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***Abstract*:** The efficacy of using 0.01% chlorophacinone on steam-rolled oat (SRO) groats applied in CA alfalfa by spot-baiting/hand baiting around burrow entrances (~11.5 g) to control free-ranging Belding's ground squirrels (*Spermophilus beldingi*) were compared in 6 randomly assigned square treatment units (TUs). Four TUs were given the rodenticide and 2 treated with placebo bait. Each TU was a 0.4 ha square surrounded by a similarly treated 5.5 ha square buffer zone. Baits were applied on May 13 and re-applied, on May 20 and May 22, after 7 days of un-forecasted cool wet weather greatly reduced their above ground activity. Pesticide (EPA SLN CA-890024) efficacy was calculated as % reduction (PR) of ground squirrels on each TUs measured directly by visual counts (VCs) and indirectly by active burrow counts (ABCs). VCs and ABCs provided mean PRs that met US EPA's 70% minimum standard efficacy threshold for field rodenticides ($\bar{x}$ = 73.5%, SD $\pm$ 13.3; $\bar{x}$ = 80%, SD $\pm$ 6.2, respectively). ANOVA results of the PRs were highly significant ($F = 29.72$, df 1/4, $p = 0.0055$ and $F = 72.92$, df 1/4, $P = 0.001$, respectively). All carcasses (38) located above ground were analyzed for pesticide and 80% had detectable levels in whole animals ($\bar{x}$ = 0.1131 ppm, SD $\pm$ 0.0928). Suggestions to improve the pesticide's efficacy and lessen its potential non-target hazards were discussed.

Key words: alfalfa, anticoagulant, Belding's ground squirrels, chlorophacinone, efficacy, rodenticide

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INTRODUCTION

Ground squirrels cause damage to agricultural crops such as alfalfa both from clipping and trampling (Sauer 1976). Warfarin was the first anticoagulant rodenticide used in California agriculture to control such ground squirrel depredations (Ball 1950). Recently, chlorophacinone (2-[(p-chlorophenyl) phenylacetyl]-1,3-indandione) (CAS No. 3691-35-8) has been successfully used as a rodenticide with both commensal rodents (Gill 1992, Advani 1992) and wild rodents (Giban 1974: Vossen and Gadd 1990, Tobin 1992, Fagerstone and Ramey 1996). Successful control of wild rodents has occurred using hand baits, bait stations, tracking powders (Advani 1992), and burrow builders (Guedon and Combes 1990). Clark (1978) and more recently Silberhorn et al. (2003) have discussed chlorophacinone's long history of use in ground squirrel control in California as a multiple dose rodenticide. Rodents usually consume the bait over a period of a week or more to produce effective control. Because it and other anticoagulants do not produce bait shyness (Marsh 1994), it can be used whenever rodents are active and consuming seeds (Passof 1974, Marsh 1994). Other favorable pesticide attributes include its low solubility in water (making it less likely to be transported through soils and plant tissue membranes), large molecular weight (which generally precludes its passage through root membranes) (Askham 1986), and decomposition into nontoxic elements when exposed to ultraviolet light (sunlight) (Askham 1986) or wet conditions (Spare 1992; Ramey et al., 2000). Sauer (1976) successfully utilized 0.005% and 0.01% chlorophacinone in bait stations placed in a grid pattern to control Belding's ground squirrels (*Spermophilus beldingi*). Marsh (1994) stated its effectiveness for ground squirrel control as a toxic grain bait was closely linked to the squirrel's life cycle. Therefore, it has been mainly used in California from mid May - July to control most species of ground squirrels when the annual wild grasses and forbs produce seeds.

The California Department of Food and Agriculture (CDFA) has had a state registration with the United States Environmental Protection Agency (US EPA) for the use of chlorophacinone in controlling California rangeland rodents including the Belding's ground squirrel, California ground squirrel (*S. beecheyi*), pocket gophers (*Thomomys* spp.), deer mice (*Peromyscus* spp.), and house mice (*Mus musculus*). The primary objective of this research conducted by the National Wildlife Research Center (NWRC) for CDFA was to determine the bait's efficacy for reregistration by the EPA for spot-baiting (i.e. hand-baiting) using 0.01% chlorophacinone on steam rolled oats (SRO) groats (EPA SLN CA-890024) to control Belding's ground squirrels in alfalfa.

