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An Overview of Recent Ground Squirrel Bait Registration Research Supported By the California Bait Surcharge Program

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AN OVERVIEW OF RECENT GROUND SQUIRREL BAIT REGISTRATION RESEARCH SUPPORTED BY THE CALIFORNIA BAIT SURCHARGE PROGRAM

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ABSTRACT: The California Department of Food and Agriculture Rodent Bait Surcharge Program is actively funding studies to develop and register safe, effective and practical ground squirrel baits. Under this program, Genesis Laboratories has conducted eight studies since 1994 designed to fulfill registration requirements for existing baits and to develop new baits. Areas of research include field efficacy, application methods and rates, non-target hazards, and residue loads in animal and plant tissues. Existing diphacinone and chlorophacinone treated oat groat baits have proven to be effective in controlling the California ground squirrel. Applications of these baits to alfalfa crops did not result in quantifiable residue loads. Preliminary studies found bromethalin treated oats may be effective against the California ground squirrel. Chlorophacinone treated cabbage bait was not effective against Belding's ground squirrel.

KEY WORDS: rodenticides, California Ground Squirrel, Belding's Ground Squirrel, diphacinone, chlorophacinone, bromethalin, efficacy, residues

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INTRODUCTION

In 1990, the California state legislature passed a bill authorizing the county agriculture commissioners to collect a surcharge of 50 cents on each pound of vertebrate pest control material sold. Monies generated by this fee are used to fund research to fulfill registration requirements of existing vertebrate pesticides, and to explore new vertebrate management products and methods.

Genesis Laboratories has conducted eight research projects under this program since 1994. Six of these projects were designed to fulfill data requirements needed to obtain Section 3 EPA registration of existing anticoagulant bait formulations. Two field efficacy studies of novel baits or formulations for ground squirrel control have also been conducted.

For many years diphacinone and chlorophacinone treated oat groat baits for ground squirrel control have been produced by various counties under a 24C (Special Exemption) label maintained by the California Department of Food and Agriculture (CDFA). Both 50 ppm and 100 ppm formulations with each active ingredient (a.i.) are currently produced. The 100 ppm products are now labeled for spot baiting only. The 50 ppm formulations are approved for spot baiting and in bait stations.

One requirement of the USEPA Pesticide Assessment Guidelines (Section G: 96-12) is that rodenticides be at least 70% effective against the target species when used according to label directions. Field efficacy tests were conducted of these existing anticoagulant baits as well as a bromethalin-treated oat bait. In addition, a chlorophacinone-treated cabbage bait was tested against Belding's ground squirrel. Non-target hazards and bait stability were evaluated in conjunction with the efficacy testing.

In addition, crop residues of the diphacinone and chlorophacinone oat baits were evaluated on alfalfa, where voles (*Microtus* spp.) are the primary target species.

FIELD EFFICACY AND NON-TARGET HAZARDS

Diphacinone-Treated Oat Baits

In 1994, field efficacy tests were conducted of the Rodent Bait Diphacinone Treated Grain oat groat baits to control the California ground squirrel (*Spermophilus beecheyi*). The results were presented at the 17th Vertebrate Pest Conference (Baroch 1996).

To briefly summarize, the baits (50 parts per million [ppm] and 100 ppm) were found to be effective in controlling *S. beecheyi* in spot baiting and bait station applications. Squirrel carcasses found on the surface of treated plots averaged about one per acre. There were a number of non-target kills from direct bait consumption by rodents and rabbits. There were no cases of secondary poisoning observed, although various avian scavengers consumed poisoned squirrel carcasses. The baits were found to decompose rapidly when applied by spot baiting.

Chlorophacinone-Treated Oat Baits

In 1995, studies were carried out using the Rodent Bait Chlorophacinone Treated Grain oat groat baits in 0.005% and 0.01% a.i. concentrations to control the California ground squirrel. The study design followed that used for the diphacinone treated oat bait conducted the year before, as described in Baroch (1996). Both concentrations of the baits were applied by spot baiting. In addition, the 0.005% bait was applied in bait stations. Field efficacy, tissue residue loads, non-target hazards, and bait degradation rates were investigated. The studies were conducted on rangeland at the San Juan Experimental Range near Fresno, California.

