# CALIFORNIA GROUND SQUIRREL FIELD EFFICACY STUDY USING 0.005% CHLOROPHACINONE BAIT

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ABSTRACT: A field efficacy study was completed using Wilco Ground Squirrel Bait (containing 0.005% chlorophacinone) to control California ground squirrels (*Spermophilus beecheyi*) in Madera County, California. Bait was applied in plastic stations at the rate of 2 lbs. per station. Two plots with buffer zones were treated (about 11.5 acres each). Visual and burrow counts were used as the census indices to determine field efficacy. Pretreatment and posttreatment census observations were conducted over three days pretest and three days posttreatment. Efficacy after 21 days was 94.4% for plot T-1 and 100% on plot T-2 using visual counts. Burrow count data was similar with the T-1 efficacy of 95.1% and T-2 of 95.8%. Tissue residue analysis was completed on muscle, liver and gut remains in recovered ground squirrels and non-target wildlife. Partial carcasses of two cottontail rabbits were found on the plots. Turkey vultures were observed on the study area daily. The numbers and size of vultures observed and the quantity of dead squirrels recorded on the ground surface would tend to indicate no significant impact on the vulture population nor on other wildlife in the treatment area.

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## INTRODUCTION

The California ground squirrel is the most destructive ground squirrel in the U.S. Losses to agricultural crops were estimated at $8 million in 1971 (Clark 1978). The squirrels compete with livestock for forage, invade small grain fields, and damage a wide variety of vegetable and nut crops. As much as one-third of the crops grown in the state incur some degree of squirrel depredation each year (Marsh 1986).

Toxic baits provide the most effective means of controlling large infestation of ground squirrels. Acute toxicants such as zinc phosphide have been used for a number of years but can be ineffective. Anticoagulant rodenticides are currently registered within the state under Special Needs Permits (24-c). None, however, have received full Section 3 EPA registration.

The main purpose of this study was to evaluate the effectiveness of Wilco Ground Squirrel Bait, containing 0.005% chlorophacinone, against California ground squirrels to meet the registration requirements stipulated by the EPA. A second objective was to recover dead ground squirrels killed by the bait and complete residue analyses to assess the potential impact of the baiting program to non-target wildlife species.

## METHODS AND MATERIALS

### Study Area

The field trial was completed on the San Joaquin Experimental Range, located approximately 16 miles north of Fresno, California. The study area lies in the lower Sierra Nevada foothills of central California. The elevation ranges from 700 to 1,700 feet on the 4,500 acre ranch. Soils are of granitic origin and have low capacity for storing water. Rainfall averages about 19 inches. The experimental range is in the plant-oak woodland type, and includes grassland savanna. At the time of the study, the grass was mature with the panicles ripe with seeds. The plots had not been subjected to livestock grazing the year prior to this project.

The material was manufactured by Wilco Distributors, Inc. and shipped directly to the study site in Madera County, California. The test substance used was a 3/8-inch pelleted bait. The bait was placed into Wilco protective bait stations in the field.

Samples from the batch of Wilco Ground Squirrel Bait manufactured for this study were sampled and analyzed by Lipha Tech Laboratory, Milwaukee, WI. Nine bait samples were analyzed for the amount of chlorophacinone in the test substance (50-90 ppm, certified limits) and uniformity of mix.

### Census Methods

Two treatment (T-1 and T-2) and two control (C-1 and C-3) census plots were located in areas of ground squirrel infestation. An estimated minimum of 20 ground squirrels per study plot was the factor determining the selection of plots. Each census plot was a minimum of 2.5 acres in size. These were marked with surveyor flagging along the perimeters. Buffer zones around each treatment plot were also delineated and marked with plastic flagging. These extended 225 feet beyond the perimeter of the census plots (approximately 9 ac.), however, were only used for bait application and non-target hazard surveys. Pretreatment and posttreatment ground squirrel population censusing was completed using visual counts (direct) and plugged-burrow opening (indirect) census methods. The visual counts followed recommendations by Fagerstone (1983). A blind was constructed within 25 yards of the edge of each plot, a minimum of one day before the census began. Ground squirrels were counted on each plot by making one systematic scan of the plot with binoculars. Counts were made between 6:15 a.m. and 11 a.m. depending on weather conditions, over three consecutive days. Each day three ground squirrel counts were completed on plots, at ten minute intervals. The highest number counted was used as the population estimate for the plot.

For both the pretreatment and posttreatment periods, all open ground squirrel burrows on the treatment and control plots were closed by shoveling loose soil over the opening. Forty-eight hours later the number of re-opened burrows were counted. This census method was initiated on the day visual counts were completed.