MATERIALS AND METHODS

Study Site

The study was conducted California's Siskiyou County, in the northeast corner of the Butte Valley. Average elevation was 1,230 m with surface soils predominantly Poman-Fordney (fine sandy loam) (USDA, 1994). The local climate was tempered by winds from the Pacific Ocean. During the 26-day study in May 1996, the average daily maximum temperature was 18.3°C and a total of 4.45 cm of precipitation fell (Table 1). The valley's location, topography, soil, and climate made it suitable for livestock grazing and the production of alfalfa, wheat, barley, oats, and potatoes (Ramey et al. 2000). On the study site, alfalfa was irrigated using a pivoting overhead sprinkler system because of concerns about rapid water loss.

Chlorophacinone Bait and Baiting

The 0.01% chlorophacinone and 0.0% placebo baits were formulated according the CDFA's Confidential Statement of Formula for EPA SLN CA-890024 as discussed in the Vertebrate Pest Control Handbook (Clark, 1986). Using a commercial supplier, the subsequent grain baits had a mean ($\bar{x}$) percent of chlorophacinone (w/w) of 0.0109% (SD $\pm$ 0.00008%) for the nominal 0.01% concentration and 0.000% (SD $\pm$ 0.0000%) for the placebo. Chlorophacinone concentration in SRO groat baits was determined according to the standardized methods later published by Primus et al. (1998). Bait formulations were the same as reported in a concurrent study by Ramey et al. (2000). The 0.01**%** chlorophacinone bait (formulated on April 15, 1996) and placebo baits were brought to the study site for the first day of baiting on May 13 (Table 1). Unused baits were returned to the Siskiyou County Department of Agriculture on May 24.

Table 1. Daily temperatures, precipitation, and study events including visual counts (VC), closing holes (CH), active burrow counts (ABC), and carcass searching (CS) with number found (X) in chronological order at the Dorris site, May 1996 (Reprinted with the authors permission, Ramey et al., 2000).

Study Day	May Date	Temperature (^{0}C) Minimum	Temperature (^{0}C) Maximum	Precipitation (cm)[a]	Study Events
1	4	-5.6	15.6	-	Treatment Unit Layout
2	5	0.0	16.1	-	Treatment Unit Layout
3	6	-2.8	18.3	-	Pre-treatment VC
4	7	-1.1	17.2	-	Pre-treatment VC
5	8	-2.8	17.2	-	Pre-treatment VC
6	9	-3.9	18.9	-	Pre-treatment CH
7	10	-2.2	22.2	-	Pre-treatment Activities
8	11	1.7	26.7	-	Pre-treatment ABC
9	12	7.2	27.8	-	Pre-treatment Activities
10	13	7.8	26.7	-	Spot/Hand Baiting & CS (0)
11	14	8.9	20.6	0.69	Carcass Searching (0)
12	15	8.3	14.4	0.91	Carcass Searching (0)
13	16	6.1	12.2	0.71	Carcass Searching (0)
14	17	6.7	12.2	0.18	Carcass Searching (0)
15	18	3.9	12.2	0.48	Carcass Searching (0)
16	19	1.1	12.8	0.13	Carcass Searching (0)
17	20	-2.2	17.8	0.03	Spot Baiting & CS (12)[b]
18	21	5.0	17.8	0.86	Carcass Searching (6)
19	22	0.0	12.2	0.05	Spot Baiting & CS (6)[b]
20	23	-2.8	11.1	-	Carcass Searching (1)
21	24	1.1	17.8	-	CS (2) & Excess Bait Returned
22	25	5.0	22.2	-	Post-treatment VC & CS (6)
23	26	5.0	22.8	-	Post-treatment VC & CS (5)
24	27	6.1	22.2	0.41	Post-treatment VC, CH & CS (6)
25	28	0.0	17.8	-	Post-treatment CS (2)[c]
26	29	-1.1	17.2	-	Post-treatment ABC & CS (0)
27	30	-1.1	17.8	-	Post-treatment CS (0)
28	31	2.2	20.6	-	Post-treatment CS (0)

