Comparison of 0.005% and 0.01% diphacinone and chlorophacinone baits for controlling California ground squirrels (*Spermophilus beecheyi*)

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**Abstract.** Diphacinone and chlorophacinone, first-generation anticoagulant rodenticides, are frequently used for control of California ground squirrels (*Spermophilus beecheyi*) in agricultural and rangeland areas in California, USA. Owing to growing concerns over the risks to non-target species associated with the use of these rodenticides, the USA Environmental Protection Agency (EPA) proposed that the concentration of baits for above-ground use should be reduced from 0.01% to 0.005% active ingredient. We conducted field trials to compare the efficacy of 0.005% and 0.01% chlorophacinone and diphacinone baits in broadcast and spot applications for control of California ground squirrels on rangeland. We found no significant difference in efficacy owing to bait type, concentration or application method. Repeat testing is needed in other habitat types (e.g. crop areas) where alternative foods might reduce the effectiveness of a 0.005% bait application.

**Introduction**

In California, USA, the first-generation anticoagulants diphacinone and chlorophacinone are commonly used for control of California ground squirrels (*Spermophilus beecheyi*). The anti-coagulants are mixed with a blue dye and applied on steam-rolled oat groats (0.01% or 0.005% active ingredient (a.i.)). The effectiveness of these baits relies on squirrels consuming a series of doses over several days to obtain a lethal dose. A supply of bait over a 6–8-day period is therefore needed in most cases. To achieve this, bait having 0.005% a.i. may be continuously supplied in bait stations, or bait having 0.01% a.i. may be applied by hand (spot baiting) or by mechanical bait spreader (broadcast baiting) near active burrow systems. For broadcast baiting, the label recommends 2–3 applications of bait at a rate of 11 kg ha<sup>-1</sup> with 48 h between applications. For spot baiting, the label recommends distributing ~45 g of bait over 3.7–4.6 m<sup>2</sup> around active squirrel burrows, with 3–4 applications, 48 h apart.

Baroch (1996) evaluated the efficacy of spot-baited 0.01% and 0.005% diphacinone and chlorophacinone in controlling California ground squirrels on rangeland. We employed a baiting strategy of two treatments, three days apart for spot and broadcast baiting (Whisson and Salmon 2002). This information is critical for making informed decisions on bait registration as well as for developing more effective baiting strategies that reduce environmental hazards, including risks to non-target species.

**Materials and methods**

The study was undertaken from 10 May to 7 July 2001. This is the time of year when baits are normally applied owing to the high acceptance of baits by ground squirrels (their diet has switched from green vegetation to mostly seed) and high level of activity of squirrels (temperatures not high enough for them to be aestivating).

**Study area and site selection**

Two study areas were selected: one was in cattle-grazed rangeland areas in the Sierra Nevada foothills of the southern San
Anticoagulant bait efficacy for ground squirrel control

Joaquin Valley (near Granite Station, ~32 km north-west of Bakersfield, Kern County), and the other in the Monterey Coastal Range (near King City, Monterey County) (Fig. 1). Both areas experience a Mediterranean climate with low rainfall and hot, dry summers. During the study period, maximum temperatures ranged from 27 to 39°C in the Sierra Nevada foothills area, and from 23 to 38°C in the Monterey County area. Dominant vegetation at all sites consisted of annual grasses and forbs. Sites in each area were seasonally grazed by cattle, but no cattle were present during the study period.

Within each area, two sites at which California ground squirrels were abundant were selected. We established 10 independent plots at each site. Each plot comprised a population census area and a buffer area. Census areas were between 0.4 and 0.8 ha and delineated according to ground squirrel activity (a minimum of 20 squirrels present), and topography suitable for viewing squirrels from a distance. We marked the perimeter of each census area with coloured flags, and mapped them using a hand-held global positioning system. We also established and mapped a 70-m-wide buffer area around the census area. This resulted in plot sizes of 3.4–4.4 ha.

Within each of the four study sites, we randomly assigned plots to one of eight different treatments or two controls (i.e. baited with non-toxic oat groats), except where steep terrain precluded the use of an all-terrain vehicle (ATV) for broadcast baiting treatments. Plots that were not suitable for broadcast baiting were spot baited, and the bait type and concentration were randomly selected. The eight different treatments represented all possible combinations of application method (spot or broadcast), bait concentration (0.01% or 0.005% a.i.), and bait type (diphacinone or chlorophacinone).

Assessment of squirrel populations

We assessed squirrel populations immediately before treatment, and 10 days after the last bait application, using a visual count index adapted from Fagerstone (1984). With our index, we conducted five counts of the number of active squirrels on a plot between 0700 and 0900 hours each day, when squirrels are most active (Linsdale 1946; Fitch 1948), for three consecutive days. The index was calculated as the mean of the highest number of squirrels on each census day.

Bait acceptance

Grain-based baits are most effective in late spring and early summer when California ground squirrels switch from eating primarily green, leafy vegetation to eating seeds (Marsh 1994). To test for bait acceptance, we placed ~8 g of untreated oat groats in a pile on bare ground near 10 different active burrows, and marked the location of each with a flag. We placed the grain in the morning and checked in the late afternoon to see whether it had been consumed. We considered that ≥75% consumption of all 10 grain piles placed in the treatment area in a day was sufficient to proceed with treatment. If <75% of the grain was consumed, the process was repeated the following day. At all sites, bait acceptance was ≥75% by the second day of testing. We conducted bait-acceptance tests concurrently with the pretreatment assessment of squirrel populations.

Treatments and bait formulation

We applied bait on the first and fifth day, following pretreatment counts to the census and buffer areas of each plot. For spot baiting, we scattered ~45 g of bait over 3.7–4.6 m² around active squirrel burrows (label recommendation). We considered burrows to be active if freshly excavated dirt or other signs (tracks, fresh trails) of activity were evident. We broadcast bait at 11.4 kg ha⁻¹ (label recommendation) using a mechanical seed spreader mounted on a four-wheel-drive ATV. Bait was broadcasted at a swath width of 9.1 m and bait applied within ~4.6 m of active burrows. We calibrated the seed spreader before field work and twice during the study (Clark 1994). We either broadcast or spot-baited control plots with untreated (clean) oats. This was to counter the possible impact of the grain component of the bait on ground squirrel behaviour.

Test materials (baits) were the anticoagulants diphacinone (CAS # 82-66-6) and chlorophacinone (CAS # 3691-35-8) in the concentrations of 0.01% and 0.005% a.i. applied to steam-rolled oat groats. Baits were prepared by Kings County (EPA Establishment # 11071-CA-01) and Fresno County (EPA Establishment # 011019-CA-001) Agriculture Commissioners. We submitted samples from each bag of bait (56.5 kg) to the California Department of Food and Agriculture for quality control analysis (Salmon et al. 2002). All bait used was within the active ingredient tolerance (±10.0%) required by the EPA for commercial baits.

Data analysis

Efficacy (E) of a treatment was calculated as:

\[
E = \frac{[(\text{pretreatment index} - \text{post-treatment index})/\text{pretreatment index}] \times 100}{1}
\]

Fig. 1. Study site locations for bait efficacy study, 2001.
Pretreatment squirrel counts for plots in each site were adjusted by the percentage change in control plots at that site, according to EPA testing protocols.

We used a three-way factorial in a randomised block design with sites as blocks, and comparing least-squares means of efficacy for bait type × application method × concentration (control plot data excluded). We used the MIXED procedure (SAS Institute 2002) with percentage efficacy (E) as the response variable. We used a significance level of $\alpha = 0.05$.

**Results**

**Efficacy of treatment**

We excluded data from all plots at one of the Sierra Nevada foothills’ sites from efficacy analysis owing to declines of 63 and 83% in the ground squirrel population index on the control plots of that site during the study period. We considered declines of 3.2–35.4% and an increase by 16.3% in indices on other control plots to be acceptable.

Efficacy was higher than the 70% level required by the EPA for registration on 22 of 24 treatment plots (Table 1). Efficacy was only 51.9% on one plot that was spot baited with 0.005% chlorophacinone, and 59.9% on another that was broadcast baited with 0.01% diphacinone.

We found no difference in efficacy between bait types ($F_{1,14} = 0.31, P = 0.5867$), concentration ($F_{1,14} = 0.99, P = 0.3357$) or application method ($F_{1,14} = 0.10, P = 0.7550$). There were no significant interaction effects: concentration × bait type ($F_{1,14} = 0.91, P = 0.3569$), bait type × application method ($F_{1,14} = 1.59, P = 0.2278$), concentration × application method ($F_{1,14} = 1.35, P = 0.2643$), or bait type × concentration × application method ($F_{1,14} = 0.49, P = 0.4968$) (Table 1).

**Discussion**

Results from our study suggest that there is no difference in efficacy between 0.005% and 0.01% anticoagulant bait for control of California ground squirrels on grazed rangeland in California. Furthermore, efficacy did not vary according to bait type (diphacinone or chlorophacinone) or application method (spot or broadcast). All combinations of treatments (bait type × concentration × application method) reduced populations by the minimum of 70% (mean of all sites) required by the EPA for registration. Our results are similar to those reported by Baroch (1996), who, in a smaller-scale study, compared concentration and application method of diphacinone bait for control of California ground squirrels on rangeland. Although these results appear to support discontinuing the registration and use of 0.01% anticoagulant bait concentrations, care should be taken to interpret them in context with the limitations of the study and further field studies are warranted before making a final registration decision.

Although we detected significant changes in population indices as a result of bait application, our index, which is frequently used for assessing California ground squirrel populations (Hazén and Poché 1992; Baroch 1996), may not have been sensitive enough to detect differences between treatments. High variation in population indices before and after treatment at control sites, and high variation in treatment efficacy between sites suggest that squirrel behaviour may have reduced the sensitivity of our population index. Activity of California ground squirrels varies hourly as well as daily as a result of factors such as stage of the breeding cycle, temperature, presence of predators, and food supply (Linsdale 1946; Stroud 1983). Our index limits the effects of daily variation of behaviour on our index by restricting squirrel counts to the hours when squirrels are most active, and using the mean of the highest of five counts per day from three days. Increasing the number of days over which counts are taken may improve the sensitivity of the index in future tests. Including control sites was important in determining effects of other factors on population indices. At one site, large decreases in the population index of control plots occurred and resulted in our excluding the results for the site from analysis. Several days of high temperatures (>35°C) following the bait treatment may have resulted in a large proportion of squirrels entering aestivation, although it is unclear as to why population declines weren’t also observed at the second site in the same area. The delayed time to death of animals after anticoagulant bait application also makes it difficult to measure anticoagulant bait efficacy. The time delay increases the potential for invasion of treatment areas from neighbouring untreated areas, for emergence of young or aestivating adults that were not present during pre-treatment population assessment, for natural dispersal, or for loss of squirrels owing to aestivation before post-treatment population assessment.

Alternative methods of deriving a population index for ground squirrels, such as active burrow counts and other indirect measures of activity, have also been used (Fitch 1948; Tietjen 1976; Baroch 1996) but generally aren’t sensitive to small changes in population size (Downey 2003). The use of radio-collars with mortality sensors is expensive and labour intensive but may provide the only accurate and sensitive measure of the effectiveness of anticoagulant bait. During this study, we attempted an active burrow count index method but found our methods ineffective owing to the extreme hardness of the soil in our plots and difficulty of filling the burrow entrances.

Our study considered squirrel control only on rangeland, where the efficacy of baiting may be higher because squirrels have relatively few alternative foods. In cropped situations or areas where there is a mosaic of different habitats, and therefore food sources, bait is likely to be less attractive. For example,

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**Table 1. Mean efficacy (percentage reduction in population index) of bait type, bait concentration, and application method in controlling California ground squirrel populations in field studies of diphacinone and chlorophacinone on California rangeland, May–July 2001**

<table>
<thead>
<tr>
<th>Bait type</th>
<th>Concentration (%)</th>
<th>Application method</th>
<th>Efficacy Mean</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Chlorophacinone</td>
<td>0.005</td>
<td>Broadcast</td>
<td>86.9</td>
<td>5.8</td>
</tr>
<tr>
<td>Chlorophacinone</td>
<td>0.005</td>
<td>Spot</td>
<td>70.6</td>
<td>11.5</td>
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<td>Broadcast</td>
<td>85.5</td>
<td>7.7</td>
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<td>Diphacinone</td>
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<td>Broadcast</td>
<td>84.4</td>
<td>6.6</td>
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<td>Spot</td>
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<td>1.8</td>
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<td>20.2</td>
<td>16.1</td>
</tr>
<tr>
<td>Control</td>
<td>0.000</td>
<td>Spot</td>
<td>7.5</td>
<td>10.0</td>
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control of ground squirrels with grain baits is notoriously difficult in and around nut crops where squirrels feed almost exclusively on nuts (Kowalski et al., in press). In these situations, where squirrels may ingest smaller quantities of bait, higher-concentration baits might prove to be more effective.

Reducing bait concentration from 0.01% to 0.005% may reduce the potential for secondary poisoning of non-target species by reducing anticoagulant bait residues in squirrels that have consumed bait (Ward 2003); however, it is unlikely to reduce the potential for primary poisoning of smaller non-target species such as meadow voles (Microtus californicus), deer mice (Peromyscus spp.) and kangaroo rats (Dipodomys spp.) that would most likely be present in treatment areas and for which only a small amount of bait is lethal. Furthermore, both primary and secondary poisoning risks may increase as a result of more frequent bait treatments using lower-concentration bait if treatments are not providing the desired level of control.

From carcass surveys on study plots (census areas only were searched), we collected 16 non-target animals (14 kangaroo rats and 2 deer mice) that died from anticoagulant poisoning. This represents a non-target poisoning rate of 0.37 carcasses ha⁻¹. Baroch (1996) reported a non-target poisoning rate of 0.5 carcasses ha⁻¹ of eight different rodent and lagomorph species after diphacinone bait treatments on rangeland. However, Baroch (1996) applied bait four times, at two-day intervals and carcass searching was conducted only on baiting days, which limited the potential to find carcasses that died after the last day of baiting. Thus, it is likely that the non-target poisoning rate reported by Baroch (1996) is an underestimate. In our study, we applied bait twice, with three days between baiting days and searched for carcasses for at least 10 days after the final day of bait distribution. Both studies resulted in efficacy above the 70% threshold required by the EPA for field studies, suggesting that a reduced baiting strategy may be equally effective as the current label recommendations and result in lower hazards to non-target animals.

Modifying baiting strategies (timing and method of application) rather than reducing bait concentration may have more effect in reducing non-target risks (Whisson and Salmon 2002). In a laboratory study, Whisson and Salmon (2002) showed that the timing of bait applications was more important than bait concentration in controlling ground squirrels. They recommended reducing the number of applications to two (label recommends 3–4) with 2–3 days between applications. In a study of seed uptake by ground squirrels, Dochtermann (2005) found that squirrels consumed grain broadcast as far as 30 m from their burrows, within seven days. Dochtermann (2005) therefore suggested that a single bait application of ~22.5 kg ha⁻¹ (about twice the current recommended rate) would take advantage of the squirrels’ exceptional ability to locate seeds and may provide effective control. A single application would have the additional advantage of reducing labour costs. Broadcast applications of bait may also reduce risks to non-target species by dispersing bait over a larger area than the spot-baiting technique. Additional research is recommended to more fully evaluate the efficacy of diphacinone and chlorophacinone baits in California ground squirrel control under varying agricultural conditions. We believe this is especially important since the rangeland sites we used for this study had limited food resources available to the ground squirrels. In most agricultural areas, other food will likely be available to compete with the bait, and this could significantly impact the efficacy of these baits and baiting strategies.

Acknowledgements
Funding was provided by the California Department of Food and Agriculture Vertebrate Pest Control Research Advisory Committee (CDFA contract 00-0471). This study was conducted under the University of California Animal Use and Care Protocol #9493. Special thanks to Ralph Phillips of Kern County Cooperative Extension for help in locating the Bakersfield study site. We thank Cathie Joughin, Tim Hearne and Bill Whitney for access to their ranches and for their willingness to manage their livestock to accommodate the study. We also thank Henry Gonzales, Bill Taylor and Pam Everett of the King City office of the Monterey County Agricultural Commissioner’s office for logistical help and identifying cooperators. We are grateful to Fred Rinder of the Fresno County Agriculture Commissioner’s office and Les Wright of the Kings County Agriculture Commissioner’s office for supplying bait, sometimes on short notice. We appreciate help with bait application and carcass searching provided by numerous personnel from the California Department of Food & Agriculture and the University of California. We thank Nick Condos and Duane Schnabel of the California Department of Food and Agriculture, who served as the initial and final Study Directors, respectively.

