture (Clark 1978). Since the cancellation of registrations for compound 1080 and strychnine for squirrel control, zinc phosphide and some of the anticoagulant compounds, such as diphacinone and chlorophacinone, have been the only baits available for squirrel control. The California Department of Food and Agriculture is seeking a Section 3 EPA registration of diphacinone treated grain bait for control of the California ground squirrel. These baits have been carried under 24(c) registrations previously. As part of the required data package field efficacy must be demonstrated, with a 70% level of control as the threshold.

This study was designed to evaluate the field efficacy of Rodent Bait Diphacinone Treated Grain, using two concentrations of active ingredient and two application methods. Degradation rates of baits placed in the field and residue loads in ground squirrel carcasses were also assessed.

## METHODS AND MATERIALS

### Study Site

The study was conducted on the San Joaquin Experimental Range, a 4,500 acre (1,790 ha) ranch located approximately 17 miles north of Fresno, California in the lower Sierra Nevada Foothills. Elevations range from 700 to 1700 feet above sea level. Winters are mild and moist and the summers hot and dry. Annual rainfall averages 19 inches. The vegetation is classified as the plant-oak woodland type, consisting of grassland, savannah, and dense stands of trees and brush (Duncan, et al. 1985). Most herbaceous plant species germinate with the fall rains, grow rapidly and set seed in the spring, drying out by mid-May (Larson, et al. 1985). This study was scheduled to present the bait at a time when the squirrel's diet is shifting from green forage to seeds, and when the young of the year are weaned and actively foraging.

Wildlife is abundant on the ranch. The open areas support large, well established populations of *Spermophilus beecheyi*. Squirrels are distributed over the entire ranch, although densities are greatest in the large open meadows.

Seventeen census plots were established on the ranch in mid-May 1994. Census plots ranged from 1.4 to 3.3 acres in size. Census plot boundaries were marked with wire surveying stakes. Buffer zones of approximately 225 feet were marked around the perimeter of each census plot receiving test substance.

Using a randomization procedure, five plots were assigned to receive the 0.005% diphacinone bait applied by spot baiting, five plots to receive the 0.01% diphacinone bait applied by spot baiting, two plots were to be treated with the 0.005% bait in bait stations, and five plots served as untreated control plots. The two geographically closest untreated plots served as controls for the bait station plots.

### Activity Determination

Two activity indices were used: visual counts and active burrow counts.

The visual count method followed the guidelines established by Fagerstone (1983). Natural or artificial blinds which offered a view of most or all of the census plot were established near each census plot boundary.

Visual counts and active burrow counts were conducted before and after bait applications. On spot baited plots, mid-treatment visual censuses were conducted for three days, beginning seven to eight days after the first bait application. This census was conducted to assess baiting efficacy and help determine the appropriate time to begin the post-treatment censusing. Mid-treatment censusing on bait station plots was conducted for three days, starting 14 days after the initial application.

On spot baited plots, post-treatment visual censusing began 10 to 11 days after the first bait applications (bait applications were staggered, with half the plots being baited one day and half the next day). Post-treatment active burrow counts were conducted 14 to 15 days after

the first bait application. Post-treatment censusing on bait station plots began 22 days after the stations were first filled.

During each visual censusing period, three counts were made on each plot for three consecutive days during peak activity periods. At 15 minute intervals, a single slow scan of the plot was made using binoculars. All visible squirrels were counted. From the nine counts conducted over three days, the highest single count was used as the population estimate.

Closed burrow censuses were conducted immediately after the visual counting was completed. All squirrel burrows were closed on the census plots.

Active burrows were counted 48 hours (±2.25 hr) after being closed. Opened burrows were marked with wire surveying stakes to prevent double counting.

Bait Analysis

Baits were manufactured by Haco, Inc. of Madison, Wisconsin. The baits are a whole oat groat coated with diphacinone and an oil soluble blue dye. Representative samples of each product were analyzed at Genesis Laboratories in Fort Collins, Colorado to determine the concentration and homogeneity of the active ingredient. Samples were analyzed before the products were applied in the field.

Bait stability under field conditions was also studied. Approximately 200 g of each bait was placed in aluminum pie pans in the field. The pans were covered with 1/4" mesh hardware cloth and staked down to prevent disturbance by animals. The samples were placed on the first day bait was applied and retrieved after nine days exposure on the spot baited plots. A bait sample was also placed in a bait station, with the openings covered with wire mesh, for 22 days and then retrieved for analysis. Diphacinone concentrations in field samples were compared with samples taken from unopened sacks of bait under storage at the field site.