[a] Precipitation is for a 24-h period starting at 12:00 am
[b] One partial carcass was not analyzed on each of two days for chlorophacinone residues
[c] Two decomposed carcasses were not analyzed for chlorophacinone residues

Spot-bait applications conformed to the label specifications and the first day of baiting occurred on May 13. Trained pesticide applicators used metal dippers (small cups with handles) to dispense ~11.5 g over 1 m^2 at burrow entrances. Additional bait applications occurred later than originally anticipated because an arctic storm brought un-forecasted cold and wet weather to the study area. This weather greatly reduced above ground squirrel activity in the alfalfa when compared to the pre-treatment counts so the study was delayed. Spot-baiting resumed on May 20 and was concluded on May 22. The weatherability of this bait has been previously reported by Ramey et al. (2000) and will only be summarized here. On May 19 (6 days after the initial baiting), a 71% chlorophacinone loss had occurred, disregarding mass changes and mold growth, under the wet conditions observed between May 13 - 19 with a daily $\bar{x}$ = 0.45 cm of precipitation. A 51% loss of chlorophacinone occurred to bait applied on May 20 following 7 days of drier conditions ending May 26 with a daily $\bar{x}$ = 0.06 cm of precipitation (Table 1) without mold growth.

Six square treatment units (TUs) each measuring 0.4 ha (1.0 ac) were established in alfalfa fields that supported large populations of Belding's ground squirrels on May 4 – 5. To reduce post-treatment ground squirrel immigration onto each TU as observed by Sauer (1976), a buffer zone was established around each TU. The buffer zone was a square area constructed by placing a parallel line out 90.5 m from the boundary of each TU. Each TU and associated square buffer zone totaled 5.9 ha (14.8 ac) in area and was referred to as a study plot. All 6 study plots were randomly selected to receive either the 0.01% chlorophacinone treatment (4 TUs) or the 0.0% placebo treatment (2 TUs). A minimum of 50.0 m separated the edge of all buffers. One criteria applied from the study of Marsh and Record (1985) in establishing the TUs was to have a fixed location outside each TU that would allow an observer to visually count the ground squirrels on each TU from vehicles.

Bait Efficacy

Efficacy was estimated using 2 different methods, directly by visual counts (VC) and indirectly by active burrow counts (ABC). Visual counts have been used for estimating the efficacy of ground squirrel pesticides since about 1945. Methods employed in this study were similar to those recommended by the U. S. Environmental Protection Agency (US EPA 1982) and Fagerstone (1983). For each TU, 5 ground squirrel counts were recorded at about the same time each morning (starting ~ 0800) with 10 minute intervals between the 5 counts for 3 consecutive days. The 15 VCs gathered pre- and post-treatment were averaged to give a mean VC pre- and mean VC post-treatment for each TU. The percent reduction (PR) in post-treatment VC was calculated as a measure of efficacy for each TU.

The ABC method, similar to O'Connell and Clark (1992), provided a second (indirect) estimate for evaluating efficacy. ABC data were obtained on the days after the pre- and post-VCs had been completed (Table 1). Burrow entrances were closed pre-treatment on May 9 and post-treatment on May 27, and the number of burrows reopened 48 hours later was recorded. These were the pre- and post-treatment ABC values used for each TU from which the PRs were calculated.

Mean PR variables (mean VC and mean ABC) were analyzed using PROC MIXED analysis of variance (ANOVA) methods (SAS Institute 1992) comparing treated to placebo TUs. ANOVAs compared each PR variable between treated and placebo units; degrees of freedom (df) were 1 and 4 for the numerator and denominator, respectively. P-values for PR for the mean ($\bar{x}$) VCs and $\bar{x}$ ABCs (i.e. open holes) were determined. Additionally, corrected PRs were not calculated after the work of O’Connell and Clark (1992), because of significant study differences which are discussed.