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Salmon, T. P., Whisson, D. A., and Gorenzel, W. P. (2002). Field efficacy studies comparing 0.005% and 0.01% diphacinone and chlorophacinone.
baits for controlling California ground squirrels (*Spermophilus beecheyi*). Unpublished report. California Department of Food & Agriculture Contract 00-0471. CA Department of Food & Agriculture: Sacramento, CA.


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AUTHOR(S):
Geraldine R. McCann

STUDY COMPLETION DATE:
February 24, 2000

LABORATORY:
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Fort Collins, CO 80521-2154

LABORATORY PROJECT ID:
QA-506
CDFA No. 94-0620

CITATION:
STATEMENT OF DATA CONFIDENTIALITY

No claim of confidentiality is made for any information contained in this study on the basis of it falling within the scope of FIFRA 10(d)1(A), (B), or (C).

Company: California Department of Food and Agriculture

Agent: Nick Condos

Title: Program Supervisor

Date: 12/13/99

Signature: [Signature]

QA-506
GOOD LABORATORY PRACTICE/QUALITY ASSURANCE STATEMENT

This study meets the requirements of 40 CFR Part 160. It was maintained on the National Wildlife Research Center (NWRC) Master Schedule. An inspection was performed on July 22, 1998, by Donald J. Elias. The results of this inspection were given to the Study Director (Geraldine R. McCann) and to the NWRC Director (Richard D. Curnow) on July 22, 1998.

Agent: Donald J. Elias
Title: Quality Assurance Officer
Date: 7/22/98

Submitter: California Department of Food and Agriculture

Agent: Nick Condos
Title: Program Supervisor
Date: 2/25/00

Sponsor: U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, NWRC

Agent: Dr. Richard D. Curnow
Title: Director
Date: 2/24/00

Study Director: Geraldine R. McCann
Title: Biological Science Technician (Wildlife)
Date: 2/24/00

Signature:

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The Vertebrate Pest Control Research Advisory Council (VPCRAC), through a cooperative agreement with the California Department of Food and Agriculture (CDFA), funded a laboratory study at the National Wildlife Research Center (NWRC) to provide efficacy data required by the United States Environmental Protection Agency (EPA) for the reregistration of 0.01% chlorophacinone oat groat bait for controlling deer mice (Peromyscus maniculatus) in California. Twenty mature deer mice (10 males and 10 females) were purchased from the University of South Carolina on November 19, 1997 to begin a breeding colony. By July 1998, the offspring reached maturity and 60 mice (30M:30F) were selected for the laboratory test. The mice were randomly assigned by weight and sex to one of three groups, each containing 10 males and 10 females. A 15-day, two-choice feeding trial was started with a control group (Group I) receiving 2 dishes of OPP rat and mouse challenge diet (0.00% control bait). The 2 treated groups (Group II and Group III) received 1 dish of OPP rat and mouse challenge diet and 1 dish of 0.01% chlorophacinone oat groat bait. The test ended at Day 9 when all 40 (100%) of the treated deer mice died. Mortality occurred as early as Day 1 and continued until Day 9, with 92% of the mice dying between Days 3 and 7. Females from Groups II and III ate more 0.01% chlorophacinone grain bait than the males from either groups. The treated bait accounted for 69% of the total consumption for female groups combined and 55% of the combined male groups total consumption. This laboratory study indicates that chlorophacinone is an efficacious field rodenticide for controlling deer mice in California. The 100% mortality exceeds the EPA standard of 90% mortality for verifying efficacy of rodenticides.
INTRODUCTION

In 1993, the EPA requested from the California Department of Food and Agriculture (CDFA) efficacy data for the 0.01% chlorophacinone oat groat bait for controlling deer mice (*Peromyscus* spp.). To meet this objective, the Vertebrate Pest Control Research Advisory Council (VPCRAC) and the CDFA, in cooperation with the National Wildlife Research Center (NWRC), conducted a laboratory feeding trial that evaluated mortality and bait acceptance among deer mice fed an oat groat bait formulated with 96.03% technical chlorophacinone. These data were requested by the Environmental Protection Agency (EPA) as partial fulfillment of the requirements for the reregistration of 0.01% chlorophacinone oat groat bait (SLN CA 890024) (Appendix I). This feeding trial was conducted in compliance with Code of Federal Regulations (CFR) 40, Part 160, Good Laboratory Practices (Sirofchuck 1996) and the appropriate Pesticide Assessment Guidelines (PAG), Subdivision G (U.S. EPA 1982). The test method conformed to the *Standard Peromyscus Species Anticoagulant Dry Bait Laboratory Test Method* as outlined in Office of Pesticide Programs (OPP) Designation 1.216 (2-25-74) (McCann 1980) (Appendix II).

The trial was conducted in July and August 1998 by NWRC and assigned study designation QA-506 (Appendix III). The null hypothesis ($H_0$) states that mouse mortality will be equal for treated deer mice feeding on the 0.01% chlorophacinone and OPP rat and mouse challenge diet, and the control deer mice feeding on the OPP rat and mouse challenge diet, alone.
MATERIALS AND METHODS

Formulation

0.01% Chlorophacinone Oat Groat Bait

The 0.01% chlorophacinone oat groat bait was formulated by Rodent Control Outfitters, Inc. Junction City, Oregon, and was prepared according to the Confidential Statement of Formula for this CDFA registration (SLN CA 890024). The 0.01% chlorophacinone grain bait was assayed by the Analytical Chemistry Unit of the NWRC using validation ACP Method 62A.

OPP Rat and Mouse Challenge Diet

The OPP rat and mouse challenge diet was prepared at the NWRC as specified by the EPA. The diet was formulated according to the instructions in OPP Designations (1.216) and was composed of the following ingredients (McCann 1980):

<table>
<thead>
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<th>Ingredients</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Cornmeal (whole yellow ground corn)</td>
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</tr>
<tr>
<td>Rolled oat groats (ground)</td>
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</tr>
<tr>
<td>Sugar (powdered ≥ 95% purity)</td>
<td>5%</td>
</tr>
<tr>
<td>Corn oil (≥ 95% purity)</td>
<td>5%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
</tr>
</tbody>
</table>

After formulation, the OPP rat and mouse challenge diet was packaged in plastic containers, tightly sealed, and maintained at -18°C or colder until used. Before being offered to test or control mice, the challenge diet and the toxic bait were brought to room temperature.

1Reference to commercial products or entities does not imply endorsement by U.S. Government.

2Mailing Address: P.O. Box 446, Junction City, OR 97448.
Test Procedures

Pre-treatment

Animal Procurement

A breeding colony of *Peromyscus maniculatus* was established in late November, 1997, at the NWRC’s Animal Research Building (ARB). Ten males and 10 females weighing 15-16 g each were purchased (certified hantavirus disease free) from the University of South Carolina, Columbia, SC  29208.\(^3\)

Quarantine

After their arrival on November 19, 1997, at the NWRC’s ARB, each deer mouse was weighed and then placed in an individual cage (27.9 x 17.8 x 12.7 cm). The mice were maintained on a commercial laboratory mouse diet and provided water *ad libitum*. They immediately began the 7-day quarantine period. Following completion of the quarantine period, the deer mice were checked by the resident veterinarian before being released to start the breeding colony.

The room temperature was maintained between 19 and 22°C. The light cycle was 12 hours dark and 12 hours light.

Breeding Colony

The breeding colony remained in the same room used during the quarantine period. The 10 males and 10 females were paired up on November 26, 1997, and the breeding colony was maintained until enough offspring were produced to conduct the required laboratory tests. From this breeding colony, 60 sexually mature offspring (30 males, 30 females) weighing between 12.1 g and 23.6 g were selected.

\(^3\) Reference to commercial products or entities does not imply endorsement by U.S. Government
Weighing, Ranking, and Assignment of Treatments

The first 60 deer mice (30 males, 30 females) that met the minimum body weight required for testing were removed from the breeding colony and moved to the breeding room annex where they were weighed and placed into individual uniquely numbered cages. Each gender was then ranked by weight into 10 weight classes, each containing 3 animals. Each weight class for both genders contributed one animal to each of the 3 groups (I, II, and III). The 3 groups of 10 deer mice, separated by gender, were randomly assigned to one of the following Groups: 0.0% control (Group I), 0.01% chlorophacinone bait (Group II), and 0.01% chlorophacinone bait (Group III). This grouping resulted in 10 deer mice per gender per group, or a total of 20 animals per group and evenly distributed the mice by body weights among the 3 groups.

3-Day Acclimation Period

The 10 mice assigned to each treatment group by sex were removed from their individual cages and grouped by sex in triple size cages (63.5 x 24.1 x 17.8 cm). Each of the 6 cages was then labeled by sex and treatment and moved into the test room. The animals were allowed to acclimate to their new groups for 3 days. This test room temperature was maintained between 19 and 22°C, under a 12 hr: 12 hr light-dark cycle.

15-Day Feeding Trial

Day 1

On the first day of testing, the regular diet was removed from all deer mice and they were offered two rectangular aluminum dishes (15.24 x 3.81 x 3.81 cm), located on opposite sides of the cage. Group I (control) received 2 dishes (one labeled O and one labeled C) each containing (in excess of 40 g) OPP rat and mouse challenge diet. Mice in Groups II and III, receiving the 0.01% chlorophacinone grain bait, were given one dish containing (in excess of 40 g) the toxicant and a second dish containing (in excess of 40 g) OPP rat and mouse challenge diet. Trays were placed beneath each cage to catch bait and OPP rat and mouse challenge diet spillage. Dishes containing the 0.01% chlorophacinone were positioned on the left and dishes containing
the OPP rat and mouse challenge diet were placed on the right, both at the front of the cage. Bottled water was available to the animals at all times and was positioned at equal distances from the bait dishes. The animal room was closed until Day 2 except for minimal and unobtrusive observation, as conditions permitted, to monitor the animals. The 24-hour period from the time the bait was issued until its removal the next morning was considered Day 1 (July 18 to July 19, 1998).

**Day 2**

Bait dishes and any spillage were removed and weighed to determine the gross weight to the nearest 0.01 g. The amount of bait consumed was recorded. Sometimes the bait weighed more than it did initially. This may be attributable to either moisture gain (from humidity) or possibly urine in the dishes. Those particular bait weights were reported as a gain and were omitted when summing the total amount of bait consumed. Dishes were then replenished with fresh bait to bring them back to their original weight. Any bait that was fouled with urine or feces was discarded. Dish positions were then reversed when returned to the cages (i.e., chlorophacinone dishes on the right, OPP rat and mouse challenge diet dishes on the left). Any mortalities were removed and weighed. The animal room was then closed as described for Day 1.

**Days 3-15**

Days 3-15 were a repeat of Day 2, except the positions of the dishes were alternated each day. Any carcasses were removed and weighed.

**5-Day Post-testing Feeding Period**

**Day 16**

All survivors were then placed on the OPP rat and mouse challenge diet for 5 days. All control animals and groups of surviving mice were offered one rectangular metal dish (15.24 x 3.81 x 3.81 cm) containing (in excess of 40 g) the OPP rat and mouse challenge diet. The position of the dish (labeled O) was alternated each day. Trays were placed beneath each cage to
catch spillage. Bottled water was available at all times and was positioned in the center of the front of the cages. The animal room was closed until Day 17 except for minimal and unobtrusive observations, as conditions permitted, to monitor the animals.

**Days 17-19**
Each morning, the OPP rat and mouse challenge diet, including any bait spillage, was again removed and weighed, following the procedure outlined for Day 16. Animals were checked for mortality.

**Day 20**
In the morning, the bait and any spillage were removed and weighed. Survivors were weighed and euthanatized with CO₂ and frozen until the carcasses could be incinerated.

**Data Recorded During the Study**
1. Daily consumption for both bait dishes for each group of deer mice.
2. Body weights of the deer mice were recorded (1) pre-treatment, (2) after death, or (3) at the end of the study.

**Amount and Proportion of Treated Bait (0.01% chlorophacinone) Consumed Compared to the OPP Rat and Mouse Challenge Diet.**
The EPA requires, upon completion of the 15-day study, that calculations be made to determine the proportion of the 0.01% chlorophacinone bait consumed compared to the consumption of OPP rat and mouse challenge diet.

**Statistics**
Bait consumption and body weights were totaled for each group, and a mean and standard deviation were computed. A student’s t-test was used to determine differences in bait consumption between the O and C dishes for male and female control deer mice.
RESULTS

Bait Analysis

Upon receipt at the NWRC, the 0.01% chlorophacinone bait was assayed by the Analytical Chemistry Unit, using validation method 62A. The bait assayed at a mean of 0.0098% (Appendix IV).

Mortality

No mortality occurred in the control group during the study. All 40 (100%) deer mice feeding upon the 0.01% chlorophacinone grain bait in Groups II and III died (Table 1) with most mortality (92.5%) occurring between days 3 and 7 of the treatment period (Appendix V, Raw Data Summary 1). There was no difference in the rate of death or total mortality between the sexes or between the two 0.01% chlorophacinone treatment cages. Figure 1 presents the cumulative mortality curves for the three treatment groups. While no animals died in the control group, mortality rates in the two groups receiving the treated bait followed the same curve.

Bait Consumption - 15-Day Feeding Trial

Control - Group I

Females

The 10 female deer mice consumed between 21.50 g and 27.81 g of the OPP rat and mouse challenge diet per day over the 9-day feeding trial. Because all 40 deer mice died on the 0.01% chlorophacinone baits by Day 9, tests with the control deer mice were terminated at this point. From the O dish, total bait consumption for the 10 deer mice was 93.75 g during the 9-day feeding trial (Table 1). Mean (SD) daily diet consumption was 10.42 g (2.16). Daily bait consumption from the O dish ranged from 7.62 g to 14.65 g (Appendix V, Raw Data Summary 2). A Student’s T-test showed the mice preferred the diet from the C dish (t = 2.9454, d.f. = 8, p = 0.0186), consuming a total of 129.10 g during the 9-day feeding trial (Table 1). Mean (SD) daily diet consumption was 14.34 g (2.23). Daily bait consumption ranged from 9.60 g to 16.96 g.
Males

The 10 male deer mice consumed between 22.7 g and 26.23 g of the OPP rat and mouse challenge diet per day over the 9-day feeding trial. Because all 40 of the deer mice died on the 0.01% chlorophacinone baits by the end of Day 9, the control deer mice were removed from further testing. From the O dish, total consumption for the 10 deer mice was 128.03 g during the 9-day feeding trial (Table 1). Mean (SD) daily diet consumption was 14.22 g (3.65). Daily consumption ranged from 10.55 g to 19.91 g (Appendix V, Raw Data Summary 3). A Student’s T-test illustrates the mice consumed statistically less from the C dish (t = 1.5558, d.f. = 8, p = 0.1584) (Table 1). Mean (SD) daily diet consumption was 10.64 g (3.24). Daily bait consumption ranged from 5.38 g to 14.66 g.