Table 1. Results of pre- and posttreatment ground squirrel censuses using visual sightings and burrow counts.

| Plot | Type | Visual Counts | | | Burrow Counts | | |
|---|---|---|---|---|---|---|---|
| | | Pre | Post | % Change | Pre | Post | % Change |
| T-1 | Treat. | 21 | 1 | -94.5 | 180 | 6 | -95.1 |
| T-2 | Treat. | 10 | 0 | -100.0 | 107 | 3 | -95.8 |
| C-1 | Control | 13 | 9 | -30.1 | 104 | 63 | -39.4 |
| C-3 | Control | 22 | 21 | -4.5 | 86 | 65 | -24.4 |

Note: to calculate the efficacy for the treatment plots, the average of the control plots was used. The following formula is to be used to adjust to a change in control plot counts (using T-1 as an example):

Visual Counts

$$1-\frac{\text{Pretreatment mean C-1+C-3}}{\text{Posttreatment mean C-1+C-3}} \times \frac{\text{Posttreatment T-1}}{\text{Pretreatment T-1}} \times 100 = 1-\frac{1}{21} \times \frac{17.5}{15} \times 100 = 94.5\%$$

## Bait Application

The bait was applied in Wilco Bait Stations at the rate of 2 pounds per station on the two treatment plots. The bait stations were positioned at about 75-foot intervals or near active California ground squirrel burrow systems on the census plots and buffer zones. An uninterrupted supply of bait was provided until signs of feeding by ground squirrels ceased. Bait stations were checked daily for bait consumption and additional bait was added as needed.

## Non-Target Hazard Survey

A search for non-target wildlife mortality was conducted each day over the three consecutive days of post treatment censusing. The area searched included the census plots, buffer zones and pasture that extended 225 feet beyond the perimeter of the buffer zones on the treatment plots, or approximately 35 acres per plot. Dead ground squirrel or wildlife carcasses found were stored in plastic bags and frozen for tissue residue analysis.

## Tissue Residue Analysis

A minimum of ten ground squirrels that died after bait application were recovered and analyzed for residues of chlorophacinone in the gut, hind quarter muscle, and liver tissues. Non-target wildlife found on the study area was also examined for residues of chlorophacinone in the same tissues if available.

The residues were determined by use of Gel Permeation Chromatography (GPC) and High Performance Liquid Chromatography (HPLC) as described by Hunter (1984, 1985 and 1988).

**Tissue Preparation**—Animals collected were initially frozen at approximately -20°C, packed on dry ice and shipped by overnight air to Milwaukee. These were immediately frozen at a minimum of -20°C. Tissues were defrosted at room temperature for analysis and processed within several hours.

## RESULTS AND DISCUSSION:

The test bait batch used in this study was manufactured by Wilco Distributors on May 18, 1990 and analyzed by Lipha Tech, Inc. on May 24, 1990 for uniformity of mix. Nine samples were taken from containers for analysis. The average concentration was 65.7 ppm (63-69) thirteen days before application in the field and the mix had a uniform mixture of chlorophacinone.

Each plot was located so that sufficient ground squirrels were observed on the surface to facilitate census work. Results of the pretreatment and posttreatment censuses are presented in Table 1.

The bait was applied on June 6, 1990 and removed from the treatment plots when the posttreatment census initiated. California ground squirrels exhibited neophobic behavior when the bait stations were placed on the plots with the test

Table 2. Census plots and the number of bait stations and amount of bait used. The buffer zones surrounding each was approximately 9 acres in size, while the total area searched during the wildlife hazard survey was about 35 acres per plot.

| Plot No. | Type | Plot Sizes (ac.) Census | Buffers | Number Bait Stations | Bait Used (lbs) |
|---|---|---|---|---|---|
| T-1 | Treat. | 2.8 | 11.5 | 103[a] | 177[b] |
| T-2 | Treat. | 2.9 | 11.5 | 97 | 95 |
| C-1 | Control | 2.5 | —— | —— | —— |
| C-3 | Control | 2.7 | —— | —— | —— |

[a]Varied depending on density of active burrow systems.
[b]Maintained 2 lbs per station during treatment period.

Table 3. The amount of chlorophacinone in bait used in bait stations on a per acre basis. The census plots and buffer zones were baited with the test substance.

| Treatment Plot | Plot Sizes (ac.) Census | +Buffers | Bait Used (lbs) | Total[a] CPN (g) | CPN per acre (g) |
|---|---|---|---|---|---|
| T-1 | 2.8 | 11.5 | 177 | 4.661 | 0.405 |
| T-2 | 2.9 | 11.5 | 95 | 2.502 | 0.218 |

[a]0.0058% chlorophacinone in Wilco Ground Squirrel Bait.