Carcass Residues

All partial and whole body ground squirrel carcasses were retrieved daily from the first day of baiting on May 13 through the completion of the study on May 30. Partial carcasses were buried on site at a depth of 1 meter. Forty two whole carcasses were retrieved and immediately frozen and stored at -20°C until assayed for chlorophacinone residues. Upon thawing at NWRC, 4 samples were not analyzed because of their extreme decomposition. Each remaining carcass (38) was weighed, skinned, and then the head and feet removed. The NWRC's Analytical Chemistry Project (ACP) analyzed the liver, whole carcass, and whole body using validated methods later published by Primus et al. (2001). Liver and serum tissue samples were chosen for chlorophacinone residue analysis because anticoagulants are accumulated and metabolized in the liver. The remaining whole body, except the appendages (head, feet, and pelt) which ACP personnel assumed did not have detectable levels of chlorophacinone, was analyzed as an additional sample. Each whole animal's residue level was estimated by adding the residues present in the liver and whole body and dividing by each animal's weight.

The chlorophacinone residue analytical methods employed in this study have been reported in more detail by Ramey et al. (2000) and are summarized below. The liver and whole body tissues were frozen separately. These tissues were homogenized with a cryogenic mill after freezing the tissue with liquid nitrogen in a stainless steel cylinder and crushing the sample with a stainless steel piston until the tissue became a powder. These powdered frozen samples were stored at -20°C until analyzed in duplicate. Chlorophacinone concentrations were determined by comparing the area of the chlorophacinone peak in the sample extract to a working standard using a High Pressure Liquid Chromatograph (HPLC). The retention time of chlorophacinone over the dates of analyses (2/7/97 to 3/27/97) varied from 15.2 to 17.5 min. To reduce the possibility of late eluting peaks appearing in subsequent chromatograms, a gradient was added to the method beginning at 18 minutes, but this gradient did not affect the retention characteristics of the analysis. Three sets of control liver and/or control whole body samples were utilized for quality control. Control animals (n = 17) were collected by CDFA personnel on August 22, 1996. Control liver and whole body tissue samples were fortified at three levels with aliquots of concentrated standards of chlorophacinone in ethyl acetate. Levels chosen for fortifying control tissues were 0.10, 1.0, and 10 ppm chlorophacinone.

RESULTS AND DISCUSSION

Pesticide Mortality

Major investigational events are presented in Table 1. Placebo and 0.01% chlorophacinone baits were formulated on April 15 and chain of custody records were maintained from manufacture through use. The average pre-treatment visual counts on all 6 TUs had decreased in the post-treatment VCs, including the 2 control TUs. Mean PR using VCs for mortality estimates on the 4 TUs treated with 0.01% chlorophacinone averaged 73.4% (Table 2). Natural mortality on the placebo TUs was 16.3. Mean PR for the 4 treated TUs using ABC was an 80.1% reduction (Table 3) and for the 2 placebo baited TUs was 43.4%. The later unexpected result is discussed later.

Table 2. Percent reductions (PR) in mean visual count over 15 observations for each treatment unit (TU) for the Dorris study site, May 1996.

Bait treatment	TU number	Belding's - Mean ± SD Pre-treatment	Post- treatment	PR	ANOVA P Value
0.01%	1	66.5 ± 7.1	9 ± 4.5	86.5	
	2	47.7 ± 10.2	16 ± 3.8	66.5	
	3	29.3 ± 5.5	5 ± 2.4	82.9	
	4	30.7 ± 7.5	13 ± 2.5	57.7	
Mean				73.4	< 0.0055
0.0%	5	64.3 ± 7.2	51 ± 6.2	20.7	
	6	22.7 ± 5.2	20 ± 6.5	11.9	
Mean				16.3	[a]

[a] No value was calculated because of the significant decrease in the number of Belding's ground squirrels on placebo TUs post-treatment because many of the avian predators were concentrated on the 2 remaining placebo TUs. A few were on the remainder of the field with lesser squirrel subpopulations that were not included in the study.