Treated - Group II

Females

The 15-day feeding trial was cut short for this group as all 10 deer mice died by Day 7. Bait consumption from the O dish on Day 1 was 10.26 g, and increased to 11.53 g on Day 2. Consumption decreased slightly on Day 3 to 9.59 g, then declined daily until Day 5. On Days 6 and 7, the dishes and bait weighed more than their original weight. Total bait consumption for the O dish was 36.36 g. The mean (SD) daily bait consumption was 7.27 g (4.60). Daily bait consumption ranged from 0.69 g to 11.53 g (Appendix V, Raw Data Summary 4). More 0.01% chlorophacinone bait was consumed from the T dish than the OPP rat and mouse challenge diet from the O dish. From the T dish, the mice consumed 12.25 g on Day 1, increased their consumption slightly Day 2 to 13.62 g, then decreased to 11.69 g on Day 3. Bait consumption dropped sharply on Day 4 to 5.14 g. Consumption then decreased daily to 0.79 g and 0.12 g on Day 5 and Day 6, respectively. On Day 7, the dish weighed more than its original weight. Total bait consumption was 43.61 g (Table 1). The mean (SD) daily bait consumption was 7.27 g (6.04). Daily bait consumption ranged from 0.12 g to 13.62 g. Overall, mice consumed 7.25 g more of the 0.01% chlorophacinone bait than the OPP rat and mouse challenge diet.
Males

The 15-day feeding trial was cut short for this group because all 10 deer mice died by Day 7. Bait consumption from the O dish was 11.49 g on Day 1 and increased to 12.80 g on Day 2 (Appendix V, Raw Data Summary 5). Consumption decreased slightly to 11.10 g on Day 3, sharply to 3.40 g on Day 4, and 0.81 g on Day 5. On Days 6 and 7, the bait dishes weighed more than their initial weight. Total bait consumption for the O dish was 39.60 g (Table 1). The mean (SD) daily bait consumption was 7.92 g (5.42). Less 0.01% chlorophacinone bait was consumed from the T dish than the OPP rat and mouse challenge diet from the O dish. Bait consumption from the T dish was 7.73 g on Day 1, and increased to 10.45 g on Day 2. Consumption decreased to 4.81 g on Day 3 and to 0.03 g on Day 4. On Day 5, the bait dish weighed more than its original weight. No bait was consumed on Day 6, and on Day 7 the bait dish weighed more than its original weight. Total bait consumption was 23.02 g (Table 1). The mean (SD) daily bait consumption was 4.60 g (4.64). Overall the mice consumed 16.58 g more of the OPP rat and mouse challenge diet than the 0.01% chlorophacinone bait.

Treated - Group III

Females

The 15-day feeding trial was cut short for this group because all 10 deer mice died by Day 9. Bait consumption from the O dish was 5.20 g on Day 1 and increased to 7.67 g on Day 2 (Appendix V, Raw Data Summary 6). Consumption decreased sharply to 1.81 g on Day 3, and decreased daily until Day 9, when 0.05 g were consumed. Days 5 and 6 the bait dishes weighed more than their original weight. Total bait consumption for the O dish was 16.32 g (Table 1). The mean (SD) daily bait consumption was 2.33 g (2.96). Daily bait consumption ranged from 0.05 g to 7.67 g. More 0.01% chlorophacinone bait was consumed from the T dish than the OPP rat and mouse challenge diet from the O dish. From the T dish, the mice consumed 17.91 g on Day 1, and they increased their bait consumption on Days 2 and 3 to 19.04 g, and 21.61 g, respectively. Bait consumption declined to 10.40 g on Day 4 and 2.88 g on Day 5. Consumption then declined daily until Day 9 when only 0.04 g were consumed. Daily bait consumption ranged from 0.04 g to 21.61 g. Total bait consumption was 75.27 g (Table 1). The mean (SD)
daily bait consumption was 8.36 g (8.98). Overall, the mice consumed 58.95 g more of the 0.01% chlorophacinone bait than the OPP rat and mouse challenge diet.

**Males**

The 15-day feeding trial was cut short for this group as all 10 deer mice died by Day 7. Bait consumption from the O dish was 8.59 g on Day 1, and decreased to 7.80 g on Day 2 (Appendix V, Raw Data Summary 7). On Days 3 and 4, consumption decreased to 2.82 g and 0.18 g, respectively. On Days 5 and 7 the bait dish weighed more than its original weight. On Day 6, consumption was 0.16 g. Daily bait consumption ranged from 0.16 g to 8.59 g. Total bait consumption for the O dish was 19.55 g. The mean (SD) daily bait consumption was 3.91 g (4.07). More 0.01% chlorophacinone bait was consumed from the T dish than the OPP rat and mouse challenge diet from the O dish. From the T dish, the mice consumed 13.50 g on Day 1, and increased their consumption on Day 2 to 16.31 g. On Days 3 and 4, bait consumption decreased to 10.36 g and 5.39 g on Days 3 and 4, respectively. On Days 5 and 6, consumption decreased daily, to 2.89 g to 0.82 g, respectively. Total bait consumption from the T dish was 49.27 g (Table 1). On Day 7, the dish weighed more than its original weight. The mean (SD) daily bait consumption was 8.21 g (6.15). Overall, the mice consumed 29.72 g more of the 0.01% chlorophacinone bait than the OPP rat and mouse challenge diet.

**Mean Bait Consumption Per Mouse Per Day**

The mean bait consumption per mouse per day was plotted for the number of deer mice alive at each feeding day during the 15-day treatment period for Groups II and III (Fig. 2).

For Groups II and III, Day 1, the average bait consumption per mouse ranged from 0.77 g to 1.79 g. Bait consumption peaked on Day 2, for Group II (both sexes) and Group III (males). On Day 3, bait consumption decreased slightly for Group II (both sexes) and Group III (males), ranging from a low of 0.48 g to 1.46 g. Whereas, Group III females consumed their most bait on Day 3. On Day 4, bait consumption decreased in Groups II and III for both sexes. Day 5, consumption decreased with all animals consuming less than 0.75 g per mouse per day. From Day 6 to the termination of the study on Day 7 (Group II) and Day 9 (Group III), bait consumption per day per animal was less than 0.6 g.
Amount and Proportion of Treated Bait (0.01% Chlorophacinone) Consumed Compared to the OPP Rat and Mouse Challenge Diet.

The total amount of OPP rat and mouse challenge diet and 0.01% chlorophacinone oat groat bait consumed over the 15-day treatment period was used to determine the bait acceptance (Table 2). Females from Groups II and III ate more 0.01% chlorophacinone grain bait (69%) than the males from both groups combined (55%). Dietary consumption of 0.01% chlorophacinone oat groat bait was calculated for males and females combined and accounted for approximately 63.1% of the total diet.

5-Day Post-testing Bait Acceptance

All 20 deer mice survived in Group I, and were tested on the 5-day post-testing bait acceptance trial.

Control - Group I

Females

All 10 female mice survived post-treatment. The total diet consumption for all 10 deer mice for the 5-day post-testing feeding period was 113.13 g, with a mean (SD) daily bait consumption of 22.63 g (0.32) (Table 2). Their daily bait consumption ranged from 22.11 g to 22.96 g (Appendix V, Raw Data Summary 8).

Males

All 10 male mice survived post-treatment. The total diet consumption for all 10 deer mice for the 5-day post-testing feeding period was 112.97 g, with a mean (SD) daily bait consumption of 22.59 g (1.09) (Table 2). This daily bait consumption ranged from 20.74 g to 23.62 g (Appendix V, Summary 8).

Body Weights Pre- and Post-treatment

When testing the deer mice, they were housed together in groups of 10. Therefore, individual mice could not be identified to allow comparison of their individual weights pre- and post-treatment. Instead, the body weight were compared by group. The mean pre-treatment
body weights for each of the males Group I, II, and III were within 1.5 g of one another, ranging from 202.7 (Group I) to 204.2 (Group III). The mean pre-treatment body weights for each of the females Group I, II and III were within 3.3 g of one another, ranging from 194.1 g (Group III) to 197.4 (Group I) (Table 4).

Control - Group I

Females
Pre-treatment, the 10 deer mice weighed 197.4 g, with a mean (SD) of 19.74 g (2.32) (Table 4; Appendix V, Raw Data Summary 9). Post-treatment, the 10 deer mice weighed 199.4 g, with a mean (SD) of 19.94 g (2.97) (Table 4; Appendix V, Raw Data Summary 10). This was a loss of 2.0 g between pre- and post-treatment body weights. Two deer mice weighed less than the lightest female pre-treatment (16.9 g), but 2 deer mice weighed more than the heaviest female pre-treatment (23.5 g). The remaining 6 mice weighed between the lightest and heaviest female pre-treatment.

Males
Pre-treatment, the 10 deer mice weighed 202.7 g, with a mean (SD) of 20.27 g (2.14) (Table 4; Appendix V, Raw Data Summary 9). Post-treatment, the 10 deer mice weighed 187.44 g, with a mean (SD) of 18.74 g (3.39) (Table 4; Appendix V, Raw Data Summary 10). This was a loss of 15.3 g between pre- and post-treatment body weights. Two deer mice weighed less than the lightest male pre-treatment (16.4 g) and one mouse equaled the weight of the lightest female pre-treatment. The remaining 7 mice weighed between the lightest and heaviest male pre-treatment.

Treated - Group II

Females
Pre-treatment, the 10 deer mice weighed 194.5 g, with a mean (SD) of 19.45 g (2.42) (Table 4; Appendix V, Raw Data Summary 9). Post-treatment, the 10 deer mice weighed 168.20 g, at time of death, with a mean (SD) of 16.82 g (2.30) (Table 4; Appendix V, Raw Data Summary 10). This was a loss of 26.3 g between pre- and post-treatment weights body weights.
Three deer mice weighed less than the lightest female pre-treatment (15.5 g). The remaining 7 deer mice weighed between the lightest and heaviest pre-treatment female.

**Males**

Pre-treatment, the 10 deer mice weighed 203.1 g, with a mean (SD) of 20.31 g (2.00) (Table 4; Appendix V, Raw Data Summary 9). Post-treatment, the 10 deer mice weighed 164.78 g, at time of death, with a mean (SD) of 16.48 g (1.23) (Table 4; Appendix V, Raw Data Summary 10). This was a loss of 38.3 g between pre- and post-treatment weights body weights. Eight deer mice weighed less than the lightest male pre-treatment (17.4 g). The remaining 2 deer mice weighed between the lightest and heaviest pre-treatment male.

**Treated - Group III**

**Females**

Pre-treatment, the 10 deer mice weighed 194.1 g, with a mean (SD) of 19.41 g (2.24) (Table 4; Appendix V, Raw Data Summary 9). Post-treatment, the 10 deer mice weighed 158.80 g, at time of death, with a mean (SD) of 15.88 g (1.23) (Table 4; Appendix V, Raw Data Summary 10). This was a loss of 35.3 g between pre- and post-treatment body weights. Seven deer mice weighed less than the lightest female pre-treatment (15.9 g). The remaining 3 deer mice weighed between the lightest and heaviest pre-treatment female.

**Males**

Pre-treatment, the 10 deer mice weighed 204.2 g, with a mean (SD) of 20.42 g (1.90) (Table 4; Appendix V, Raw Data Summary 9). Post-treatment, the 10 deer mice collectively weighed 167.29 g, at time of death, with a mean (SD) of 16.73 g (2.11) (Table 4; Appendix V, Raw Data Summary 10). This was a loss of 36.9 g between pre- and post-treatment body weights. Nine deer mice weighed less than the lightest male pre-treatment (17.8 g). The remaining mouse weighed between the lightest and heaviest pre-treatment male.
All 40 deer mice in the treated groups consumed sufficient amounts of the 0.01% bait to cause 100% mortality. Because of the 100% mortality among the treated groups and because no mortality occurred among the 20 control deer mice (Group I), the null hypothesis was rejected. The 100% mortality among the deer mice consuming the 0.01% chlorophacinone grain bait exceeded the 70% minimum standard for mortality established by the EPA for verifying efficacy of rodenticides (U.S. EPA 1982).

Our inability to compare individual post-treatment body weights of deer mice with their pre-treatment body weights has made interpretation of post-treatment body weight gain or loss difficult. Among the control mice, the average weight gain per mouse was 0.20 g for females and 1.76 g for males. Four control males weighed less than the lightest pre-treatment male (18.75 g) and no control males post-treatment exceeded the body weight of the heaviest male mouse pre-treatment (23.1 g). Two females post-treatment weighed less than the lightest female (16.9 g) pre-treatment and two post-treatment females weighed more than the heaviest pre-treatment female (23.5 g). The mice in the 2 treatment groups most likely lost body weight during treatment. Twenty-eight (70%) of the 40 definitely lost body weight, as they weighed less than the lightest mouse weighed pre-treatment. Of the other 12 mice, none weighed more than the heaviest pre-treatment animal in its group.

Based on the plotted data for the mean amount of 0.01% treated bait ingested per mouse per day, the deer mice readily accepted the chlorophacinone oat bait on Day 1 (Fig. 2). The mean amount of bait consumed per mouse on Day 1 ranged from 0.77 g to 1.79 g. On their first day of exposure, they consumed 26.88% of the total amount of the 0.01% treated bait consumed. On Day 2, both Groups II and III increased their consumption of the 0.01% treated bait over Day 1. Maximum bait consumption occurred on this day, bringing their cumulative consumption to 57.96% of the total amount of 0.01% treated bait consumed. On Day 3, both Groups II and III decreased their consumption of the 0.01% treated bait, by which time the mice had consumed 79.13% of the total amount of 0.01% treated bait consumed. On Day 4, four mice from both Groups II and III decreased consumption of the 0.01% treated bait. The mice had consumed...
90.10% of the total amount of the 0.01% treated bait consumed. On Days 5-9, the remaining mice consumed less than 0.6 g per mouse and they consumed the remaining 9.90% of the total amount of the 0.01% treated bait consumed.

During the 15-day feeding trial, the deer mice consumed more of the 0.01% chlorophacinone bait than the OPP rat and mouse challenge diet. The magnitude of the difference in bait consumption between the two bait types could not be measured. Had the experimental design of the Standard House Mouse Anticoagulant Dry Bait Test Method (1.216) specified that the second dish for the control animals contain 40 g of sham treated oat groats (oat groats with all ingredients except the toxicant) instead of the OPP rat and mouse challenge diet, any differences in consumption between the two bait types could have been measured.

In this study, the females in Group II and both sexes in Group III preferred the 0.01% chlorophacinone bait over the OPP rat and mouse challenge diet. They consumed a total of 240.38 g of both bait types, of which 168.15 g (70%) was the toxic bait. The males in Group II consumed a total of 62.62 g of both bait types, of which 23.02 g (37%) was the toxic bait. This high consumption of the toxic bait contrasts to the toxic bait consumption in previous similarly designed laboratory studies with both house mice (Mus musculus) and white Norway rats (Swiss-Webster strain). The house mice were exposed to both the 0.01% chlorophacinone oat groat bait and the OPP rat and mouse challenge diet. Of the total consumption of both bait types (580.67 g), 446.92 g (77.0%) was the OPP rat and mouse challenge diet and 133.75 g (23.0%) the toxic bait (McCann and Matschke 1999a). Thirty-seven (94.8%) of the 39 house mice tested died. In a similar study (McCann and Matschke 1999b), white rats were exposed to the same two bait types (0.005% chlorophacinone oat groat bait and OPP rat and mouse challenge diet). They consumed a total of 8,137.80 g of both bait types, 98.73% of which was the OPP rat and mouse challenge diet and 1.26% the toxic bait. Of the rats tested, 15 of 40 (37.5%) died.
Summary of Conclusions

1) Among the 40 deer mice receiving the treated bait, 100% died; 92.2% of the mice died between days 3 and 7.

2) High initial acceptance by the deer mice of the 0.01% chlorophacinone bait occurred during the first three days of testing; overall, more of the 0.01% treated bait was consumed than the OPP rat and mouse challenge diet. The consumption of the treated bait (63.1%) more than meets the 33% consumption required by EPA standards as the "amount" acceptable.

3) The 100% deer mouse mortality exceeds the minimum 90% standard established by the EPA for verifying the efficacy of rodenticides.
REFERENCES


Table 1. Summary of mortality and total bait consumption of deer mice during the 0.01% chlorophacinone concentration, 15-day, 2-choice, feeding trial.

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<td>N = 10</td>
<td>(100%)</td>
<td>Mean</td>
<td>2.33</td>
<td>8.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>2.96</td>
<td>8.98</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>10/10</td>
<td>Sum</td>
<td>19.55</td>
<td>49.27</td>
<td></td>
</tr>
<tr>
<td>N = 10</td>
<td>(100%)</td>
<td>Mean</td>
<td>3.91</td>
<td>8.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>4.07</td>
<td>6.15</td>
<td></td>
</tr>
</tbody>
</table>

*aDish contained the OPP rat and mouse challenge diet.

*bDish contained either the OPP rat and mouse challenge diet or the 0.01% chlorophacinone bait depending on group.

#cAll mice were dead after Day 7.

cAll female mice were dead after Day 9, and all male mice were dead after Day 7.
Table 2. Amount and proportion of treated bait (0.01% chlorophacinone) consumed compared to the OPP rat and mouse challenge diet during the 15-day feeding trial.