Each bait concentration was applied by spot baiting to five replicated plots. Ground squirrel activity on a central area of approximately 2.5 acres in each plot was evaluated before and after application of the baits. In addition, squirrel activity on five untreated control plots of approximately 2.5 acres each was evaluated. A

direct index for estimating activity, visual counts, and an indirect index, active burrow counts, were used to evaluate the bait efficacies.

Spot baited plots were treated every other day for four applications immediately following the pre-treatment censusing. Bait was replenished only as needed on each occasion after the first application. The 0.005% bait was applied at a rate of 10.0 pounds per acre. The 0.01% bait was applied at a rate of 9.4 pounds per acre.

The 0.005% a.i. bait was applied on two plots in PVC plastic bait stations for 24 days, starting immediately after the pre-treatment censusing. Bait stations were checked every third day and bait was replenished as needed to maintain a continuous supply. The bait was applied at a rate of approximately two pounds per bait station. Bait stations were placed at approximately 75 foot intervals near active burrows.

Consumption on the treated plots varied from 16.9 to 18.8 pounds per acre. Regular carcass searches were made of all treated plots and surrounding areas. Carcasses of ground squirrels and non-target species were collected. Whole carcass tissues of 10 ground squirrels retrieved from plots in each treatment were analyzed for chlorophacinone residues. Non-target carcasses were retrieved and examined for evidence of test substance ingestion.

On spot-baited plots, squirrels were exposed to the test substances for 12 to 13 days between pre-treatment and post-treatment censusing. Squirrel activity on the plots treated with the 0.005% bait decreased 84.3% according to visual counts and 85.3% according to active burrow counts. Squirrel activity on plots treated with the 0.01% bait decreased by 92.4% using visual activity counts and 78.0% using active burrow counts. Activity reductions were not significantly different statistically between the two bait concentrations.

On bait station plots, squirrels were exposed to the test substances for 24 days between pre-treatment and post-treatment activity counts. Bait efficacy was 93.3 to 100.0% according to the visual index and 89.4 to 90.0% according to active burrow counts. A total of 86 dead ground squirrels (1.21 carcasses/acre treated) were found on the plots spot baited with 0.005% bait. A total of 78 dead ground squirrels (1.07 carcasses/acre) were found on the plots spot baited with the 0.01% bait. Carcasses of six other rodents and lagomorph species were found on the spot baited plots. Necropsies confirmed test substance exposure in some, but not all, non-target species found.

Twenty-five (25) dead ground squirrels (0.77 carcasses/acre) were found on the bait station plots. Carcasses of five other rodent, avian, and herptile species were also found.

No secondary poisoning cases were observed. Turkey vultures (*Cathartes aura*) found and consumed dead squirrels on the treated plots. In some cases they eviscerated the carcasses, leaving behind the entrails which contain the highest concentration of the active ingredient.

Analysis of whole carcass tissue residues in squirrels recovered from spot baited plots found mean residue loads of 0.19 mg of chlorophacinone in squirrels exposed to the 0.005% bait and 0.62 mg of chlorophacinone in squirrels exposed to the 0.01% bait. Analysis of whole carcass tissue residues in squirrels recovered form bait station plots found mean residue loads of 0.162 mg of chlorophacinone in squirrels exposed to the 0.005% bait (n = 10).

Both baits were analyzed and found to be within certified limits before being applied in the field. Analysis of test substance samples exposed in simulated spot baiting applications for nine days found the 0.005% (nominal) bait had degraded to 0.0035% chlorophacinone. The 0.01% (nominal) bait degraded to 0.0078% chlorophacinone when exposed for the same period.

Analysis of samples of the 0.005% bait showed the bait was stable when exposed to field conditions inside bait stations for 24 days.

Chlorophacinone-Treated Cabbage Bait

In the early spring of 1996, a trial was conducted to evaluate the potential of chlorophacinone-treated cabbage bait to control Belding's ground squirrel (*Spermophilus beldingi*) in alfalfa fields in northern California. This species can occur at very high densities, and yield reductions in alfalfa/grass crops of up to 61% have been documented (Sauer 1976). Some populations of this species have historically been reluctant to accept grain based baits (Wright 1982).