A high performance liquid chromatography (HPLC) method was used to determine the concentration of diphacinone in the baits. The method employs a reversed phase column, UV detection, and internal standard quantification.

Bait Application: Spot Baiting

Baiting began immediately following the closed burrow censusing. Bait was first applied on May 22, 1994. Plots were baited on a staggered schedule. Five plots received the first application on May 22. The other five plots were first baited on May 23. The final application was on May 29, 1994.

Bait was spread in the grass near active burrows at a rate of 1/3 cup (approximately 45 grams) per placement. Applications were repeated every second day until each plot had received four applications. Placements were replenished only as needed to maintain a continuous supply. The blue dye enabled applicators to readily estimate consumption in the field.

Bait Application: Bait Stations

Bait stations were constructed of 4 inch diameter white PVC pipe joined in a "T" shape. The bait stations were placed in the field four days before bait was applied. Each station was placed in an inverted position, and fastened to a stake. This arrangement provides two entrances and visibility through both ends for squirrels. A cap covered the reservoir. Bait stations were filled on the first day with 7 cups of bait each, so each station contained about 900 grams or 2 pounds of bait. Stations were checked every third day and replenished as needed. Usually bait was added if it appeared that 50% or more of the initial quantity had been consumed. After June 4 (12 days), no more bait was applied to either plot. Stations with high activity were replenished by transferring bait from less active stations.

Baiting Efficacy

Baiting efficacy was calculated by the following formula if there was no decrease in the control plot population index during the period:

$$\text{Efficacy} = \frac{\text{Pre-treatment Census} - \text{Post-treatment Census}}{\text{Pre-treatment Census}} \times 100$$

If the control plot population index declined during the treatment period, the following formula was used to adjust for the change:

$$\text{Efficacy} = 1 - \frac{\text{Post-treatment T-1}}{\text{Pre-treatment T-1}} \times \frac{\text{Pre-treatment C-1}}{\text{Post-treatment C-1}} \times 100$$

Analysis of variance was used to compare efficacy between and within test plots. T-tests were used to test for significant differences between treated and control plots, except in the case of the two bait station plots, which were simply compared to results on the two nearest control plots.

Carcass Searches

Census plots were cleared of carcasses before baiting began as part of the burrow closing procedure. Carcass searches were usually conducted once each day on each treated census plot and buffer zone during the baiting period.

Specimens of ground squirrels found on the surface were collected until a total of 8 to 10 animals had been recovered from each set of treatment plots. Ground squirrel carcasses were analyzed by a GS/MS method. Non-target mammal specimens were examined for signs of the test substance ingestion and symptoms of anticoagulant poisoning.

RESULTS AND DISCUSSION

Plots Sizes, Bait Applications

Census plot areas ranged from 1.9 to 3.9 acres. With the addition of a 225' buffer zone to treated plots, the treated plot areas ranged from 11.5 to 18.4 acres.

Baiting rates ranged from 10.3 to 12.6 pounds per acre on spot baited plots. The baiting rate was only 5 pounds per acre on the bait station plots (Table 1). The baiting rates for the bait station plots represent the consumption, whereas the figures for the spot bait plots represent the amount of bait dispersed.

Table 1. Baiting rates on spot baited plots. Spot baited plots were baited four times, every other day treated. every third day for 22 days. Control plots did not receive placebo bait.

| Treatment | Pounds | Acres | Pounds/ Acre | DPN/acre (g) |
|---|---|---|---|---|
| Spot Baited: | | | | |
| 0.005% | 837.4 | 66.2 | 12.6 | 0.287 |
| 0.010% | 758.3 | 73.8 | 10.3 | 0.470 |
| Bait Stations: | | | | |
| 0.005% | 205.7 | 32.9 | 6.3 | 0.143 |
| Control: | None | 12.1 | None | None |

The bait application pattern illustrated in Figure 1 corresponds well with field observations of bait consumption. Spot baited placements were readily consumed after the first and second applications, with most of the bait being gone within 24 hours. The consumption rate decreased sharply following the third application. It was estimated that roughly 50% of the third application was taken within 48 hours. Much of the fourth application remained uneaten.