<table>
<thead>
<tr>
<th></th>
<th>Total Consumption (g)</th>
<th>Percent of 0.01% Bait in Total Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OPP Diet</td>
<td>0.01% Bait</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>36.36</td>
<td>43.61</td>
</tr>
<tr>
<td>Group III</td>
<td>16.32</td>
<td>75.27</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>39.60</td>
<td>23.02</td>
</tr>
<tr>
<td>Group III</td>
<td>19.55</td>
<td>49.27</td>
</tr>
<tr>
<td>Total</td>
<td>111.83</td>
<td>191.17</td>
</tr>
</tbody>
</table>

Proportion of treated bait in diet: \( \frac{191.17 \text{ g}}{303.00 \text{ g}} = 0.631 (63.1\%) \)
Table 3. Summary of consumption of the OPP rat and mouse challenge diet by surviving deer mice (Group I) during the 5-day post-testing feeding period.

<table>
<thead>
<tr>
<th>Bait consumption (g)</th>
<th>Group I Females</th>
<th>Group I Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum</td>
<td>113.13</td>
<td>112.97</td>
</tr>
<tr>
<td>Mean</td>
<td>22.63</td>
<td>22.59</td>
</tr>
<tr>
<td>SD</td>
<td>0.32</td>
<td>1.09</td>
</tr>
<tr>
<td>N = 10</td>
<td>N = 10</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Summary by groups of pre- and post-treatment body weights of deer mice.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Summary</th>
<th>Pre-treatment body weight (g)</th>
<th>Post-treatment body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Group I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Sum</td>
<td>197.4</td>
<td>199.4</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>19.74</td>
<td>19.94</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.32</td>
<td>2.97</td>
</tr>
<tr>
<td></td>
<td>N = 10</td>
<td></td>
<td>N = 10</td>
</tr>
<tr>
<td>M</td>
<td>Sum</td>
<td>202.7</td>
<td>187.44</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>20.27</td>
<td>18.74</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.14</td>
<td>3.39</td>
</tr>
<tr>
<td></td>
<td>N = 10</td>
<td></td>
<td>N = 10</td>
</tr>
<tr>
<td>0.01% Chlorophacinone Group II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Sum</td>
<td>194.5</td>
<td>168.20</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>19.45</td>
<td>16.82</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.42</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td>N = 10</td>
<td></td>
<td>N = 10</td>
</tr>
<tr>
<td>M</td>
<td>Sum</td>
<td>203.1</td>
<td>164.78</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>20.31</td>
<td>16.48</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.00</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>N = 10</td>
<td></td>
<td>N = 10</td>
</tr>
<tr>
<td>0.01% Chlorophacinone Group III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Sum</td>
<td>194.1</td>
<td>158.80</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>19.41</td>
<td>15.88</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.24</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>N=10</td>
<td></td>
<td>N=10</td>
</tr>
<tr>
<td>M</td>
<td>Sum</td>
<td>204.2</td>
<td>167.29</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>20.42</td>
<td>16.73</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.90</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td>N = 10</td>
<td></td>
<td>N = 10</td>
</tr>
</tbody>
</table>
Figure 1. Cumulative deer mouse mortality (♀ and ♂ combined) during the 15-day 0.01% chlorophacinone concentration feeding trial. Group I (control) and Groups II and III (0.01% chlorophacinone oat groat bait).
Figure 2. Mean daily bait consumption per mouse by sex for deer mice, Groups II and III (0.01% chlorophacinone concentration) during the 15-day feeding trial.

Group II

Group III

(Number) = number of animals alive

FEMALES

MALES
Appendix I. 0.01% Chlorophacinone Oat Groat Bait Label
BAITING: POCKET GOPHERS, Thomomys spp.
A hand probe should be used to locate main burrow system of gophers. Carefully rotate probe to enlarge opening. Using a funnel, place ½ cup of treated bait into burrow. Cover hole with clod or dirt. Do not disturb bait. Bait should be placed in two locations per gopher system. Repeat treatment as necessary. Additional information is available from the agricultural commissioner's office.
Appendix II. Standard Peromyscus Species Anticoagulant Dry Bait Laboratory Test Method (1.216)
STANDARD PEROMYSCUS SPECIES ANTICOAGULANT DRY BAIT
LABORATORY TEST METHOD
OPP Designation: 1.216 (1-1-75)

1. Scope

1.1 This method is designed to determine effectiveness of ready-to-use anticoagulant dry bait rodenticide products used for Peromyscus spp. (white-footed or deer mice) control. It is applicable in connection with registration and enforcement procedures under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended.

2. Test Animals

2.1 All mice used in this test shall be outbred Peromyscus spp. (white-footed or deer mice). They shall be healthy, active, sexually mature, and fall within the minimum weight class of 15 g; maximum of 40 g; and have a maximum acceptable difference in average weights between the sexes of 5 g.

2.2 Ectoparasite control with appropriate concentrations of carbaryl, malathion or pyrethrum dusts is permissible if applied externally to both test and control animals not less than seven days prior to start of test.

3. Apparatus

3.1 The mice should be placed in solid-bottomed all-metal group cages designed to hold laboratory mice and having a bottom surface area of 17,000 to 25,000 cm$^2$ (18.3 to 26.9$^2$).

3.2 Metal or ceramic feeders, designed so that test mice may not nestle or wallow in diet, should be used.

4. Pretest Holding Conditions

4.1 All mice used in this test method must be held, sexes separate, for observation in the laboratory for a period of at least one and not more than four weeks prior to testing, the last seven days of which shall be under laboratory conditions (i.e., temperature, humidity, lighting, etc.) comparable to those of the animal testing room if not actually in the testing room. The test animals must NOT be fasted prior to testing. Water and a commercial mouse diet must be available to them at all times. Do NOT use the standard OPP field rodent challenge diet for pretest feeding.
5. **Holding and Test Conditions**

5.1 **Temperature**

20 to 25°C. Strong air currents from heaters or air conditioners shall not blow directly onto test animals.

**Relative humidity**

50 to 55%

**Light**

12 h artificial light per day, not to exceed 2153 lx (200 ft candles) at cage location. Total reversing of the natural photoperiods of the test animals by timed lighting is not recommended.

5.2.1 The standard OPP field rodent challenge diet shall be composed of:

- Commercial rodent laboratory diet 50% by weight
- Rolled oat groats (ground) 50% by weight

Combine dry ingredients together and thoroughly mix. Be certain the mixing utensils are clean of contamination before preparing diet.

5.2.2 The particle size for the commercial rodent laboratory diet (for instance, Charles River, Wayne or Purina) shall conform to the following specifications. Seventy-five percent (± 5 percent) of the ground diet shall be small enough to pass through a No. 10 screen (10 meshes to the inch) and 50 percent (± 10 percent) large enough to be retained by a No. 20 screen (20 meshes to the inch). The remainder may either be larger or smaller than the screens mentioned.

5.2.3 The oats shall be steam rolled oat groats (oat seed with the hulls removed) coarsely ground after the rolling process. Seventy-five percent (± 5 percent) of the ground oats shall pass through a No. 5 screen (5 meshes to the inch) and 50 percent (± 10 percent) be retained by a No. 20 screen (20 meshes to the inch). The remainder may be either larger or smaller than the screens mentioned.

5.2.4 The standard OPP field rodent challenge diet may be stored under refrigeration if it is to be used within three days of preparation. If it is to be held for longer periods the diet shall be packaged in plastic containers 2.2 to 4.5 kg (5 to 10 lb) per container, tightly closed or sealed, and maintained at -18°C or below until it is to be used. It shall be at room temperature when offered to test or control animals. Challenge diets shall not be prepared and stored for longer than six months.
OPP 1.216

6. Procedure

6.1 A test group consists of a minimum of 20 mice (10 males, 10 females), group caged. Include one untreated control test group of 20 mice (sexes equal), group caged, in each test. If a series of tests is being conducted at the same time on the same species, only one untreated control test group need be included. Acclimate all animals to test conditions for three days prior to exposure to toxicant, immediately following pretest holding period (4.1).

6.2 Water must be available to each animal at all times. Glass water bottles equipped with ball-type watering tubes are recommended. Gravity fed automatic or open-cup waterers are not recommended.

6.3 Ready-to-use rodenticide-treated food and the standard OPP field rodent challenge diet are each offered to test mice in separate containers (3.3) on opposite sides of the cage to provide in excess of the daily food requirement of 10 grams per animal per day, minimum. The two containers must be identical in type and size and the food offered in each container should be equal and consistent throughout the test. The untreated control test group is offered only the OPP field rodent challenge diet. The gross weight of each container and its contained food are determined daily and returned to the starting weight by addition of the given food. If food becomes fouled by urine or feces, replace food in each container. Record each day the quantity of each food consumed during the preceding 24 h. Weighing accuracy must be at least to the nearest 0.1 gram. Spilled food shall be recovered and weighed to establish exact food consumption data. Where food spillage is damp it shall be dried to approximately its original moisture content before weighing.

6.4 Reverse the position of the bait and standard OPP field rodent challenge diet containers in the cages every 24 h to counter any feeding position preference of the mice. The test mice must have a free choice between treated and untreated food.

6.5 Animals on test should not be subjected to undue or unnecessary stress from noise or human activities (i.e., movement). Human activity within the animal test room shall be minimal.

7. Test Period

7.1 Maintain test period for 15 days, unless a 100% mortality of test mice is recorded prior to that time.

7.2 Remove dead mice daily, or more frequently as observed.

7.3 Remove toxicant-treated food at the end of the 15-day test period, leaving and maintaining the untreated food.
7.4 More than a 10% mortality in the control group negates the test, even if a 100% mortality has been achieved in the test group.

7.5 This laboratory efficacy test should be replicated at least once.

8. Test Period Follow-Up

8.1 Maintain observation on surviving mice for a minimum of five days following test period.

8.2 Continue feeding OPP field rodent challenge diet.

8.3 Describe unusual activities of test and control mice in report of test and posttest periods.

9. Calculation and Evaluation of Results

9.1 Record date, weight, and sex of each mouse dying during the test and of survivors in both the test and control groups, and amount of treated and untreated food consumed during the test and posttest periods. Retain original laboratory test records for future reference. Grouped data averages should be used for efficacy evaluation.

9.2 The product is considered satisfactory if a minimum mortality of 90% of test animals is obtained.
Appendix III. Study protocol QA-506, Amendments, and SOPs
STUDY PROTOCOL

I. STUDY PROTOCOL TITLE:

Chlorophacinone and Diphacinone: Standard Mouse Anticoagulant Dry Bait Laboratory Tests

II. SPONSOR:

California Department of Food and Agriculture (CDFA)

USDA/APHIS/ADC/DWRC/NWRC

III. STUDY DIRECTOR AND PARTICIPANTS:

Geraldine R. McCann* and George H. Matschke (Supervisor)

IV. OBJECTIVE/HYPOTHESES:

Our objective is to determine the efficacy of chlorophacinone and diphacinone grain baits for controlling house mice (Mus musculus). We will test the following null hypotheses (Ho): mortality will be the same for treated and control house mice feeding on the diphacinone baits and mortality will be the same for treated and control house mice feeding on the chlorophacinone baits.

* Study Director
V. JUSTIFICATION AND BACKGROUND:
The CDFA has entered into an agreement with the
USDA/APHIS/ADC/DWRC/NWRC/Ecological Effects Unit to conduct this laboratory
study. This study will evaluate the efficacy of chlorophacinone and diphacinone baits
against laboratory mice as a partial fulfillment for the reregistration of chlorophacinone
and diphacinone commensal rodent labels.
The study will be conducted in compliance with Code of Federal Regulations (CFR) 40,
Part 160, Good Laboratory Practices (EPA 1993) and the appropriate Pesticide
Assessment Guidelines (PAG), Subdivision G (EPA 1982).

VI. ANIMAL CARE AND USE:
A. Where applicable, the number, body weight range, sex, source of supply, species,
strain, substrain, and age of the test system.
Sixty-six sexually mature house mice (Mus musculus) of the Swiss-Webster genotype
(33M:33F) weighing between 15–35 g will be purchased from Simonson Laboratories, Inc., 1180 Day Road, No. C, Gilroy, CA 95020.
B. The procedure for identification of the test system:
The cage of each animal group will be given a unique number (SOP WRC-216).
C. Identification of chemicals with chemical abstract number (CAS), materials, or
devices to be used or tested.
1. Chemical: Chlorophacinone (CAS No. 3691-35-8) and diphacinone (CAS No.
82-66-6)
2. Material: Steam rolled crimped oat groats (squirrel type thickness)
3. Device: None
D. Rationale for involving animals, the appropriateness of the species, and the number of
animals to be used.
Animals are required for testing because no alternative system exists that would test
the efficacy of the chlorophacinone and diphacinone baits. The species and the
number of animals to be tested have been specified by the EPA [Guideline Reference
Number (GRN) 96-10].
E. Source:
See VI-A.
F. Trapping:
NA
G. Handling/Restraint:
See SOP WRC-528.
H. Transport:
Simonson Laboratories, Inc., will ship the house mice via air to the Denver International Airport then Animal Care will ground transport them to the NWRC/ARB (SOP WRC-294.R2) (SOP WRC-395.R1).

I. Housing/Maintenance/Diet:
The house mice will be housed by sex in groups in stainless steel cages (70.8 × 24.1 × 17.8 cm) where they will be fed a rodent laboratory chow diet. Water will be available at all times. They will be maintained per SOP WRC-528.

J. Quarantine:
The mice will be held a minimum of 7 days in quarantine (SOP WRC-232.R1).

K. Euthanasia:
Survivors at the end of the study will be euthanatized with CO₂ gas (SOP WRC-128.R5).

L. Disposition of Animals:
All animals will be incinerated upon conclusion of the study (SOP WRC-233.R3 and WRC-436).

M. Provide written assurance that the activities do not unnecessarily duplicate previous experiments. This must illustrate a good faith on the part of the researcher to find if this experiment duplicates previous experiments.
In April 1996, the Denver Wildlife Research Center (DWRC) library conducted a literature review, searching the following 17 databases for articles on chlorophacinone or diphacinone on house mice (Mus musculus):
Biosis Previews (R). 1969-1996/Mar W4
CAB Abstracts. 1972-1996/Feb
Pascal. 1973-1996/Jan
Medline (R). 1966-1996/May W4
CA Search (R). 1967-1996/UD=12414
Agricola. 1970-1996/Apr
AGRIS International. 1974-1995/Dec
Zoological Record Online (R) 1978-1996/V132P13
World Transl. Index 1979-1996/Feb
Oceanic Abst. _1964-1996/Apr
Aquatic Science Abstracts _1979-1996/Apr
CRIS/USDA 1996/Feb
GEOBASE (TM) _1980-1996/Mar
Pesticide Fact File_1995
A total of 6 published articles on anticoagulants were located, 2 on chlorophacinone, 3 on diphacinone, and 1 article included both compounds. All 6 of the articles were received and reviewed including one in French that was translated but was not applicable to this study.

**Chlorophacinone** - Under laboratory conditions, Lund (1971) fed 0.025% chlorophacinone oat groats bait to groups of 20 mice for each test. His tests consisted of an array of feeding periods varying from 1 day to 21 days. When the feeding period was 1 to 5 days, mouse mortality was 5 to 75%; whereas, when the feeding period was increased to 6, 10 and 21 days, mortality was 80, 90, and 95% respectively.

**Diphacinone** - Marshall (1981), in a laboratory situation, fed 3 differently prepared 0.005% diphacinone wax baits to house mice. Twenty mice individually housed were placed on each of the 3 bait types. The wax baits and a non-toxic alternative diet were fed for 15 days. The percentage of wax baits accepted by the house mice varied greatly, 3.0%, 17.4%, and 38.6%. The highest house mouse mortality, 100%, occurred on the wax bait having 38.6% acceptance. The other 2 wax baits both had 85% mortality.

Advani (1992) evaluated the efficacy of 0.005% diphacinone wax bait blocks on house mice in apartment buildings located in New York City, NY. He monitored the mouse population pretreatment before placing the bait blocks for 4 monthly treatments. Monthly, the mouse population was monitored 7 to 10 days after placement of the baits. The highest percent control (77.7%) occurred after 4 months when comparing the difference between pre-control census (October) and the post-treatment census (December).

Arends et. al. (1984) reported that two separate baiting periods of diphacinone pellet bait (concentration not given) were required to achieve greater than 70% mortality among house mice inhabiting chicken houses on a broiler breeder farm. The first baiting period began after determining the number of active mouse burrow systems. Six bait applications occurred (43g/10 active burrow systems), with a bait application every other day. According to a posttreatment census, the number of active burrows declined only 32.7%. A second baiting period was then conducted, with 4 bait applications, one every other day. The second posttreatment census showed the number of active burrows declined 74.8%. Caching of the bait by the mice may explain the low mortality during the first baiting period.