Table 3. Percent reduction (PR) in active burrow mean counts for each treatment unit (TU) for the Dorris study site, May 1996.

Bait treatment	TU number	Number of active burrows Pre-treatment	Post- treatment	PR	ANOVA P Value
0.01%	1	251	55	78.1	
	2	244	62	74.6	
	3	178	19	89.3	
	4	74	16	78.4	
Mean				80.1	< 0.001
0.0%	5	126	74	41.3	
	6	246	134	45.5	
Mean				43.4	[a]

[a] No value was calculated because of the significant decrease in the number of Belding's ground squirrels on placebo TUs post-treatment because many of the avian predators were concentrated on the 2 remaining placebo TUs. A few were on the remainder of the field with lesser squirrel subpopulations that were not included in the study.

The population indices of ground squirrels measured by PRs in visual counts did not correlate well with the PRs in ABCs partly because the data were collected on different days. However, both efficacy PR estimates from the VCs and ABCs were each above the EPA's minimum standard efficacy threshold of 70% mortality for field rodenticides (US EPA 1982). ANOVAs showed that average mortality post-treatment using mean visible counts decreased significantly ($F = 29.72$, $df = 1/4$, $p = 0.0055$), and the mean active burrow counts excluding natural mortality also decreased significantly ($F = 72.92$, $df = 1/4$, $p = 0.001$) from pre-treatment values.

Data from the VCs and ABCs were not used to calculate corrected percent reductions as was done in Ramey et al. (1999), because upon further review this study had 3 significant differences when compared with the investigations of O'Connell and Clark (1992): (1) the emergence of young-of-the-year, (2) the concentration of avian predators on placebo study plots following treatment, and (3) an increasing preference for other forage like dandelions (*Taraxacum officinale*) over the grain baits. First, we observed the emergence of young ground squirrels after the pre-treatment counts had concluded. This event precipitated a significant increase in avian predators. Our study design did not identify age classes or individuals; therefore, our general observation about the mortality increases among mainly the emerging newborn Belding's ground squirrels is antidotal. For instance, emerging young seemed to demonstrate more youthful exuberance, and they often did not heed alarm calls about the presence of avian predators: eagles, gulls, hawks, vultures, and ravens. Emergent young also seemed to run all over the study area both inside and outside of the TU boundaries, associated buffers, and beyond. These movements probably led to higher natural mortality among the emerging young on all TUs; however, some of them certainly survived that were not included in the pre-VC and pre-ABC counts but were included in the post-VC and post-ABC counts. Thus, decreasing our mortality estimates by some unknown amount.

Second, during the post-treatment observational periods, natural mortality was definitely disproportionately higher on the control plots than the treated plots. Probably because chlorophacinone use on the treated study plots had been very effective resulting in a significant decrease in their average ground squirrel prey pool with mortality $\bar{x} >$ 72%. As the prey population decreased on the chlorophacinone treated TUs, many avian predators moved initially from there to the placebo TUs to forage and a few other areas not included in the study. This unexpected concentration of avian predators primarily on the placebo TUs during post-treatment certainly influenced our estimates of natural mortality on them (Tables 2 and 3). Other areas of the fields and adjacent fields did not have a Belding's ground squirrel problem because of much lower densities as monitored prior to the study and during the study. Obvious clipping of alfalfa was limited to study site selected. Also, adjacent areas were a railroad right-of-way with weeds, sagebrush, and wheat. The emerging young had more extensive movements than previously reported by other authors including O'Connell and Clark (1992). Their information had been utilized in designing our study and particularly in the establishing the size of our buffer zones. The 5.9 ha study plots (0.4 ha TU and surrounding buffer) weren't large enough to accommodate the movements of some juveniles. For example, some juveniles were observed to move from one TU to another, while some others were observed to move from areas outside the study plots onto the treated TUs. Although all study participants had observed this occurrence during the 10 minutes "down time" between post-treatment VCs counts, it was not quantified. Finally, the extent of emerging ground squirrels survivorship interacting with the artificial concentration of avian predators is not known. In future studies, we would recommend the use of telemetry to estimate ground squirrel movements, survival and the need for larger buffer zones for investigations conducted during the spring.