Efficacy

Efficacy was well above the EPA standard of 70% for both concentrations of bait and both application methods. Both activity indices found a greater than 90% decline in activity on spot baited plots (Table 2, Figures 2 and 3). Both baits reduced populations by over 90%. There was no significant difference between performance of the different bait concentrations. The bait exposure period was 10 to 11 days.

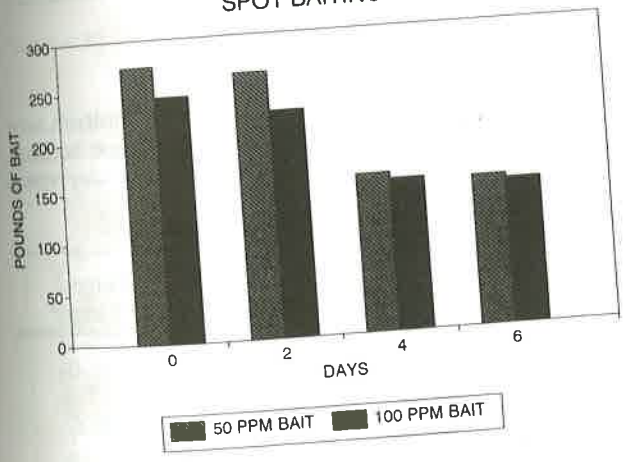


Figure 1. Spot baiting applications. Day 0 represents the initial application. Bait was replenished every other day to maintain a constant supply.

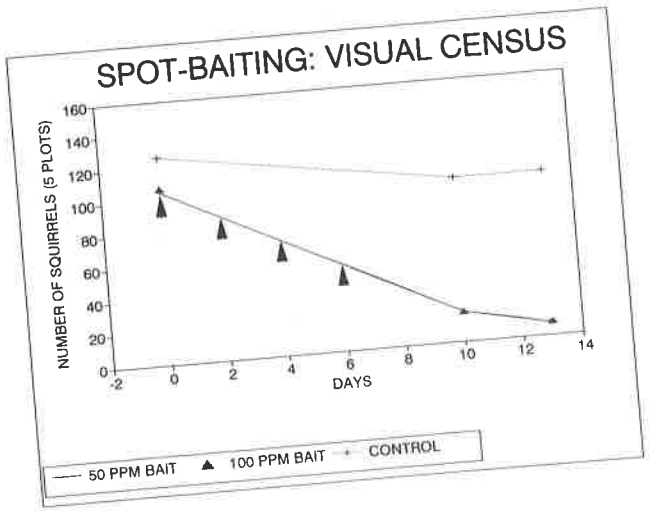


Figure 2. Results of visual activity counts on spot baited plots. Arrows indicate bait applications.

Evidence of squirrels was not seen using the bait stations until four to five days after the bait was first applied. Consumption then picked up. About one-half of the bait dispensed was retrieved when stations were collected following 22 days exposure.

Tables 3 and 4 present the results of the activity counts on the bait station plots. The bait exposure period was 22 days. The efficacy was somewhat lower on bait station plots: 84.0 to 92.2% according to visual counts, and 81.8 to 87% according to active burrow counts. The lower efficacy is largely attributable to lower active

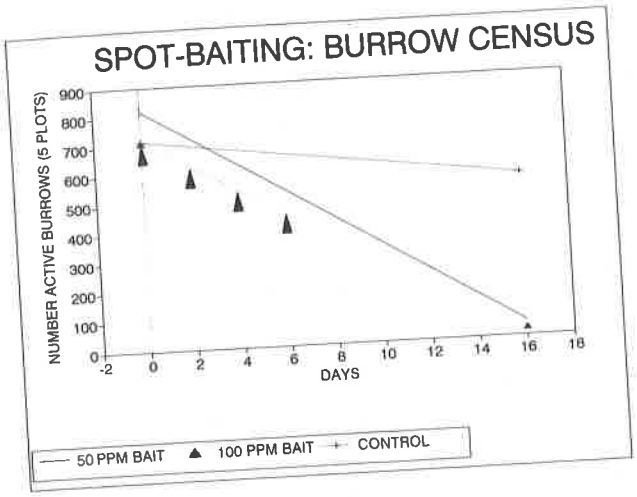


Figure 3. Results of active burrow counts on spot baited plots. Arrows indicate bait applications.

burrow counts on the control plots. As illustrated in Figure 4, visual activity counts increased on plots 11 and 14 during the bait station study, while active burrow counts (Figure 5) declined each time. This method may not be suitable for using more than twice in a short time period.