**Chlorophacinone - Diphacinone** - Rowe and Redfern (1968) measured mortality and bait acceptance among house mice given both chlorophacinone and diphacinone oat baits. Four no-choice tests were conducted, each one lasting either 3, 7, 14, or 18 days. The concentrations tested were as follows: chlorophacinone 0.005% and...
0.025% and diphasictrone 0.0125% and 0.025%. Mortality was as follows: for the 3
day tests: less than 50% of the animals died on all four concentrations; for the 7 day
test, 100% mortality occurred on the 0.025% chlorophacinone concentration, but 50
to 80% mortality occurred at the 3 other concentrations. For the 14 day test, 100%
mortality occurred again on the 0.025% chlorophacinone, and the mortality for the
other three concentrations increased to 80-90%. For the 18 day test only the
0.0125% diphasictrone concentration failed to achieve 100% mortality. On this
concentration, 2 of the mice survived and consumed a total of 255 and 466 mg/kg of
diphasictrone. In the bait acceptance study, the mice were placed on test for 2 days
and given a two-choice test. They received untreated pinhead oatmeal in addition to
one of the 4 toxic concentrations. For both toxicants, the mice on the 0.025%
concentration consumed significantly less (p < 0.001) bait than the untreated bait. No
significant difference (p < 0.005) occurred between the consumption of either of the
two lower concentrations (0.0125% or 0.05%) or the untreated oat bait.

After reviewing these articles none would support the reregistration requirements
established by the EPA for maintaining the CDFA's chlorophacinone and diphasictrone
labels for controlling house mice because none of these publications reported on two-
choice feeding tests where either chlorophacinone or diphasictrone were offered at the
0.01% concentration along with the EPA challenge diet to house mice.

N. In regard to potential pain of animals for this experiment, provide written statements
addressing each area:

1. That you have considered alternatives to any painful procedures and, if
unavailable, you have indicated the principal sources that have been consulted in
considering the alternatives (e.g., Biological Abstracts, the Animal Welfare
Information Center).
Domestic house mice need to ingest lethal doses of chlorophacinone or
diphasictrone to provide the required efficacy data. Analgesics are unacceptable
due to the possible distortion of normal bait ingestion and interference with
chlorophacinone and diphasictrone's mode of action and could subsequently effect
the toxicity data in this species. With anticoagulants, it would appear reasonable
that more than slight pain would be expected in this study.
2. When more than slight pain is reasonably expected, and sedatives or analgesics will not be used, the reasons for this procedure are scientifically justified.

More than slight pain may be expected, but sedatives or analgesics will not be used because they could alter the toxicity of the chlorophacinone and diphenacinone on the test species.

3. That procedures, which cause more than slight or momentary pain, MUST involve during the planning phase a consultation with the attending veterinarian of the Denver Wildlife Research Center.

A discussion between the Study Director and the DWRC Veterinarian occurred on June 20, 1996, regarding care and welfare of the house mice.

DWRC Veterinarian: 

Initials: [Initials]  

Date: [Date]

4. If the animals experience severe or chronic pain that cannot be relieved, they will be euthanatized at the end of the procedure or, if appropriate, during the procedure.

If any animals appear to experience severe or chronic pain they will be euthanatized. The decision for euthanasia will be made by the Center veterinarian. All survivors will be euthanatized on Day 20 (SOP WRC-128.R5).

VII. METHODS:

A. Protocol:

The protocol for this study has been outlined by the EPA in their Standard House Mouse Anticoagulant Dry Bait Laboratory Test Method from the Office of Pesticides Programs (OPP Designation: 1.204) (EPA, 1982).

1. Pretest Holding Conditions:

All mice used in this test method must be held, sexes separate, for observation in the laboratory for a period of at least 1 week and not more than 4 weeks prior to testing. They will be under laboratory conditions (i.e., temperature, humidity, lighting, etc.) comparable to those of the animal testing room - if not actually in the testing room. The test animals will NOT be fasted prior to testing. Water and a commercial mouse diet must be available to them at all times. The standard OPP rat and mouse challenge diet will NOT be used during the quarantine period or pretesting period.
2. Pretreatment Procedure

Following the quarantine period, 60 animals (30M:30F) will be randomly selected from the total population using a computer generated random number program (SOP WRC-368.R1). These animals will be weighed and the average body weight of the two sexes will be calculated, using 5 g as the maximum acceptable weight difference between the sexes. If an acceptable difference in body weights exists between the sexes at that time, the mice of each sex will be ranked by weight into 10 weight classes, each containing 3 animals (SOP WRC-59.R3). The groups of ten mice (sexes separate) will be randomly assigned to one of the two concentrations (0.01% or 0.00%) for a total of 10 animals per sex per each concentration, or a total of 20 animals per each concentration. The chlorophacinone and diphacinone will not be tested simultaneously.

Day 1 of Testing

On the first day of testing, the 15-day two-choice feeding study will begin by removing the regular diet. Each group of 10 mice will then be offered two dishes, which have been placed on opposite sides of the cage. Every group receiving the toxicant diet will be given one dish containing in excess of 20 g of the 0.01% chlorophacinone or diphacinone grain bait and a second dish containing an excess of 20 g of the standard OPP rat and mouse challenge diet. The two dishes will be of equal weight. Each group of mice receiving the control diet will be given two 20 g dishes of the standard OPP rat and mouse challenge diet. On Day 1, the toxic dishes will be positioned on the left, and the challenge dishes will be placed on the right in the front of the cage. EPA does not require that separate test dishes measuring moisture gain or loss be used. Bottles of water will be available to all animals. The bottle will be positioned at equal distances from the feed dishes. The animal room will be closed and not reentered until Day 3.

Day 2

The dishes will be removed to determine the gross weight to the nearest 0.5 g of each container and its contents. At that time fresh bait will be added to the dishes and the dish weighed. If food becomes fouled by urine or feces, it will be discarded and replaced. The position of the dishes will be reversed when replaced in the cages; i.e., toxic dishes will be placed on the right and the challenge dishes will be set on the left in the front of the cage. The animal rooms will be closed and not reentered until Day 3.
Days 3-15

Days 3-15 will be a repeat of Day 2, except the position of dishes will be reversed daily. Animals will be checked for mortality and dead animals will be removed and weighed.

3. Posttesting Period

Day 16

Animals will be checked for mortality and the dishes will be removed and the remaining bait will be weighed. Each survivor on the chlorophacinone or diphascinone treatment will be given one 20-g dish of the challenge diet.

Days 17-19

Animals will be checked daily for mortality, the dishes will be removed, and the remaining bait will be weighed, and fresh bait added.

Day 20

All dishes will be removed, and the remaining bait will be weighed. The survivors will be euthanatized with CO₂ (SOP WRC-128.R5).

B. Analytical Chemistry: Briefly summarize the analytical chemistry portion of the experimental design.

The chlorophacinone and diphascinone grain baits will be assayed by DWRC's Analytical Chemistry Unit. Chemists will use validated Method 62A for both chlorophacinone and diphascinone.

Analytical Chemistry Project Leader: [Signature] 7/23/96

Initials

C. Bait Formulation:

The bait will be formulated by the Rodent Control Outfitters of Junction City, OR. The baits will be prepared according to the Confidential Statement of Formula for the 0.01% chlorophacinone and 0.01% diphascinone oat baits.

The standard OPP rat and mouse challenge diet shall have the following composition (% by weight):

- Cornmeal (whole yellow ground corn) 65
- Rolled oat groats (ground) 25
- Sugar (10X powdered or confectioners, 95% + purity) 5
- Corn oil (95% + purity) 5

Combine dry ingredients, add oil, and mix thoroughly.
After formulation, the OPP rat and mouse challenge diet will be packaged in plastic containers, tightly sealed, and maintained at -18°C or below until it is to be used.

When offered to the test or control animals the challenge diet and the test bait will be at room temperature.

D. Location of Work:
All research will be conducted at the NWRC/ARB facilities, Colorado State University Campus, Fort Collins, CO 80524-2719

E. Cooperators and Consultants:
USDA/APHIS/ADC/DWRC/NWRC/EEU
California Department of Food and Agriculture

F. Related Study Protocols:
None

G. Justification for selection of the test systems:
See VI-D.

H. A description and/or identification of the diet used in the study as well as solvents, emulsifiers, and/or other materials used to dissolve or suspend the test or control substances before mixing with the carrier. The description shall include specifications for acceptable levels of contaminants that are reasonably expected to be present in the dietary materials and are known to be capable of interfering with the purpose or conduct of the study if present at levels greater than established by the specifications.
An oil will be added to the oats as an adhesive for the technical chlorophacinone and diphacinone. The technical chlorophacinone or diphacinone will not be dissolved or suspended in any substance before they are applied directly to the oats treated with the oil adhesive.

I. The route of administration and the reason for its choice:
The route of administration will be orally by an oat groat bait. Grain baits treated with chlorophacinone or diphacinone will be used as if applied in an operational control program.

J. Each dosage level, expressed in milligrams per kilogram of body weight or other appropriate units of the test or control substances to be administered, and the method and frequency of administration.
The 0.01% chlorophacinone and diphacinone concentrations will be assayed. The mg/kg intake will depend upon the quantity of bait consumed by each mouse. The bait will be placed in metal dishes, from which the mice will be fed for 15 days.
K. A description of the experimental design including the method for the control of bias: Animals will be randomly selected for testing. Bias will be controlled by randomly assigning each group of mice to one of the 3 different treatments (0.0%, 0.01% chlorophacinone or diphacinone, and 0.01% chlorophacinone or diphacinone) (SOP WRC-59.R3).

L. Statistical Analyses:

1) A 3-factor repeated measures ANOVA would compare treatment, sex, and days on test with days being the repeated factor in the analysis.

2) A 3-factor repeated measures ANOVA would compare consumption of control bait for treatment and sex groups, until the first mortality occurs.

3) A 3-factor repeated measures ANOVA would be conducted on the proportion of total consumption that was represented by the treated bait.

4) Contingency table methods will be used to compare overall survival between the treated groups and control groups for both sexes.

5) Kaplan-Meier survival curves will be used to compare treatment (diphacinone or chlorophacinone) sex groups if appropriate.

M. Environmental Conditions of the Study:
Temperature of the animal rooms will be recorded daily
Light (12 h light:12 h darkness cycle)

N. Accountability of the Test Substance:
All bait concentrations will be under control of personnel from the Biological and Chemical Effects Unit of NWRC's Product Development Section. Just before testing, these baits will be forwarded to the Ecological Effects Unit, which will maintain the chlorophacinone or diphacinone bait in a locked storage cabinet at ambient temperature until disposal. The quantity of the bait types used each day will be recorded on end use product chain of custody forms (SOP A19R.2). The EPA Challenge diet will be maintained under refrigeration at -18°C.
O. The Records to be Maintained:

- Purchase of mice
- Shipping of mice
- Quarantine animal care records
- Postquarantine animal care records
- Formulation of 0.01% chlorophacinone or diphacinone grain baits
- Assay of the 0.01% chlorophacinone or diphacinone grain baits
- Weighing and ranking records
- Random allocation of the groups of 10 mice to the treatments
- Bait formulation records
- Daily bait consumption
- Date of death, weight, and sex
- Descriptions of unusual activities displayed by test and control animals in report on test and posttest periods.

P. Authority and Permits:

- NA

Q. Standard Operating Procedures (SOP's):

- SOP A-19R.2 Chain of Custody
- SOP A-31.R1 Personal Protective Equipment
- SOP WRC-59.R3 Small Mammal Ranking for Testing
- SOP WRC-128.R5 Animal Euthanasia with CO₂ Gas
- SOP WRC-216 Animal Handling Procedure to Maintain Identifications
- SOP WRC-232.R1 Quarantine Procedures for All the Animals at DWRC
- SOP WRC-233.R3 Incinerator Use and Maintenance
- SOP WRC-294.R2 Transporting Small Mammals by Air
- SOP WRC-368.R1 Computer Generated Random Numbers
- SOP WRC-436 Incineration of Animal Carcasses and Tissue
- SOP WRC-465.R1 Hazard Communication
- SOP WRC-528 Laboratory mouse handling and Maintenance

VIII. COMPLIANCE WITH ENDANGERED SPECIES ACT (SECTION 7):

- NA

IX. COMPLIANCE WITH THE NATIONAL ENVIRONMENTAL POLICY ACT:

Does the study, as proposed, have the potential for significant impact on the environment? Yes _____ No X
X. **EMPLOYEE SAFETY:**
USDA/APHIS/ADC/DWRC/NWRC/BEU safety regulations will be followed. Routine safety procedures will be followed, and appropriate gloves will be worn by employees while handling house mice. Similarly, appropriate protective clothing and equipment will be used while preparing and handling the grain baits (SOP A-31.R1 and WRC-465.R1).

XI. **SCHEDULE:**

<table>
<thead>
<tr>
<th></th>
<th>Chlorophacinone</th>
<th>Diphacinone</th>
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<td>Proposed experiment starting date:</td>
<td>October 1996</td>
<td>August 1996</td>
</tr>
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<td>Proposed experiment completion date:</td>
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<td>September 1996</td>
</tr>
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<td>Study completion date:</td>
<td>March 1997</td>
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XII. **STAFFING:**

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</thead>
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<tr>
<td>Biological Technician (WL)</td>
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XIII. **COST ESTIMATE FOR EACH FISCAL YEAR:**

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<tr>
<td>Salaries and benefits</td>
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<tr>
<td>Bait analysis</td>
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<tr>
<td>Animal purchase</td>
<td>182</td>
<td>182</td>
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<tr>
<td>Supplies</td>
<td>50</td>
<td>50</td>
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<tr>
<td>Animal care</td>
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<td>44</td>
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<tr>
<td>Baits (Grain Bait)</td>
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<tr>
<td>1. 0.01% Chlorophacinone</td>
<td>63</td>
<td>52</td>
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<tr>
<td>2. EPA Challenge Diet</td>
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<tr>
<td>Direct costs</td>
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<td>$6,757</td>
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<td>Indirect costs</td>
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<tr>
<td>Total</td>
<td>$7,861</td>
<td>$7,848</td>
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</table>

* All expenses are paid by CDFA Accounting Code 67374-03124

XIV. **QUALIFICATIONS OF STAFF:**

Study participants have documentation supporting education, experience, and special classes which qualify them for the work they will be performing in this study.

XV. **ARCHIVING:**

All raw data, documentation, protocol, and final report will be transferred to the archives at the close of the study (which is the day that the final report is signed).
XVI. REFERENCES:


Title: Chlorophacinone and Diphacinone: Standard House Mouse Acute Dry Bait Laboratory Test

SUBMITTED BY:                           8/19/96
(Study Director)                        Study Initiation Date

REVIEWED BY:                           7/31/96
(Ken Dunn)                             Date

(Statistician)                         7/24/96

Date

CONCURRENCE:

Y. Clay Mitchell                       8/6/96
(Quality Assurance Officer)           Date

William D. Deenberry                  7/31/96
(Animal Care and Use Committee)       Date

APPROVED BY:                           8/2/96
Kathleen A. Fagerstone                Date
(Section Chief)                       

Richard Braggan                       8/4/96
(Director's Office)                   Date

Any changes in, or deviations from, this protocol will be documented on the Study Protocol Amendment Form, signed and dated by the Study Director, Section Chief, Animal Care and Use Committee (if applicable), and Quality Assurance Officer. This amendment will be distributed to all participants.
Chloropacinone and Diphacinone: Standard Mouse

Study Title: Anticoagulant Dry Bait Laboratory Tests
Study Director: Geraldine R. McCann
Study Sponsor: the USDA/APHIS/ADC/NWRC

Date: March 5, 1997
Study Protocol No.: QA-506
Amendment/Revision No.: 1

Protocol Item(s) to be Changed:


Study Protocol Amendment/Revision USDA/APHIS/ADC/NWRC/PDS

Page 1. Sponsor: Add California Vertebrate Pest Advisory Council

Page 1. Objective/Hypothesis: Change the grain bait to wax bait.

Page 2. Justification and Background: Add California Vertebrate Pest Advisory Council right after CDFA and change the bait to wax.

Page 2. Animal Care and Use: A.: Change the number of animals to be used to eighty-six and 43M:43F.

Page 2. Animal Care and Use: C.3.: Change the device to wax.