Succulent carriers such as chopped cabbage and dandelions have been used effectively in the past, with compound 1080 (sodium fluoroacetate) as the toxicant. Acceptance of these succulent baits is good in early spring when the population has just emerged from hibernation and other food resources are limited. By late spring, when green vegetation is available, bait acceptance declines.

Since compound 1080 is no longer available, chlorophacinone was tested on fresh chopped cabbage as an alternative. Preliminary small plot trials by CDFA personnel indicated that it might be feasible to control the squirrels with one or two relatively heavy applications of chlorophacinone treated cabbage. Because of the expenses and labor involved in preparing and applying such a bait, it was felt that more than two applications would be prohibitively expensive for growers. Accordingly, the study was designed to compare the efficacy of a single heavy application or two lighter applications at a two-day interval. Experience with compound 1080 treated chopped cabbage and with chlorophacinone treated oat groat baits used to control *S. beecheyi*, suggested that a total baiting rate of about 10 to 12 pounds/acre might be sufficient. Fifteen (15) test plots were established in northern Modoc County in March 1996. Squirrel activity on the plots was determined using visual counts and closed burrow counts prior to bait applications. Bait was prepared just before application by hand mixing 200 pound lots of freshly chopped cabbage treated with 0.28% Rozol (chlorophacinone) Mineral Oil Concentrate (LiphaTech, Inc., Milwaukee, Wisconsin). The target concentration was 0.005% a.i., or 50 parts per million (ppm). The actual concentration of a.i. as determined by laboratory assay was 43.6 ppm.

Bait was applied by crews walking the plots and spreading bait on the ground near active burrows. Five plots received a single application, which averaged 11.94 pounds/acre. Five plots were treated with two lighter

applications at a two-day interval, with an average application rate totaling 15.67 pounds/acre. Five additional plots served as untreated controls.

Bait consumption began almost immediately. Most of the bait from both treatment regimes was gone within 24 hours of application. Dead squirrels with signs of anticoagulant poisoning began appearing on the surface within three days of the initial applications.

Plots were searched daily for carcasses. This area is along a major migration route for raptors moving north in the spring, including many golden eagles (*Aquila chrysaetos*) and bald eagles (*Haliaeetus leucocephalus*).

Forty-five (45) poisoned ground squirrel carcasses were found on the plots receiving a single bait application. A total of 81 poisoned ground squirrel carcasses were found on the surface of plots receiving two bait applications. Tissues were analyzed from 10 whole squirrel carcasses recovered from each type of treatment plot. Carcasses from plots receiving one application carried a mean residue load of 0.078 milligrams (mg) of chlorophacinone. Carcasses from plots receiving two applications carried a mean residue load of 0.126 milligrams (mg) of chlorophacinone.

In spite of good bait acceptance and the presence of poisoned squirrels on the surface, efficacy was very low for both treatments. When corrected for changes on the control plots, efficacy ranged from 0 to 13% according to both activity indexes.

Either the amount of toxicant or the pattern of applications were not sufficient to control these test plot populations. A higher concentration of bait, or perhaps a pulsed baiting approach, or both, may be required to give adequate control with this toxicant.

Buffer zones of about 100 meters were baited around the activity census plots. Wide-ranging movements by the squirrels may have confounded the results. A few radio-collared squirrels in this study were found to travel great distances in a short time. One individual, a male, moved 1,065 meters from the capture site in one day, then was back at the capture site the next day.

It is recommended that in future studies plots be blocked by treatment type. Plots receiving different treatments should be widely separated, rather than randomly assigning treatments to nearby plots as was done in this case. Treated buffers should be extended as far as is practicable. Finally, the requirements of the visual activity index method (see Fagerstone 1983) used in this study were difficult to meet due to the very unsettled weather which is typical in Modoc County in the early spring. The use of radio telemetry to monitor efficacy is suggested.

Bromethalin-Treated Oat Baits

In the spring of 1996, a trial was conducted with bromethalin-treated oat groats to evaluate the efficacy against *S. beecheyi*. The trial was conducted on rangeland in the eastern Sierra Nevada foothills near Porterville, California. Bromethalin is a promising candidate as a field rodenticide because it does not require repeated applications, and is unlikely to cause nontarget secondary poisoning.