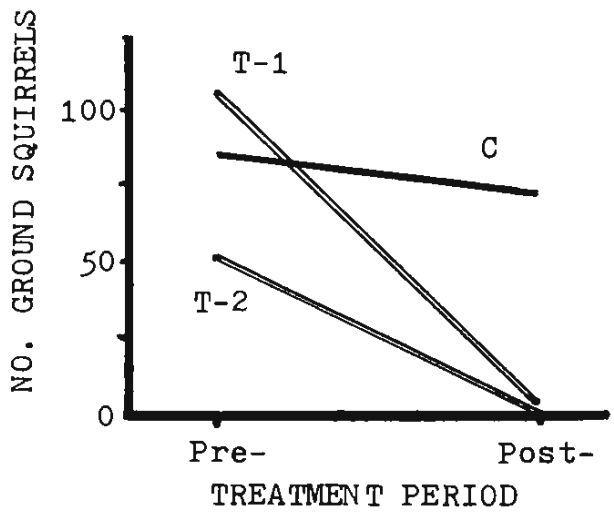


Figure 1. Results of the ground squirrel visual count census from the control plots and plots treated with Wilco Gound Squirrel Bait containing 0.005% chlorophacinone.

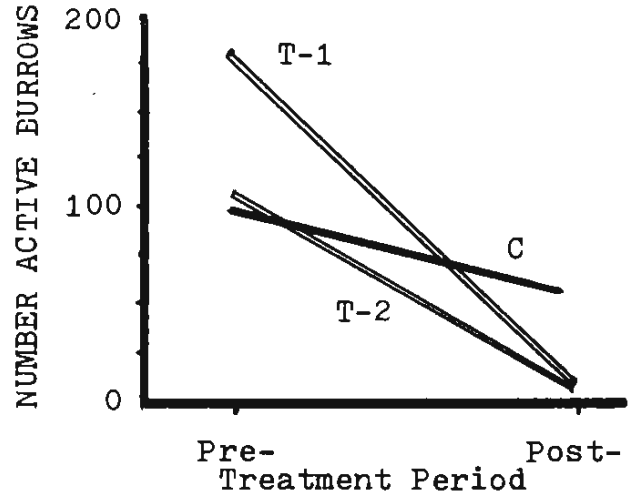


Figure 2. The number of active ground squirrel burrow systems from two treatment plots (T-1 and T-2) and the control plots three weeks after treatment with Wilco Ground Squirrel Bait, containing 0.005% chlorophacinone.

substance. Stations were not entered by squirrels for the first several days and bait consumption did not initiate appreciably until 5 days after application. Four days after presentation of the bait, about one third of the bait stations showed signs of squirrel usage. On day six, 15 pounds of bait was added to bait stations.

Table 2 presents details on plot size, number of bait stations used and amount of test substance used per plot. T-1 received 177 lbs of chlorophacinone bait and T-2, 95 pounds. This is the equivalent to 7.16 grams of chlorophacinone used on both test plots. An average of 0.405 grams of chlorophacinone per acre was used on plot T-1 and 0.218 grams per acre on T-2 (Table 3). On day 7 of the study, 4 stations were empty on plot T-1 and about half on T-2. Bait consumption continued consistently for about another week.

A mid-baiting visual census was taken on June 16-18 to assess the status of the baiting program. Treatment plot number T-1 had a reduction of 42.8% in squirrel numbers while control plot C-3 had no change in the population size. The posttreatment census work initiated on June 24, 1990 and after three days revealed an excellent reduction in ground squirrel numbers after use of the Wilco Ground Squirrel Bait. The visual counts for plots T-1 and T-2 showed population reductions of 94.5% and 100% respectively (Fig. 1). The burrow count data displayed similar results with an efficacy for T-1 of 95.1% and T-2 at 95.8% (Fig. 2).

## Non-Target Hazards

During the study numerous wildlife species were observed on and near the study area including mule deer, bobcat, coyote, Audubon cottontail, turkey vulture, red-tailed hawk, scrub jay, California quail, mourning dove, crow, and numerous songbird species.

A turkey vulture roost was located about 0.75 miles northwest of the treatment plots. About 50 of the scavengers routinely cruised over the study area each day in search of food. Nine days after the test substance application, vultures were observed feeding on squirrel carcasses on the test plots. Over the next 12 days vultures were seen either perched on trees on the study area or consuming ground squirrel carcasses. As many as 12 birds were seen feeding on a dead squirrel. An estimated 10 ground squirrel carcasses were consumed by vultures, based on actual sightings.