Page 3. Animal Care and Use: H.: Change the first sentence to read that the animals will be shipped by ground transport to Animal Care at the Animal Research Building at the NWRC.

Page 3. Animal Care and Use: L.: Add to the first sentence: unless designated to be used for further research and their designation/disposition will be documented.


Page 7. Methods: A. 2.: In the first sentence change the number of animals being used to 80 animals (40M:40F). In the third sentence change the weight class numbers from 3 to 4, and in the fourth sentence, change 0.01% to 0.005%.

Page 7. Methods: A. 2.: Day 1 of Testing: Change the toxicant bait to wax bait block of 0.005% and a second dish containing the standard OPP rat and mouse challenge diet will be in excess of 40 g.


Page 8. Methods: B.: Change the grain baits to wax bait blocks.


Page 8. Methods: L.: Change the ingredients of the grain baits to wax bait blocks.

Page 8. Methods: J.: Change the ingredients of the grain baits to 0.005% wax bait blocks.
Page 8. VII: Methods: K.: Change the ingredients of the grain baits to 0.005% wax bait blocks.

Page 8. VII: Methods: O.: Change the records to be kept from grain baits to 0.005% wax bait blocks.

Proposed Protocol Revision:

Page 1. IV: Objective/Hypothesis: Our objective is to determine the efficacy of chlorophacinone and diphacinone wax baits for controlling house mice (Mus musculus). We will test the following null hypotheses (Ho): mortality will be the same for treated and control house mice feeding on the diphacinone wax baits and mortality will be the same for treated and control house mice feeding on the chlorophacinone wax baits.

Page 2. V: Justification and Background: The CDFA and the California Vertebrate Pest Advisory Council have entered into an agreement with the USDA/APHIS/ADC/DWRC/NWRC/Ecological Effects Unit to conduct this laboratory study. This study will evaluate the efficacy of chlorophacinone and diphacinone wax baits against laboratory mice as a partial fulfillment for the reregistration of chlorophacinone and diphacinone commensal rodent labels.

Page 2. VI: Animal Care and Use: A.: Eighty-six sexually mature house mice (Mus musculus) of the Swiss-Webster genotype (43M:43F) weighing between 15-35 g will be purchased from Simonson Laboratories, Inc., 1180 Day Road, No. C, Gilroy, CA 95020.


Page 3. VI: Animal Care and Use: H.: Simonson Laboratories, Inc., will ship the house mice via air to the Denver International Airport then the animals will be shipped by ground transport to Animal Care at the Animal Research Building of the NWRC (SOP WRC-294.R2) (SOP WRC-395.R1).

Page 3. VI: Animal Care and Use: L.: All animals will be incinerated upon conclusion of the study (SOP WRC-233.R3 and WRC-436) unless designated to be used for further research and their destination/disposition will be documented.

Page 6. VII: Methods: A. Protocol: The protocol for this study has been outlined by the EPA in their Standard House Mouse Anticoagulant Dry Bait Laboratory Test Method from the Office of Pesticides Programs (OPP Designation: 1.214) (EPA, 1982).

Page 7. VII: Methods: A. 2.: Following the quarantine period, 80 animals (40M:40F) will be randomly selected from the total population using a computer generated random number program (SOP WRC-368.R1). If an acceptable difference in body weights exists between the sexes at that time, the mice of each sex will be ranked by weight into 10 weight classes, each containing 4 animals (SOP WRC-59.R3). The groups of ten mice (sexes separate) will be randomly assigned to one of the two concentrations (0.00% or 0.005%) for a total of 10 animals per sex per each concentration, or a total of 20 animals per each concentration.

Page 7. VII: Methods: A. 2.: On the first day of testing, the 15-day two-choice feeding study will begin by removing the regular diet. Each group of 10 mice will then be offered one dish and a wax bait block, which have been placed on opposite sides of the cage. Every group receiving the toxicant diet will be given one wax bait block of 0.005% chlorophacinone or diphacinone bait and a second dish containing an excess of 40 g of the standard OPP.
rat and mouse challenge diet. Each group of mice receiving the control diet will be given two 20 g dishes of the standard OPP rat and mouse challenge diet. On Day 1, the wax bait block will be positioned on the left, and the challenge dishes will be placed on the right in the front of the cage. EPA does not require that separate test dishes measuring moisture gain or loss be used. Bottles of water will be available to all animals. The bottle will be positioned at equal distances from the OPP diet and the wax bait block.

Page 7. VII: Methods: A. 2.: Day 2: The dishes and wax bait block will be removed to determine the gross weight to the nearest 0.5 g of each container and its contents. At that time fresh bait will be added to the dishes and the dish weighed. If food becomes fouled by urine or feces, it will be discarded and replaced. The position of the dish and wax bait block will be reversed when replaced in the cages; i.e., wax bait blocks will be placed on the right and the challenge dishes will be set on the left in the front of the cage. Change the toxicant bait to wax bait block.

Page 8. VII: Methods: A. 3. Posttesting Period: Day 16: Animals will be checked for mortality and the dishes and wax bait blocks will be removed and the remaining bait will be weighed. Each survivor on the chlorophacinone or diphacinone treatment will be given one dish in excess of 40 g of the challenge diet.

Page 8. VII: Methods: B.: The chlorophacinone and diphacinone wax bait blocks will be assayed by the DWRC/NWRC Analytical Chemistry Unit.

Page 8. VII: Methods: H.: The 0.01% chlorophacinone or diphacinone grain baits will be suspended in paraffin wax before they are fed to the mice.

Page 8. VII: Methods: L.: Grain baits treated with chlorophacinone or diphacinone suspended in wax will be used as if applied in an operational control program.

Page 8. VII: Methods: J.: The 0.005% chlorophacinone and diphacinone wax baits will be assayed. The mg/kg intake will depend upon the quantity of bait consumed by each mouse. The bait will be placed on plastic plates, from which the mice will be fed for 15 days.

Page 8. VII: Methods: K.: Bias will be controlled by randomly assigning each group of mice to one of the 4 different treatments (0.0%, 0.005% chlorophacinone or diphacinone wax bait blocks, and 0.005% chlorophacinone or diphacinone wax bait blocks) (SOP WRC-59.R3).

Page 8. VII: Methods: O.: Formulation of 0.005% chlorophacinone or diphacinone wax baits

Assay of the 0.005% chlorophacinone or diphacinone wax baits

Signatures:
Study Director

Section Chief

Date March 5, 1997

Date 3/5/97

QA Officer

Animal Care & Use Committee

Date March 5, 1997

Date 3/5/97
Study Protocol Amendment/Revision
USDA/APHIS/ADC/NWRC/PDS

Chlorophacinone and Diphaeinone: Standard Mouse
Study Title: Anticoagulant Dry Bait Laboratory Tests
Study Director: Geraldine R. McCann
California Department of Food & Agriculture (CDFA) and
Study Sponsor: the USDA/APHIS/ADC/NWRC

Date: March 5, 1997
Study Protocol No.: QA-506
Amendment/Revision No.: 2

Protocol Item(s) to be Changed:


Proposed Protocol Revision:


Reason(s):
The protocol is being amended to clarify finishing the statistical analysis, final report writing, and archiving all records for each segment of each test. The completion process was delayed because of the many different species being tested in the same protocol and the 2 bait types, which add additional facets to each test in the same protocol.

Signatures:
Study Director
Date 22 March 1997
Section Chief
Date 3/27/97
Animal Care & Use Committee
Date 4/17
Item(s) to be Changed:
Page 1. IV: Objective/Hypotheses: Change the species to be tested to deer mice (Peromyscus sp.).

Page 2. VI: Animal Care and Use: A.: Change the species to deer mice raised in our own laboratory and eliminate Simonsen Laboratories, Inc.

Page 2. VI: Animal Care and Use: H.: Change transport information to Not Applicable.

Page 3. VI: Animal Care and Use: I.: Change the SOP to WRC-548.

Page 5. VI: Animal Care and Use: N.: Delete Domestic house in the first sentence.


Page 12. XI: Schedule: Change completion dates to accommodate further testing with chlorophacinone and dipheacinone and writing of final report to December 1999.

Revision
Page 1. IV: Objective/Hypotheses: Our objective is to determine the efficacy of chlorophacinone and dipheacinone grain baits for controlling deer mice (Peromyscus sp.).

Page 2. VI: Animal Care and Use: A.: Sixty-six sexually mature deer mice between 15 and 40 g will be selected out of the in-house breeding population instead of shipping laboratory mice.

Page 2. VI: Animal Care and Use: H.: NA.

Page 3. VI: Animal Care and Use: I.: The mice will be housed in groups by sex in stainless steel cages (70.8 X 24.1 X 17.8 cm) where they will be fed a rodent laboratory chow diet. Water will be available at all times. They will be maintained per the SOP WRC-548.

Page 5. VI: Animal Care and Use: N.: Mice need to ingest lethal doses of chlorophacinone and dipheacinone to provide the required efficacy data.

Page 6. VII: Methods A. Protocol: The protocol for this study has been outlined by the EPA in their Standard Peromyscus Species Anticoagulant Dry Bait Laboratory Test Method (OPP Designation: 1.216) (EPA, 1982).
Methods A. Protocol: Q:

Standard Operating Procedures (SOP's):

- SOP A-19R.73 Chain of Custody
- SOP A-31-R1/15004.00 Personal Protective Equipment
- SOP WRC-59.R3 Small Mammal Ranking for Testing
- SOP WRC-128.R5 Animal Euthanasia with CO₂ Gas
- SOP WRC-216 Animal Handling Procedure to Maintain Identifications
- SOP WRC-232.R1 Quarantine Procedures for All the Animals at DWRC
- SOP WRC-233.R3 Incinerator Use and Maintenance
- SOP WRC-368.R1 Computer Generated Random Numbers
- SOP WRC-436 Incineration of Animal Carcasses and Tissue
- SOP WRC-465.R1 Hazard Communication
- SOP WRC-548 Deer Mouse Maintenance

Schedule:

- Chlorophacinone
  - Proposed experiment starting date: October 1996
  - Proposed experiment completion date: November 1998
  - Study completion date: December 1999

- Diphenacine
  - Proposed experiment starting date: August 1996
  - Proposed experiment completion date: December 1998
  - Study completion date: December 1999

Reason(s): The protocol is being amended to accommodate testing of another species, finish statistical analyses, final report writing, and archiving all records for each segment of each test. The completion process has been delayed because of the many different species being tested in the same protocol and the 2 bait types, which add additional facets to each test in the same protocol.
NATIONAL WILDLIFE RESEARCH CENTER  
Ft. Collins, Colorado

Date Prepared: 10/02/89 caf  
Date Revised: 01/18/91 gem  
Date Revised: 03/25/91 gem  
Date Revised: 12/03/96 gem  
Page 1 of 4  
(w/attach.)

STANDARD OPERATING PROCEDURE

CHAIN OF CUSTODY

I. PURPOSE:

A. All Test and Control Substance (TCS) materials ordered for NWRC and its field entities will be handled in the manner specified by this SOP.

B. Definition - TCS Materials

All technical grade chemicals, manufacturing-use products (e.g. concentrates), dyes and markers, purchased end-use products (e.g. spray concentrates and formulations), and reference standards involved in registration activities will be referred to as TCS materials in this SOP.

C. 1. Analytical, bioassay, field, and other data gathered using TCS materials have the potential of being used as evidence in legal proceedings and/or in interagency communications involving APHIS registration activities. Thus, it is important that all NWRC personnel follow strict custody criteria for such materials from the time received until final disposal. Deficiencies in TCS custody will jeopardize the validity of data gathered using these materials.

2. Chain of custody procedures are necessary for identification, tracking, inventory, and troubleshooting purposes, as well as serving to document material integrity and physical security.

D. This SOP does not include storage of materials formulated by NWRC or others (such as baits) or for storage of samples collected in the field. In addition, candidate compounds (pilot studies, screening materials) do not go through the chain-of-custody until they are past the laboratory and preliminary field testing stage and are being pursued for potential registration.
II. PROCEDURE:

A. Sample Custodian/Alternate - Duties and Responsibilities

1. The NWRC Sample Custodian/Alternate will be supervised by the Chemical Development/Registration Section and will be responsible for overseeing the handling of all TCS materials ordered by NWRC personnel. Both will be fully aware of the custodian requirements and potential hazards of chemicals and pesticide formulations.

2. The Sample Custodian/Alternate will have an assigned room with good ventilation and temperature control as well as vented cabinets (for ambient storage), freezers, and refrigerators. This room and its contents will be locked and accessible only to the Sample Custodian/Alternate. An emergency key will be retained by the Quality Assurance Officer.

3. The Sample Custodian/Alternate is responsible for officially receiving all TCS materials, assigning a unique reference number to each (custodian number), proper storage, submitting a sample to NWRC Analytical Chemistry Section for initial assay if applicable, and preparation and archiving of documentation as long as the TCS materials are under NWRC custody. The Sample Custodian/Alternate is also responsible for other related duties such as the preparation, numbering, documentation, and archiving of sub-samples, the maintenance of records, and TCS disposal according to SOP No. A-17 (every 5 years or upon expiration date).

4. TCS related documentation will be stored in a file as well as backed up on a computer disc, all in a secure area.

5. The Sample Custodian/Alternate will provide the library with yearly inventory/status reports of all TCS materials.

B. Ordering TCS Materials

TCS materials will be ordered or procured by the Study Director. The Sample Custodian/Alternate is not responsible for ordering the TCS. All Study Directors will check with the Sample Custodian/Alternate for existing supplies BEFORE ordering any TCS material.

C. Receiving TCS Materials

1. In most cases (including field stations), newly ordered TCS materials will be delivered directly to the Sample Custodian/Alternate for logging-in on the TCS Worksheet (Figure I), weighing, sampling, assay, etc. Exceptions to this procedure will be approved in writing by the Sample Custodian/Alternate. The only exceptions at this time are the receipt of 55-gallon drums at field stations and reference standards specifically for the Analytical Chemistry Section (ACS; see Section E-1, page 4).

2. The TCS Worksheet will be completed in permanent ink by the NWRC Sample Custodian/Alternate only, and will be retained in a secure area with copies provided to the study director when the material is checked out.
3. Upon receipt of TCS materials, the containers will be inspected by the Sample Custodian/Alternate for their overall condition. Any leakage or other evidence of damage will be noted, and a photograph will be taken. In cases of damage, the Sample Custodian/Alternate will inform the Study Director, who will contact the appropriate sources and make a decision regarding analysis or sample disposition.

4. Copies of freight bills or other documentation related to the incoming shipment will be initialed, dated, assigned the unique reference number (custodian number) associated with the TCS, and retained by the Sample Custodian/Alternate.

5. Each storage container shall be labeled by name, chemical abstract service number (CAS) or code number, batch number, date of receipt, expiration date (if any) custodian number, initialed, and storage conditions given (if appropriate).

6. Except for ACS reference standards, a 5 gram aliquot of the TCS will be removed by the Sample Custodian/Alternate for archiving, assigned the custodian reference number, and retained by the Sample Custodian/Alternate throughout the life of the registration. The TCS Worksheet will be completed.

7. An aliquot will be removed for assay (except for dyes; markers, and ACS materials), numbered, and sent to the ACS, Analytical Services Project, with a request for analysis. Except for those chemicals actually received in the field or by the ACS, materials will not be checked out before the assay is completed. The Study Director will provide the Sample Custodian/Alternate with the Confidential Statement of Formula or Certificate of Analysis for the TCS when this information is available. If the assay result is more than 10% below the nominal value, the TCS material will not be checked out to users until the discrepancy is investigated by the Sample Custodian/Alternate. If the ACS does not have an analytical method for a TCS, the assay value(s) stated on the manufacturers statement or certificate will be used.

8. The TCS may then be checked out for use, and the Study Director will receive copies of the TCS Worksheet and a Chemical Tracking Form (Figure II) from the Sample Custodian/Alternate.

9. The Sample Custodian/Alternate is solely responsible for preparing sub-samples from TCS materials. When a sub-sample is prepared from an existing supply, it will be placed in a new container, labeled with the unique reference number (custodian number), weighed, etc., and a Chemical Tracking Form will accompany the sub-sample.