Bromethalin is an acute rodenticide which causes death by uncoupling oxidative phosphorylation in the central nervous system. The LD_{50} ranges from 2.01 to 8.13 in laboratory mice and rats. The compound also acts as an appetite suppressant. Therefore, after the initial exposure, no more feeding takes place. Death typically occurs in two to three days if a lethal dose has been consumed. Recovery from consumption of sub-lethal doses is possible. Because of the mode of action and the small amount of bait consumed, there appear to be few secondary poisoning hazards (Jackson et al. 1982).

The field applications were made in May 1996. Because there was no previous information on necessary dose levels for ground squirrels in the wild, baits were formulated at two widely different concentrations. Nominal concentrations of 0.01% and 0.10% bromethalin-treated oat groat baits were formulated at the Fresno County Agricultural Commission bait mixing facility in Fresno, California. The field trial was conducted in Tulare County, California. Bait concentration analysis was performed by PM Resources, Inc., Bridgeton, Missouri. Tissue residues in recovered squirrel carcasses were analyzed at Genesis Laboratories, Inc., Fort Collins, Colorado.

The baits were applied to 10 plots ranging in size from 11.0 to 13.9 acres. Each bait concentration was applied to five plots. Ground squirrel activity on a central area of approximately 1.7 acres in each plot was evaluated before and after application of the test substances. In addition, squirrel activity on five untreated control plots was evaluated. A direct index of activity, visual counts, and an indirect index, active burrow counts, were used to estimate the bait efficacies.

The baits were applied in 3-inch diameter "T" shaped PVC bait stations placed at approximately 75 foot intervals. The 0.01% bait was consumed at rate of 1.8 pounds per acre. The 0.10% bait was consumed at a rate of 0.8 pounds per acre.

Squirrels were exposed to the baits for 12 to 13 days before post-treatment censusing began. Squirrel activity on the plots treated with the 0.01% bait decreased 60.4% according to visual counts and 37.3% according to active burrow counts. Squirrel activity on plots treated with the 0.10% bait decreased by 64.5% using visual activity counts and increased by 20.5% using active burrow counts.

Squirrel activity on the untreated control plots decreased 30.3% using visual counts and 59.5% according to active burrow counts. Activity decreases on the control plots were attributed to very hot weather during the post-treatment census period. Efficacy calculations for treated plots were corrected for the decreased activity on control plots. Based on analysis of variance, visual activity on treated plots was significantly different from that on the control plots. Visual activity changes were not significantly different between the two treatments. Analysis of active burrow counts only found significant differences between the 0.10% a.i. treated plots and the control plots.

The author believes actually efficacy may have been above the 60 to 65% levels indicated by visual counts. Some squirrels on treated plots were fitted with radio transmitter collars to facilitate retrieval of carcasses. Twenty-one (21) of 27 radio-collared squirrels were found

dead on the treated plots within five days of bait placement. Efficacy in this group was 78%.

Regular carcass searches were made of all treated plots. Fourteen (14) dead ground squirrels were found on the surface of plots treated with 0.01% bait. A total of five dead ground squirrels were found on the plots treated with the 0.10% bait. Carcasses of three other rodent and lagomorph species were found on the treated plots. Necropsies confirmed test substance exposure in some but not all non-target species found. No secondary poisoning cases were observed, although a coyote scat containing bait was found.

Whole carcass tissues of 12 ground squirrels retrieved from 0.01% baited plots and eight ground squirrels retrieved from the 0.10% baited plots were analyzed for bromethalin residues. Non-target carcasses were retrieved and examined for evidence of bait ingestion but were not analyzed.

Analysis of whole carcass tissue residues in recovered squirrels found mean residue loads of 1.01 mg (3.18 ppm) of bromethalin in squirrels exposed to the 0.01% a.i. bait and 4.35 mg (11.2 ppm) of bromethalin in squirrels exposed to the 0.10% a.i. bait.