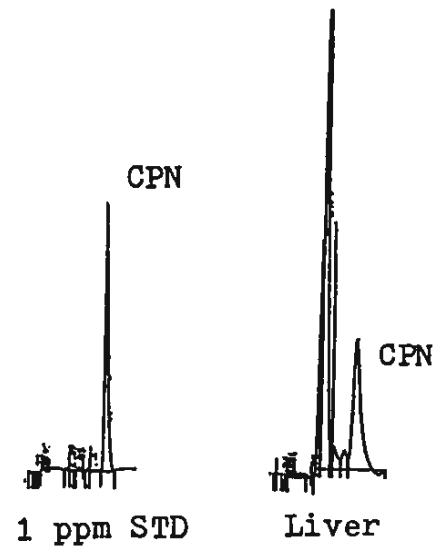


Figure 3. Chromatograms of chlorophacinone from 1 ppm analytical standard and ground squirrel liver.

The searches completed during the post treatment census period revealed one probable non-target death which was directly attributable to the ground squirrel bait. On June 21, 1990 the partial carcass of an Audubon cottontail (tissue no. T-2-9) was recovered from feeding vultures on plot T-2. On June 24 the hind legs and gut of a second cottontail (tissue no. T-2-11) were retrieved from scavenging vultures. It was not possible to discern if due to the high cottontail population and absence of cover in the areas, if the vultures were preying directly on the cottontails or feeding on dead carcasses. Residue analysis for chlorophacinone revealed a trace of chlorophacinone (0.011 ppm) in the gut remains of cottontail T-2-9 and 1.160 ppm in the gut remains of T-2-10. Table 4 presents the results of the HPLC analysis of the ground squirrel and cottontail tissues.

Table 4. Amount of chlorophacinone (ppm) found in selected tissues of ground squirrels and Audubon cottontails collected from the study area.

| Sample No. | Tissues Selected | | |
|---|---|---|---|
| | Liver | Muscle | Gut |
| Ground Squirrels | | | |
| T-1-1 | .277 | .082 | .202 |
| T-1-3 | ND | ND | 1.085 |
| T-1-4 | .038 | ND | .177 |
| T-1-6 | 2.023 | .004 | .099 |
| T-2-1 | 1.343 | .034 | 2.510 |
| T-2-2 | ND | ND | .284 |
| T-2-3 | 1.256 | .031 | 13.628 |
| T-2-4 | 1.175 | ND | ND |
| T-2-7 | .150 | .013 | .152 |
| T-2-8 | .040 | .012 | .277 |
| Cottontails | | | |
| T-2-9 | — | — | .011 |
| T-2-10 | — | ND | 1.160 |

The vulture roost was monitored for one week after baiting was completed and there were no signs of anticoagulant induced bleeding, vulture mortality, or aberrant behavior.

Ground squirrel number T-2-3 had the highest concentration of chlorophacinone in the gut, 13.628 ppm, while squirrel T-1-6 had 2.023 ppm in the liver. Little was detected in the muscle tissue, since the majority of ingested anticoagulants are eliminated within a short period of time, and the remaining amount is concentrated mainly in the liver.

The ground squirrels were found near or in crevices of rock outcrops, near the ground surface within burrow entrances, and under trees. Plot T-2 had more brush along the borders and had a higher cottontail population.

The application rate of about 0.405 grams of chlorophacinone in Wilco Ground Squirrel Bait was effective in significantly reducing California ground squirrel numbers. The use of bait stations was effective in limiting non-target wildlife exposure to the test material. In this test situation, the turkey vulture would appear to be the wildlife species with the most risk to secondary hazard exposure. A study by Mendenhall and Pank (1980) revealed the potential for secondary poisoning to birds of prey with the use of anticoagulant rodenticides. In a recent publication, Askham and Poché (1992) noted no effect on red-tailed hawks fed chlorophacinone killed voles over a period of seven consecutive days.

With raptors, the home range covers numerous square miles. The birds consume a variety of prey species over a large area other than only those on the treated plots. As was noted in this study, there was no observed effect on vultures consuming on dead ground squirrels.

The dead cottontail may have consumed bait dropped by ground squirrels during caching activity or during squirrel use of the bait stations.

GPC cleanup prior to use of HPLC of selected tissues by use of the methods produced acceptable and reproducible extraction, quantification, and chromatography of chlorophacinone. The minimum detection level achievable using this method was 10 ppb. Figure 3 presents examples of the chromatography achieved by the use of the methods described herein.

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