E. Exceptions

1. EPA Primary Reference Standards and other reference standards for the Analytical Chemistry Section (ACS) are the exception. No aliquots will be removed from EPA Primary Reference Standards because of the limited quantities available. When these materials are ordered by or for the ACS, they will be on permanent check-out to an ACS-designated alternate custodian until disposal. ACS will maintain their own records.
2. For 55-gallon drums, a Field Alternate Sample Custodian (the Study Director) may be designated to allow receipt of TCS materials at field stations. In this case, arrangement will be made before ordering between the NWRC Sample Custodian/Alternate and the respective Study Director. The Field Alternate Sample Custodian must be aware of the rules for shipping hazardous materials, obtain shipping materials, and follow the Sample Custodian's SOP for removing an archive aliquot and an assay aliquot from the TCS. The Field Alternate Sample Custodian will keep records (documentation) including the TCS Worksheet and Chemical Tracking Form, all of which must be returned to the Sample Custodian/Alternate at the NWRC as soon as possible.

F. TCS Material Use

1. Only the Sample Custodian/Alternate will aliquot and number any sub-samples of TCS materials. The Sample Custodian/Alternate will distribute the TCS materials to the user. The unique identification number (custodian number) assigned to the TCS material by the Sample Custodian/Alternate will be used to reference the TCS at all times. The TCS material received from the Sample Custodian/Alternate will not be renumbered, renamed, or sub-sampled.

2. The user is responsible for tracking TCS material use in analyses, tests, or studies, in a manner designed to preclude the possibility of contamination, deterioration, damage, or loss. The Chemical Tracking Form will be used for this purpose. The Chemical Tracking Form will accompany the TCS material, will be maintained throughout the use of the material, and the completed Chemical Tracking Form will be returned to the Sample Custodian/Alternate with the remainder of material or the empty container for archiving of paperwork and disposal of the chemical or container. The amount of chemical used will be accounted for, and the "purpose of use" entry must be specific enough to identify how the material was used, when, by whom, etc. The sample must be trackable from this description.

3. The user will have the TCS material re-assayed before use in all cases unless the initial Sample Custodian/Alternate assay has been performed within the past 90 days. This re-assay can be performed by the ACS or a contract laboratory. If the material is known to be unstable or visually appears contaminated, the material will be assayed regardless of the date of the most recent assay. The Sample Custodian/Alternate will be notified immediately if any material has deteriorated or has been contaminated. Material that is determined to be unusable must be returned with a completed Chemical Tracking Form to the NWRC Sample Custodian/Alternate for documentation and disposal.

I have read this document and approve of its contents. I certify that it will be made available for all personnel to whom it applies.

Richard Bruggen
acting Director

Date 10/4/96
### TEST AND CONTROL SUBSTANCES (TCS) WORKSHEET

**Chemical Name:**

<table>
<thead>
<tr>
<th>DRC No.</th>
<th>Company Code No.</th>
<th>Batch/Lot No.</th>
<th>CAS No.</th>
<th>Appearance</th>
<th>Nominal Amt. Received</th>
</tr>
</thead>
</table>

**Source:**

<table>
<thead>
<tr>
<th>Certified Assay (from ACS or Certificate):</th>
<th>(Circle One)</th>
</tr>
</thead>
</table>

**Storage Location:**

<table>
<thead>
<tr>
<th>Room No.</th>
<th>Cabinet No.</th>
</tr>
</thead>
</table>

**Storage Conditions:**

- Ambient
- Refrigerator
- Freezer
- Hood

**Gross Weight of Container on Arrival:**

<table>
<thead>
<tr>
<th>Balance Serial No.</th>
</tr>
</thead>
</table>

**Stability/Expiration Date:**

**Other Comments:**

**Quantitative - Analytical Tests:**

**ORIGINAL ASSAY:**

<table>
<thead>
<tr>
<th>Date:</th>
<th>Initials:</th>
<th>Method No.:</th>
<th>Invoice No.:</th>
</tr>
</thead>
</table>

**Weight of Container at Disposal:**

<table>
<thead>
<tr>
<th>Balance Serial No.</th>
</tr>
</thead>
</table>

**Sample Disposal:**

<table>
<thead>
<tr>
<th>Initials:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Weight</td>
</tr>
<tr>
<td>------</td>
<td>--------</td>
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<td></td>
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</tbody>
</table>

CF. 08/17/89 Form, container, and remaining contents should be returned to the custodian when anticipated use is completed. All material must be accounted for.

ASSAY RESULTS:

<table>
<thead>
<tr>
<th>Date Returned</th>
<th>Amount Returned</th>
<th>User Init.</th>
<th>Custodian Init.</th>
</tr>
</thead>
<tbody>
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QA-506
1.0 PURPOSE

1.1 To ensure proper personal protective equipment (PPE) is provided, appropriately used, and maintained in a reliable condition to effectively protect employees from hazards present in their work environment.

2.0 AUTHORITY


3.0 PROCEDURES

3.1 Adequate and safe protective equipment for eyes, face, head, extremities, clothing, and respiration will be provided, used, and maintained in a sanitary and reliable condition wherever it is necessary to prevent injury or impairment from absorption, inhalation, or physical contact. To ensure maximum protection, the materials used in protective equipment will be specific for the type, intensity, and duration of potential hazards. Defective or damaged equipment will not be used. Employees will notify their supervisor of any specific concerns or personal needs regarding protective equipment.

3.2 Supervisors will be responsible for ensuring work areas are assessed to determine the actual and potential hazards which may reasonably exist, along with the proper PPE to be used in that area to protect employees from those hazards. Workplace hazard assessment documentation will include the identity of the work area, the date the assessment was performed, and a certification by the evaluator to verify the workplace was assessed (Attachment 1). Contact the NWRC Safety Officer for guidance concerning the assessment of hazards or the selection and use of PPE.

3.3 All PPE will be maintained for maximum effectiveness against the hazard(s) for which it is designed. Periodic inspections and preventive maintenance should be performed to ensure the equipment is in proper working condition (see instruction manual for specific procedures). Protective equipment which is defective, worn, or damaged to the extent that which it is no longer effective will be taken out of service for repair or replacement. All protective equipment to be used for emergency situations will be kept in a highly visible and readily accessible location.
3.4 Training

3.4.1 Supervisors will be responsible for ensuring that each employee who wears PPE is trained (documentation required) and demonstrates an understanding of the following:

a. When PPE is necessary.
b. What PPE is necessary.
c. How to properly put-on, take-off, adjust, and wear PPE.
d. The limitations of the PPE.
e. The proper care, use, maintenance, useful life, and disposal of the PPE.

3.4.2 Training documentation will include the names of the employees trained, the date of the training, a list of the subjects taught, and a certification by the instructor to verify that those employees have received and understood the required training. Re-training will occur whenever new forms of PPE or hazards are brought into the work area. Contact the NWRC Safety Officer for training materials or guidance.

3.5 Requirements for Specific Types of PPE:

3.5.1 Appropriate eye and face protection will be selected to protect against the specific hazard(s) which may be encountered in the work area (e.g., chemical splashes, vapors, flying particles, dust, sparks, or intense light). Minimum requirements for filter lenses must be worn during different types of welding operations. The employer must accommodate those employees who wear corrective lenses with either prescription eye protection or appropriate protection to cover prescription lenses. All protective equipment for the eyes and face must comply with American National Standard Institute (ANSI) minimum specifications.

3.5.2 Protective helmets will be worn in the working area whenever there exists a reasonable potential for falling objects, or where the head may be exposed to overhead electrical hazards. All protective equipment for the head must comply with ANSI minimum specifications.

3.5.3 Protective footwear will be worn in work areas where there exists a reasonable potential for falling or rolling objects, objects capable of piercing the sole, or where the feet may be exposed to ground level electrical hazards. All protective equipment for the feet must comply with ANSI minimum specifications.

3.5.4 Protective equipment for the hands or skin will be worn in work areas where there exists a reasonable potential for skin absorption of harmful substances, severe cuts, lacerations, abrasions, punctures, chemical burns, or temperature extremes. Employees will use only those protective gloves and clothing which provide the maximum protection against the specific hazard(s) being handled.

3.5.5 Employees who work in atmospheres which contain chemical concentrations above the "Permissible Exposure Limits" (see 29 C.F.R. §1910.1000), will be equipped with respiratory protection. Respirator cartridges are specific for individual or groups of chemicals. Employees should read all manufacturer's instructions and warnings before use. See NWRC Standard Operating Procedure HS 003 "Respiratory Protection".

3.5.6 Employees who work in areas where there exists a sustained noise at 85 decibels or above for 8 hours a day or at a time weighted average equivalent will use appropriate hearing protection and participate in periodic hearing exams.

4.0 ATTACHMENTS

Attachment 1: APHIS form 270-R: APHIS Hazard Assessment Form
APHIS HAZARD ASSESSMENT FORM

In accordance with 29 Code of Federal Regulations, Part 1910, Subpart I, Personal Protective Equipment, APHIS facilities are required to perform health hazard assessments to identify those work practices and areas which require personal protective equipment. The assessments must be documented and should be performed in conjunction with required safety and health inspections. This form is intended to provide managers and supervisors with documentation of the assessments. The

Name of Inspector: ____________________________  Date of Inspection: ____________
Position or Title: ____________________________  Type of Work Activity: ____________
Work Location(s): ____________________________

1. Eye and Face Protective Equipment: Equipment includes safety glasses, goggles, face shields, welding helmets, etc. In addition to other areas, eye protective devices are required where employees work with corrosive chemicals or other hazardous substances, machine or hand tools, welding, cutting, soldering, or grinding equipment, woodworking equipment, or are potentially exposed to ultraviolet radiation, infra-red radiation, or hazardous gases, mists, fumes, or dust.

Identify Areas or Work Practices Requiring Eye or Face Protection and the Type of Equipment Needed:

2. Head Protective Equipment: Equipment includes hard hats, bump caps, and liners. Head protective equipment is required to protect workers from impact or penetration from falling or flying objects, overhead hazards, and from limited electric shock and burn hazards. Common areas requiring head protection include maintenance and work areas where low ceilings, beams, or overhead hazards exist, construction sites, etc.

Identify Areas or Work Practices Requiring Head Protective Equipment and the Type of Equipment Needed:

3. Foot Protection: Equipment includes steel-toe boots, work rubbers, overboots, shoe chains, metatarsal guards, foot guards, toe guards, etc. Foot protective equipment is required in areas where there is a danger of foot injuries due to falling or rolling objects, or objects piercing the sole, or where electrical hazards exist.

Identify Areas or Work Practices Requiring Foot Protection and the Type of Equipment Needed:

4. Hearing Protection: Equipment includes ear plugs, canal caps, and ear muffs. The attenuation characteristics of a particular hearing protector must be considered before it is used for a specific application. When selecting a hearing-protective device, the supervisor or manager should also consider the frequency of exposure to excessive noise. If exposure is relatively infrequent (once a day or once a week), an insert or plug will probably satisfy the requirement. However if the noise exposure is relatively frequent the ear muff protector might be preferable. Facilities must comply with the requirements of 29 CFR, 1910.95, Hearing Conservation if employees are exposed to noise above allowable limits.

Identify Areas or Work Practices Requiring Hearing Protection and the Type of Equipment Needed:
5. Protective Clothing: Includes chemical splash suits, disposable clothing, protective aprons, lab coats, insulating workwear, etc. Protective clothing may be required for employees who work with hazardous chemicals or substances, or when working in extreme environments, such as extremely cold conditions.

Identify Areas or Work Practices Requiring Protective Clothing and the Type of Equipment Needed:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

6. Respiratory Protection: Equipment includes air-purifying respirators, disposable respirators, airline respirators, self-contained breathing apparatus, and emergency escape apparatus. Respirators are used as protection against contaminants where engineering and administrative controls are not feasible. Respiratory protection is required to reduce or eliminate injuries caused by breathing air contaminated with harmful dusts, fogs, fumes, mists, gases, smokes, sprays, or vapors. Work practices typically requiring respiratory protection include laboratory work, welding, cutting or brazing, handling hazardous chemicals or substances, and during pesticide application or fumigation.

See Chapter 11, Section 3 of the APHIS Safety and Health Manual and 29 CFR, Section 1910.134 for additional requirements.

Identify Areas or Work Practices Requiring Respiratory Protection and the Type of Equipment Needed:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

7. Electrical Protective Equipment: Equipment includes rubber insulating gloves, rubber matting for use around electric apparatus, rubber insulating blankets, hoods, hoses, and sleeves. Equipment is required for electrical workers. Equipment must conform to the requirements established by the American Society for Testing and Materials, and the American National Standards Institute.

Identify Areas or Work Practices Requiring Electrical Protective Equipment and the Type of Equipment Needed:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

8. Other Protective Devices: Other items such as antidote kits, poison kits, and portable first aid kits are also considered protective devices.

Identify Areas or Work Practices Requiring Other Protective Devices and the Type of Equipment Needed:

________________________________________________________________________
________________________________________________________________________
I. Purpose:

A. To standardize the ranking of small mammals by weight for laboratory testing.

B. To assure the closest possible mean weight distribution of each animal to a treatment group.

C. To insure random assignment of each animal to a treatment group.

II. Procedure:

A. Randomly select a sample population for testing from the entire population available using the computer generated random number program (per SOP WRC-368.R1).

B. Weigh the test sample population using the Mettler PE 3600 (procedure found in WRC SOP-138.R1). The weight for the individual animals should be recorded in a permanent log (WRC SOP-52.R2).

C. All persons handling small mammals should wear leather gloves, a respiratory filter mask, a laboratory coat, and the doors should be closed to the area while working with the animals.

D. Select enough weight classes to include all animals with the number of treatment groups equaling the number of animals in each class. For example:

<table>
<thead>
<tr>
<th>Weight Class</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>96g-105g</td>
<td>1.</td>
</tr>
<tr>
<td>106g-115g</td>
<td>2.</td>
</tr>
<tr>
<td>116g-125g</td>
<td>1.</td>
</tr>
<tr>
<td>126g-135g... etc</td>
<td>1.</td>
</tr>
</tbody>
</table>

The weight classes selected depend on the species being tested and 2 treatment groups would equal a total of 20 animals being tested.
E. In a container, place enough numbers (1 and 2 for this example) to represent the animals in the weight classes. In another container, place numbers to represent the treatment groups (also 2 for this test example).

F. Randomly select one number from each container to assign treatment groups to each animal in the weight class.

G. Assign the treatment group selected to the animal selected from each class taking care to note other identification numbers to prevent mix-ups.

III. Equipment List:

- Pair of leather gloves for those handling small mammals
- Lab coat
- Net for capture of loose animal
- Closed weighing container of adequate size
- Respiratory filter masks
- Mettler PE 3600 electronic balance

IV. Specifications:

A. Closed containers used for weighing should be adequate to temporarily hold a small mammal as per SOP-WRC 397.R2.

B. All persons handling small mammals should wear leather gloves, a respiratory filter mask, and a laboratory coat.

C. Close all doors while working with small mammals.
I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

Kathleen A. Ferguson
(Section Chief)

4/13/94
(Date)

S. Clay Mitchell
(Quality Assurance Officer)

4/10/94
(Date)

Donald J. Chas
(Animal Care Committee)

April 14, 1994
(Date)
STANDARD OPERATING PROCEDURE

Title: Animal Euthanasia With CO₂ Gas

I. Purpose:
   A. To standardize animal euthanasia procedure.

II. Procedure:
   A. Operation, if CO₂ is used from a cart held cylinder
      1. Insure that CO₂ cylinder is properly secured to cart.
      2. Turn the secondary valve (located on the regulator) counterclockwise until it is loose. This indicates the valve is shut.
      3. Turn the primary valve on top of the tank counterclockwise. This will open flow of gas to the regulator. The right gauge (closest to primary valve) will indicate the remaining cylinder pressure.
      4. Open the exhaust valve on the box and position the tubing from the exhaust valve next to a window or exhaust vent. The exhaust valve on the box is located on the front.
      5. To start the gas flow, slowly turn the secondary valve clockwise. The left gauge indicates the line pressure out of the regulator. When the gas starts to flow, slowly adjust the secondary valve for the desired flow rate which is 20 lbs. for 60 seconds, this should be sufficient to fill the box. To measure this flow, use the red, inside indicator circle. It is labeled CO₂.
      6. To stop the flow, turn the secondary valve counterclockwise.
      7. Close the exhaust valve on the box.
To release the regulator pressure, make sure the primary valve is shut (turn clockwise), then open the secondary valve (turn clockwise) until both gauges indicate zero pressure.

9. The primary and secondary valves are to be shut, and pressure released from the system when the tank is not in use. This minimizes the chance for inadvertent loss of cylinder pressure.