Both baits were analyzed for concentration of the active ingredient immediately after mixing and again after field exposure. The nominal 0.01% bait assayed at 0.0088 to 0.0091% bromethalin initially. After 14 days exposure in bait stations, three samples assayed at 0.0087% bromethalin, representing a 4.4% decline. The nominal 0.10% bait assayed at 0.0875 to 0.0914% bromethalin initially. After 14 days exposure in a bait stations, three samples assayed at 0.0797% bromethalin, a decline of 8.9%.

Bromethalin shows promise as a field rodenticide due to the comparatively low amount of bait needed, and reduced secondary hazards.

ANTICOAGULANT RESIDUES ON ALFALFA

Ground squirrels (*Spermophilus* spp.) and voles (*Microtus* spp.) can be serious pests of alfalfa (*Medicago sativa*) at times. Rodent Bait Chlorophacinone Treated Grain 0.01%, and Rodent Bait Diphacinone Treated Grain 0.01% are available in California under a 24C label for controlling rodent pests in non-crop areas. The California Department of Food and Agriculture wishes to obtain a label claim for use of this bait against voles and other rodents in alfalfa crops. However, potential residue loads in treated crops have not yet been examined.

Much of the alfalfa grown in California supports the dairy industry. These studies were designed to determine the residue loads that dairy cattle or other livestock might be exposed to at proposed application rates, and at rates exceeding the label directed rates.

The bait was applied at two sites representing different regions of California. The first site was in Modoc County in northeastern California, where three cuttings per season are typical. The second site was in San Joaquin County, near Stockton, where there may be six or more cuttings a year. Sites were selected based on the suitability for test applications and sampling, regardless of current rodent infestations.

Applications were made at different times in order to represent a variety of conditions under which the bait might be used. In Modoc County, the applications were made in early May, soon after the crop had broken dormancy and 42 days prior to cutting. The crop had begun to leaf out and, therefore, presented a greater opportunity for residue capture on the foliage. In San Joaquin County, the applications were made in early September, just after the fifth cutting of the year and 25 days prior to cutting.

The bait was applied by a truck mounted broadcast seeder. Two applications were made at two-day intervals at each site. A constant supply must be available for several days for the product to be effective against the target species. One plot at each site received a nominal application rate of 10 pounds bait/acre/application. A second plot at each site received a nominal application rate of 20 pounds bait/acre/application. A placebo bait containing all inert ingredients was applied to control plots at the same rates.

It has been estimated that crimped oat groats such as the type used here, uniformly broadcast at a rate of 10 pounds/acre will result in 4.1 kernels/square foot (Clark 1994). This is based on an estimated 18,000 kernels per pound of grain. Therefore, two applications at 10 pounds per acre would result in 8.2 kernels/square foot, and two applications at the 2X rate of 20 pounds/acre would only result in 16.4 kernels per square foot.

Chlorophacinone and diphacinone are only sparingly soluble in water (The Pesticide Manual 1991). Therefore, the primary means of plant exposure is through residue capture on foliage and stems during application. Samples of alfalfa representing three crop fractions—stems and new growth, mature foliage, and hay—were collected for analysis.

Alfalfa samples were collected prior to treatment, immediately after treatment, and about every two weeks until harvest. Cut hay samples were also collected.

Samples were analyzed for chlorophacinone and diphacinone residues by Genesis Laboratories, Inc. in Wellington, Colorado, using validated High Performance Liquid Chromatography (HPLC) methods.

The chlorophacinone method limit of detection for all crop fractions is $\leq$ 77.3 parts per billion (ppb). The limit of quantitation is defined as 10X the limit of detection. Possible residues were detected in only two samples from the Modoc County site plot treated twice at the 20 pounds/acre rate, 26 days post-treatment. These residues were near the limit of detection and well below the limit of quantitation. No residues were detected on any other samples in the study. No detectable residues were found on any of the other samples.

The diphacinone method limit of detection for all crop fractions is $\leq$ 76.4 ppb. No detectable residues were found on any of the samples.

The results are consistent with what might be expected considering: 1) the low rate of bait kernels per square foot applied; and 2) the relatively rapid degradation rate of these compounds when exposed to weathering, as established in the earlier field efficacy studies reported above. The use of these baits for rodent control in growing alfalfa should not present a hazard to livestock.

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