Bait Degradation

Concentrations of diphacinone in baits placed in open locations (spot baited plots) declined by approximately 50% during the 9 day exposure period. Concentrations of diphacinone in bait retrieved from bait stations and bait stored in the original containers degraded by about 10% during 22 days (Table 5, Figure 6).

Carcasses

The number of squirrel carcasses found on treated plots was approximately 1 per acre, regardless of the bait concentration or application method (Table 6.) Mean total diphacinone in whole squirrel carcasses ranged from 0.45 to 0.48 milligrams. There appears to be no advantage in using the higher concentration of bait to reduce numbers of squirrel carcasses on the surface, as was suggested by previous studies (Clark 1978).

A total of 30 carcasses of eight other rodent species and lagomorphs were found on the spot baited plots (0.2/acre). A total of nine non-target carcasses of four rodent and lagomorph species were found on the two bait station plots (0.3/acre). Most non-targets had indications of bait ingestion. This design of bait station does not appear to provide any benefits in reducing non-target hazards compared to spot baiting.

No secondary poisoning cases were observed, although predators were common in the area. Vultures (*Cathartes aura*) were observed eviscerating squirrel carcasses found on the plots. This behavior has been noted before in vultures (Hazen and Poché, 1992) and in golden eagles (Record and Marsh, 1988).

Table 2. Results of visual activity and active burrow counts on spot baited plots. The highest number of squirrels seen during pre-treatment and post-treatment counts was used as the population estimate. The bait exposure period between censusing was 10 or 11 days. All burrows were closed on the census plots immediately after the three day visual census. Open burrows were counted 48 hours later.

| | Number of Plots | Treatment (ppm DPN) | Pre-treatment | Post-treatment | Percent Change* |
|---|---|---|---|---|---|
| VISUAL | 5 | 50 | 105 | 7 | -91.6 |
| | 5 | 100 | 107 | 8 | -90.6 |
| | 5 | Control | 126 | 100 | -20.6 |
| BURROW | 5 | 50 | 820 | 50 | -92.2 |
| | 5 | 100 | 709 | 24 | -95.7 |
| | 5 | Control | 713 | 555 | -22.2 |

*Analysis of variance showed both treatments differed significantly from the control plots (P=0.05%). T-tests found

Table 3. Results of visual activity counts on bait station plots. The baiting period was 22 days. Of the five control plots used in the spot baiting study, the two closest to the bait station plots were used as controls. Mid-treatment counts were conducted 14 to 16 days after bait was applied.

| Plot No. | Treatment (ppm a.i.) | Visual Activity Counts | | | Percent Change |
|---|---|---|---|---|---|
| | | Pre-treatment | Mid-treatment | Post-treatment | |
| 17 | 50 | 25 | 11 | 4 | -84.0 |
| 18 | 50 | 14 | 4 | 1 | -92.2 |
| 11 | Control | 28 | 20 | 36 | +28.6 |
| 14 | Control | 27 | 24 | 22 | -18.5 |

Table 4. Results of active burrow counts on bait station plots. The baiting period was 22 days. Of the five control plots used in the spot baiting study, the two closest to the bait station plots were used as controls here. Control plots were censused "mid-treatment" as part of the post-treatment census of spot baited plots.

| Plot No. | Treatment (ppm a.i.) | Active Burrow Counts | | | Percent Change |
|---|---|---|---|---|---|
| | | Pre-treatment | Mid-treatment | Post-treatment | |
| 17 | 50 | 156 | n/a | 15 | -81.8 |
| 18 | 50 | 131 | n/a | 9 | -87.0 |
| 11 | Control | 158 | 113 | 49 | n/a[1] |
| 14 | Control | 157 | 129 | 83 | -47.1 |

[1]Unable to complete activity count due to livestock on the plot.