Third, the decreasing acceptance of grain baits by Belding's ground squirrels during the study because of their ever increasing preference for dandelion flowers was unexpected and previously not reported in the literature during May. This observed change in foraging habits increased during May on all study plots, but especially those with the most dandelions (not quantified except through photographs). In contrast, the ground squirrel preferential dietary shift to consuming more seeds during May and June which had been observed by Marsh (1994) was not observed; however, the opposite was true with a shift to dandelions.

Finally, a general observation should be at least discussed before future studies are pursued. We found CDFA's baiting regimen appeared to provide more bait availability following each baiting day than may have been needed for efficacious control, even though non-target mortality was not observed. This may have been in part due to the weatherability characteristics of the bait with a significant decrease in the chlorophacinone concentration of the 0.01% bait during both the wet and dry conditions we encountered (Ramey et al. 2000). However, a feasible alternative baiting strategy may prove to be even more efficacious. One we would recommend exploring is the use of less bait around each burrow opening (amount to be determined) during each baiting day with our belief that baiting for 3 days each ~ 48 hrs apart may prove to be the most efficacious. We believe this would certainly decrease the effect of weather on the exposed bait while providing ample bait for treatment. Finally, we concur with a result previously reported by Stimman and Clark (1981) that the mortality risk to non-target birds and mammals from eating the bait was negligible. In this study, zero non-target deaths were recorded even though hundreds of personnel hours were spent on the study site following the first baiting until the cessation of the study looking for carcasses.

Carcass Residues

Carcass searches were conducted daily over the 6 study plots from the first day of baiting (May 13) until May 30. Forty six ground squirrel carcasses were found between May 20 and 28 (Table 1). Of these, 2 partial carcasses were found on the May 20, and 2 decomposing carcasses located on May 28. Of these, 1 carcass was located on a control TU No. 6 on May 20, 1996 during the second baiting. Although ACP personnel viewed its liver chlorophacinone residues (0.047 ppm) and whole body chlorophacinone residues (0.222 ppm) as questionable because of chromatographic interference, it was included in all analyses because it was an emerging newborn that could have traveled from a treated study plot after the first baiting on May 13. On May 20th, we completed our second baiting before noon and during our carcass searches just before dusk, we located 12 carcasses at the entrance to their burrows. Additional carcasses were predominately found between 24 – 48 hrs after the May 20th baiting and 48 – 96 hrs following the May 22nd baiting (Table 1).

We estimated a mean mortality of 484 deaths on each treated study plot (5.9 ha) based on an average mortality of 32.8 deaths on each treated TU (0.4 ha) from pre-treatment visual counts. Although we calculated the average mortality on treated TUs, we searched the entire treated study plots for carcasses before determining the pesticide's hazard. We found only 46 carcasses (2.4%) mainly in burrow entrances on the 4 TUs and associated buffer zones out of an estimated 1,935 deaths. Located carcasses averaged 1 per 1.39 ha over the 19-day study. With these results, we assumed that most of the ground squirrels died underground. This belief was supported by a later NWRC investigation conducted VerCauteren et al. (2002). Using a camera system, they found most rodent deaths occurred underground in the burrow system within ~1 meter of the burrow entrance. In conclusion, we observed no secondary mortality in birds or mammals during our investigations during nearly 1,000 man hours spent in the study area starting from the first day of baiting through the last day of the study.

Of the 46 Belding's ground squirrel carcasses we retrieved, only 42 were weighed, sexed, and frozen for later chlorophacinone analysis. The 4 partial carcasses found in the TU and buffer were noted and buried on site at a minimum depth of 12 cm. The "Methods Limit of Detection" (MLOD) for chlorophacinone analyses was established as the mean of each of the 15 analysis days using 15 control Belding's ground squirrels (i.e. one per day). The resulting mean MLOD of 0.031ppm (SD = 0.017) for the liver and mean MLOD of 0.025ppm (SD = 0.009) for the whole body. The mean percent recovery of spiked quality control samples for liver (n = 15) and whole body (n = 15) were 83.1% (SD = 17.2%) and 73.5% (SD = 10.0%) respectively.