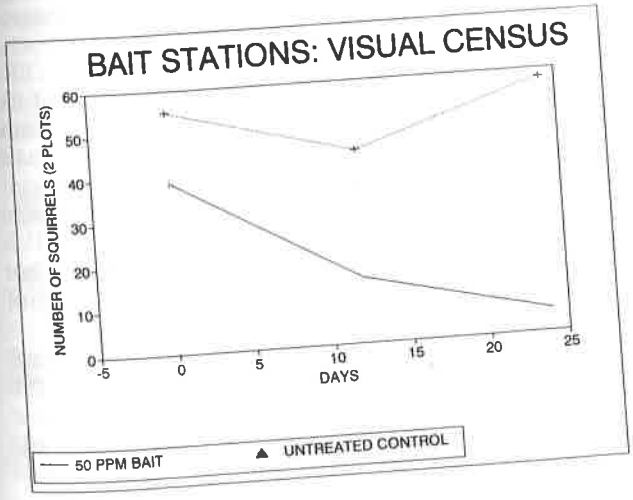


Figure 4. Results of visual activity counts on bait station plots.

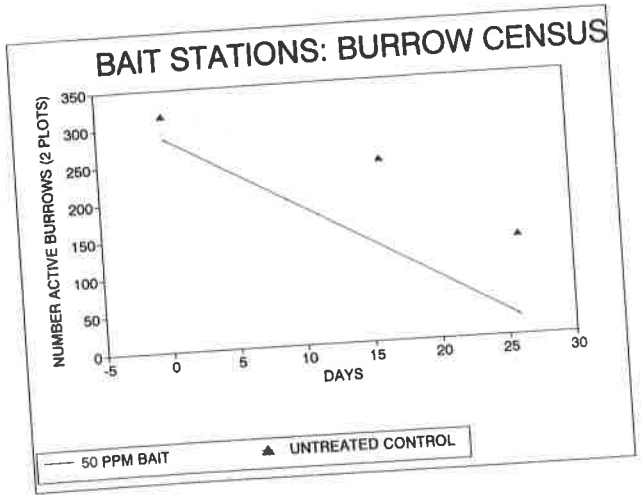


Figure 5. Results of active burrow counts on bait station plots. This method was used on the control plots three times, but only twice on treated plots. Note decline in index on control plots each time this method is repeated.

Table 5. Bait degradation rates. Baits were analyzed before and after application in the field. Samples from the initial application were retrieved from spot baited plots and from bait stations. These were compared with samples kept in storage at the field site. All values are ppm diphacinone.

| Nominal | Initial | Spot Baiting[1] | Bait Station[2] | Storage |
|---|---|---|---|---|
| 50.0 | 48.2 | 13.5 | 45.0 | 45.9 |
| 100.0 | 95.9 | 45.4 | n/a | 93.0 |

[1]Based on 9 days exposure in the field.
[2]Based on 17 days exposure in a bait station.

Table 6. Squirrel carcasses found above ground on treated plots. No carcasses of squirrels or other animals were found outside of the treated areas. Residues based on n = 8-10/treatment.

| Treatment | *S. beecheyi* Carcasses | Carcasses/ Acre | Mean DPN (ppm) | Mean Total DPN (mg) |
|---|---|---|---|---|
| Spot Baiting: | | | | |
| 50 ppm | 76 | 1.1 | 1.4 | 0.48 |
| 100 ppm | 67 | 0.9 | 1.4 | 0.46 |
| Bait Stations: | | | | |
| 50 ppm | 26 | 0.8 | 0.9 | 0.45 |

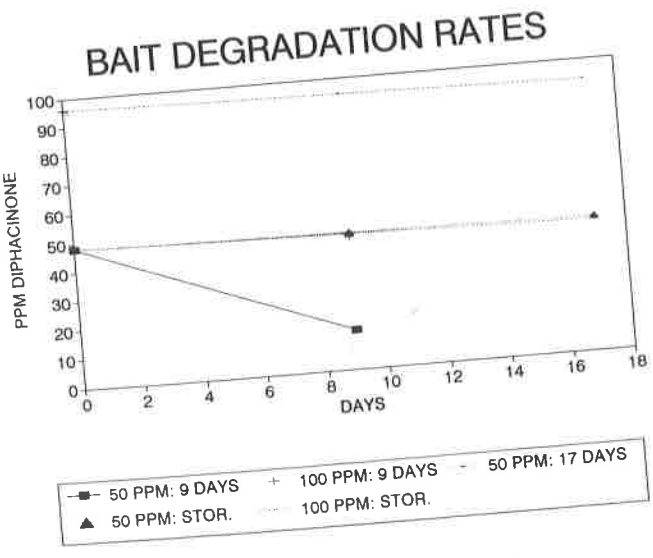


Figure 6. Bait degradation rates for bats retrieved from spot baited plots, bait stations, and bait stored in the original containers.

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