Of the 42 whole carcasses saved for chlorophacinone analyses, 4 could not be analyzed after thawing due to their extreme decomposition. The remaining 38 ground squirrels were analyzed for chlorophacinone, and 32 (86%) had detectable levels in either their whole bodies (minus the head, appendages, pelt, and liver) and/or livers. The chlorophacinone results from the other 6 were less than (<) the MLOD. Of these, 29 had detectable chlorophacinone in their livers and the other 9 did not (i.e. < than the MLOD). Chlorophacinone in whole bodies ranged between the MLOD of 0.025 ppm (SD = 0.009) to a high of 0.546 ppm, and in livers it ranged between MLOD of 0.031 ppm (SD = 0.017) to a high of 0.648 ppm. The mean level of chlorophacinone in the whole body of ground squirrels was 0.1594 ppm (SD $\pm$ 0.1409 ppm, n = 32) and in the liver 0.1279 ppm (SD $\pm$ 0.1314 ppm, n = 29). The residue concentration of chlorophacinone was estimated for the whole animal by combining the absolute amount of chlorophacinone in the liver and whole body tissues analyzed and dividing by the animal's weight. This calculation assumes little or no chlorophacinone was present in the pelt, appendages, and head. The concentration of chlorophacinone in the whole animal based on chlorophacinone in both the liver and whole body averaged 0.1131 ppm (SD ± 0.0928, n = 29).

The importance of these chlorophacinone residue data to area scavengers is that few (2.4%) poisoned ground squirrels died above ground and only some of them posed a secondary hazard depending on what tissues were eaten by which scavenger. Non-target and secondary hazards were viewed as negligible with no such deaths documented during the more than 1,000 man hours spent in the study area with diverse activities including daily carcass searches. Our carcass data presented above was incorporated with our permission into the publication of Primus et al. (2001) evaluating chlorophacinone residues in rangeland rodents. They used a common approach from Urban and Cook (1986) for evaluating non-target hazards to mammals and birds by calculating a risk quotient (RQ). Using a RQ, Primus et al. (2001) concluded that the secondary hazard posed to potential avian predators or scavengers was minimal to negligible. Similarly, Silberhorn et al. (2003) reviewing the literature of the secondary hazard posed to golden eagles (*Aquila chrysaetos*) from CDFA's rodenticides including chlorophacinone, concluded that mortality was unlikely. This conclusion was also supported with independent data sent to the state and federal agencies indicating no incidents involving golden eagles have been reported in California or any other state using with chlorophacinone baits (US EPA 2002). In addition, Silberhorn et al. (2003) stated that no national or state incidents have been reported in red-tailed hawks (*Buteo jamaicensis*), ravens, crows (*corvus, spp*), or magpies (*pica, spp*) from chlorophacinone use. Likewise, we observed no deaths or sub-toxic effects among the avian predators observed in our study area.

CONCLUSIONS

Spring spot baiting/hand baiting using 0.01% chlorophacinone on SRO oat groats in alfalfa was demonstrated to be an effective rodenticide for Belding's ground squirrel control with most deaths (est. > 97%) occurring underground. Non-target and secondary mortality, as well as sub-lethal pesticide effects were not observed. However, we found the timing of chlorophacinone use was more critical than previously reported in the literature. Therefore, chlorophacinone applicators should be cognizant of the importance of the timing with the spring use of this rodenticide knowing that various results may occur among the interactions of the animal's life cycle, weather changes, appearance of young-of-the-year, concentration of avian predators, and preferred alternative forage. In summary, we recommend the 0.01% chlorophacinone SRO bait for ground squirrel control be used: (1) as soon as the adults emerge from their burrows, (2) before the appearance of naive young and the associated increase in avian predators, and (3) before preferred alternative forage appears.

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