B. Operation: if CO₂ is used from the tank room utilizing the medical gas system.

1. Ensure the CO₂ metering device is securely connected to the wall mounted CO₂ outlet. Remove the protective cover on the CO₂ outlet, press the metering device firmly into the receptacle and finger tighten the DISS connector.

2. Attach the long connector of the black hose to the chamber first. The reason this must be done as the first step is because the long connector does not swivel and the hose must be free to turn as the connection is made.

3. Attach the opposite end (with the DISS connector which does swivel) to the base of the CO₂ metering device.

4. Adjust the flow of CO₂ by turning the knurled knob of the wall mounted metering device. Clockwise to reduce the flow, counter clockwise to increase the flow. Arrows on the knurled knob indicate the proper direction.

C. Introduction of animals to CO₂ atmosphere.

1. Open the exhaust valve on the box and position the tubing from the exhaust valve next to an exhaust vent. The exhaust valve on the box is located on the front.

2. Using proper animal handling techniques, place the animals one at a time into the CO₂ rich atmosphere of the already filled container. Follow the specific instructions for the euthanasia chamber being used. For example, if the unit has two compartments, animals can be added to first one compartment and then the other to speed the task. Add CO₂ as needed by adjustment of flow of CO₂ from the regulator and open the exhaust valve on the box as needed to ensure death is as painless as possible. When more than one animal is to be euthanized, maintain a continuous flow of CO₂ and leave the exhaust valve open.

3. The time required for death is dependent upon the species. Visually observe the animal for movement or respiration. CO₂ is heavier than air and most will remain in the box even when opened. Nonetheless, USE CAUTION WHEN REMOVING ANIMAL TO AVOID BREATHING CO₂.
4. Death by euthanasia is confirmed by loss of heartbeat and/or no reaction when the area about the eye is touched.

5. After all animals have been processed, secure the cylinder or the wall mounted equipment and clean the container.

6. Properly dispose of animal carcass according to intended use: incineration, residue analysis, freezer storage, etc.

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

[Signatures and dates]

Unit Chief

quality Assurance Officer

Animal Care & Use Committee
DENVER WILDLIFE RESEARCH CENTER
ANIMAL CARE SECTION
LAKEWOOD, COLORADO

Date prepared: 3/24/89 AJB
Date revised: 

STANDARD OPERATING PROCEDURE

Title: Animal Handling (General) so that secure and accurate identification is maintained.

I. Purpose: To standardize animal handling methods so that cage identification may serve as an accurate means of individual animal identification

A. Wild caught animals must be securely identified for research purposes, and yet remain free of artifacts which traditional identification methods may introduce.

II. Procedure:

A. Removal of animals from individual cages.

1. Never undertake to remove any animal or change any cage unless you have an exact plan on how to proceed.

2. Always bear in mind, the cage number is your only means of identification. Each animal must be matched to their cage at all times.

3. Never involve more than one animal and one cage in any procedure.

4. Complete procedure, (record, examine, treat and etc.) and replace animal before moving to next animal and next cage.

5. At any time, for whatever reason, if any confusion exists, consult your supervisor immediately.

6. Be certain identifying number is securely attached to cage.
B. Replacement of cages

This is where the greatest possibility of error lies. All of the above rules apply, especially #3 and #6.

1. Do not allow yourself to be distracted by conversation.

2. If for any reason, you are distracted. Halt what you are doing, note exactly where you are in your procedure, and attend to the distraction.

3. Do not hurry during this period. Be precise and methodical.

4. At any time, for whatever reason, if any confusion exists, consult your supervisor immediately.

C. Transport of cages.

1. As each cage is removed from the rack, be certain the cage is securely identified and closed.

2. If the cage is of the suspended type, without a top, slide a transport top over the cage as the cage is removed from the rack.

3. Do not stack loose cages.

4. Always bear in mind, the cage number is your only means of identification. Each animal must be matched to their cage at all times.

Emergency Numbers:

Al Dale 494-0411
Phyllis Harris 986-0644
or Call principal investigator as listed on the testing protocol

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

Al Dale  
Chief, Animal Care  
Section Chief  
7/20/89  
Date

Jay Mitchell  
Quality Assurance Officer  
7/21/89  
Date
Title: Quarantine procedures for Animals at the National Wildlife Research Center

I. Purpose:
   A. To standardize Quarantine methods.

II. General:

1. All incoming animals, except those collected for immediate euthanasia will be placed in quarantine for an observation and stabilization period.

2. Normally, the quarantine period for wild caught animals will be 14 days. This period may be extended as conditions dictate.

3. Normally, the quarantine period for laboratory purchased animals will be 7 days. This period may be extended as conditions dictate.

4. Prior to arrival of the animals, the room and cages will be prepared to receive the animals.

5. Only animals of the same specie will be quarantined together, and as well as can be determined, only animals trapped in the same area or received from the same source will be quarantined together.

6. Cages will be labeled with index cards or tape with the animal number. Unless instructed by a supervisor to house in a different manner, the animals will be housed individually. (See SOP WRC-216)
7. Animal records shall be stored in designated area at the close of each day.

8. Except those animals kept under natural lighting conditions, the lighting cycle will be a 12 hour light, dark cycle.

9. Feeding, watering, and care will conform to the appropriate WRC-SOP for the specie involved.

III. Procedure

1. Complete entry Form

2. Complete Pre-Quarantine Forms if required. (Such as waiting to assemble a group)

3. Complete Quarantine Form. (Entry and Release)

4. Release to Study Protocol or Post-Quarantine.

5. Ensure you have obtained all necessary signatures.

6. Complete records each day.

Emergency Numbers: Al Dale 494-0411
Phyllis Harris 986-0644
or call Principal investigator as listed on the testing protocol.

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

[Signatures and dates]

Unit Chief

Date

Quality Assurance Officer

Date

Animal Care and Use Committee

Date
Title: Incinerator Use and Maintenance

I. Purpose:
To standardize incinerator operation and disposal of material from the incinerator:

A. Standard Procedures
1. Keep door to incinerator room locked when not in use.
2. Do not add additional material to the unit while the burn is taking place.
3. If equipment malfunction is suspected advise your supervisor.

B. Standard Incinerator Operation
1) Record time from counter, and enter the time, date, and the weight of the amount you intend to burn in the Incinerator Record Book. This record is required to comply with our permit to operate this incinerator. Forms are provided for this purpose. The form in use (the present month) will be maintained in the room with the incinerator. After completion, the monthly summaries are to maintained in the section office.

2) Wear a mask, and be certain the ashes are cool, before removing the ashes. Take care that the ash pull tool does not make heavy contact with the incinerator interior. Store the removed ash in a metal trash container which has been lined with a plastic bag.

3) Clean out all air ports.

4) Turn system to on. A Red light appears on the panel and the air activated louvers will open.

5) Turn control timer past stop. This will initiate the pre-heat cycle. A Green light appears on the panel when the control burner fires. The Red light will stay on until the
pre-set temperature (1400 degrees F, 760 degrees centigrade) has been reached. The red light will go off, and a blue light will appear on the panel when the pre-set temperature listed above is reached. When this temperature is reached, and the blue light appears, the combustion blower will start.

To summarize:

a. The green light on the panel should stay on throughout the cycle. This indicates the control burner is firing.

b. The red light indicates the control burner is below 1400 degrees.

c. The blue light indicates the control burner is above 1400 degrees.

6) During the above pre-heat cycle (step 4), and while waiting for the pre-heat temperature to be reached, load incinerator, taking care the opening for the burner at the distal end of the incinerator chamber is not blocked. When closing door, simply snug the latch. If the door is tightly closed the heat will make the door nearly impossible to open.

7) When the pre-set, pre-heat temperature is reached (the red light turns off, and the blue light is on) the refuse chamber burner is ready to ignite and to begin the burn. To initiate the firing of the refuse chamber, set the timer of the refuse chamber (lower chamber) past stop. Generally it should be set to at 5 and 1/2 hours. (A yellow light will appear to indicate the refuse burner has fired).

8) The timer of the control chamber (upper chamber) should always be set for 30 minutes longer than the refuse chamber. Therefore, generally this will be 6 hours.

9) The following morning the incinerator will have automatically shut off at the time chosen, but the control fan will still be operating. Turn the system to off, and this will turn off the fan.

C. Ash Disposal

1) Dispose of the ashes when the metal container is one half full.

2) Wearing a mask, remove the plastic bag containing the ashes and place into a cardboard box of suitable size.

3) Tape the cardboard box shut and place the box in the dumpster.
Additional Information:

Even if the refuse (lower) chamber timer is turned to on, the refuse burner will not ignite until the control (upper) chamber has reached its pre-set temperature.

The magahelic gauge should always show a negative draft.

The fan (built into the North wall) is controlled and activated by the thermometer on the South wall (next to the louvers). For instance, if this thermometer is set at 70 degrees, the fan will turn on when the temperature in the room reaches 70 degrees, and turn off when the temperature drops below 70 degrees.

If the system fails to operate, the electronic controls may require manual reset. To do this, open the main front control panel to find the reset switch.

The temperature of the upper and lower chambers are pre-set.

The door latch operates a mechanical-electrical switch located on the lower left side of the door. Opening and closing of the door engages this switch. The purpose of the switch is to provide operator safety by disengaging the primary burner and the fan if the door is opened.

Emergency Numbers:  
- Al Dale 494-0411  
- Phyllis Parker 986-0644

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

[Signature]
Unit Chief

[Signature]
Quality Assurance Officer

[Signature]
Animal Care and Use Committee

Date: 4/17/96
Date: 4/18/96
Date:

Page 85 of 109
DENVER WILDLIFE RESEARCH CENTER
Chemical Development/Registration Section
Denver, Colorado

Date Prepared: 07/19/90 grm
Date Revised: 04/08/94 grm

STANDARD OPERATING PROCEDURE

Title: Computer generated random numbers.

I. Purpose:

A. To provide guidelines for computer generated random numbers.

II. Procedure:

A. Enter the Epistat program by Tracy L. Gustafson, M.D., and specify the random number generator program called: "Randomiz".

1. Select a survey sample from a population to generate a random number set.

2. Enter the print mode: either screen viewing or printed.

B. Enter the smallest number, the largest number and how many random numbers between the smallest and largest numbers are needed.

C. This program will allow multiple randomizations by asking "Do you want to perform another randomization?". Enter y for yes and n for no.

III. Specifications:

A. The random sample generator in the statistical program called "Epistat" aids in the selection of random samples for several purposes.

B. It can provide a random subset of a larger population or it can assign cases randomly to independent or paired groups.
I have read this document and approve of its contents. I certify that it will be made available to all appropriate personnel.

Kathleen A. Fagerstone
Section Chief

4/14/94
Date

S. Clay Mitchell
Quality Assurance Officer

4/15/94
Date
STANDARD OPERATING PROCEDURE

Title: Incineration of Animal Carcass and Tissue

I. Purpose:
   A. To provide guidelines and standardize a method of disposal for animal carcasses from field and laboratory testing and spent tissue samples.

II. Specifications:
   A. Tissues will be inventoried and recorded in a permanent log for archiving. Specimens will then be placed in plastic bags adequate for transport and sealed.
   B. The package to be incinerated should be double bagged before leaving the storage area to avoid any leakage and contamination in transport.
   C. Disposable latex gloves and laboratory coat are recommended apparel for safety.
   D. See WRC-233.R2 for other safety tips and incinerator operation.

III. Procedure:
   A. Coordinate with Animal Care Section as to when incinerator will be available.
   B. See WRC-233.R2 for incinerator use and operation.
   C. Transport packages to be incinerated as soon as possible after double bagging and preparation for transport.
I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

[Signature]

(Section Chief)

[Signature]

3-19-92

(DATE)

H. May Mitchell

(Quality Assurance Officer)

[Signature]

3/20/92

(DATE)

Donald P. Bliss

(Animal Care Committee)

30 March, 1992

(Date)
STANDARD OPERATING PROCEDURE

HAZARD COMMUNICATION

I. PURPOSE:
To ensure the chemical hazards present within the laboratories of the Denver Wildlife Research Center are evaluated and the information concerning those hazards is transmitted to the employee as specified by the Occupational Safety and Health Administration (OSHA) in 29 CFR 1910.1200 (b)(3).

II. PROCEDURE:
A. Labels
1. All containers of incoming chemicals shall be checked for a visible and legible label which should contain the following information in English:
   a. The chemical name (and common name if available).
   b. The name and address of the manufacturer or distributor.
   c. The appropriate hazard warnings.
2. Chemicals received without a label shall not be used and must be returned to the distributor or manufacture as soon as possible.
3. The label or the information contained on the label will not be removed, covered, obscured, altered, or defaced.

B. Material Safety Data Sheet (MSDS)
1. A MSDS must exist for all chemicals on hand. If an MSDS does not exist, then one must be requested from the distributor or manufacturer, or created according to the criteria in 29 CFR 1910.1200(g).
2. No chemical shall be dispensed or used without an MSDS on hand.

3. A collection of MSDS's for every chemical on hand must be kept in a readily accessible location.

C. Employee Awareness

1. Supervisors are responsible to ensure employees are informed of any chemical hazards which may exist in the workplace and are trained on how to work safely with those chemicals. Contact the DWRC Chemical Hygiene Officer for training materials or guidance. Training shall include:
   a. How to read and understand a chemical label.
   b. How to read and understand an MSDS.
   c. How to select and use protective equipment.
   d. What to do in case of an emergency.
   e. The location of the chemicals, MSDS's, fire extinguishers, fire alarms, spill kits, safety equipment, personal protective equipment, emergency eye washes and showers, and emergency escape routes.

D. Shipping Chemicals

1. The shipment of chemicals from a laboratory shall be in compliance with the same regulations which govern chemical shipments from manufacturers or distributors:
   a. Containers shall be labeled with the same information listed in part II.A.1 of this SOP (in accordance with 29 CFR 1910.1200(f)(1)).
   b. Recipients of the shipment shall be provided an MSDS which has been prepared in accordance with 29 CFR 1910.1200(g). The MSDS shall be either placed in the shipment container or sent directly to the recipient at the time of shipment.

I have read this document and approve of its contents. I certify that it will be made available for all personnel to whom it applies.

[Signature]
DWRC Quality Assurance Officer

[Signature]
Date
STANDARD OPERATING PROCEDURE

Title: Deer Mouse Maintenance

I. Purpose:

A: Safety Measures:

The possible existence of Hantavirus (Sin Nombre) infection in these animals is a serious concern. For this reason special safety rules will apply.

1. Respirators must be worn in the room, and the principles for cleaning and care of respirators apply.

2. Each day a freshly laundered lab coat will be used while providing care. Each day the lab coat used will be stored for washing in a specially labeled container in the laundry room. A respirator will be worn when the lab coats in this container are placed in the machine for washing.

3. Rubber gloves will be worn when working in the animal room, and these gloves will be disposed of in the room in a double bagged container before exiting. Periodically, this bag will be closed and delivered to the incinerator for immediate incineration, or placed in the freezer for incinerator storage.

4. Rubber footwear must be worn in the animal room, and cleaned in a disinfectant bath upon leaving.

5. When cages are changed, the bedding will be moistened with disinfectant prior to removal of the cage to the cage wash room.

6. Prior to being removed from the room, racks will be sprayed with disinfectant.

7. After caging equipment is removed to the cage wash room, a lab coat, respirator and gloves will be worn while the bedding, moistened with disinfectant, is emptied from the cages into a double bagged container using the Baker DS-400.
8. The lab coat, respirator, and gloves can be removed after the refuse is closed in a double bagged manner.
9. As soon as practical, the soiled cages or the rack will be placed in the cage wash unit for washing. No prewashing will occur at the prewash station.
10. As soon as practical, the double bagged refuse will be delivered to the incinerator for immediate incineration or placed in the freezer for incinerator storage.
11. The Baker DS-400 will be sprayed with disinfectant and later cleaned of all debris at the prewash station.

B. To standardize mouse maintenance.
1. Records on animals shall be stored in designated area at the close of each day.
2. Feeding schedules, light schedules, and room temperatures will be maintained without change throughout testing periods. To improve health status of the animals, adjustments to these schedules may occur at other times. Ensure the proper schedules are in place.
3. Cages will be labeled with index cards or tape with the ID# of the animal.
4. If an animal is found dead, securely attach the ID # to the animal, or to the bag in which the animal is placed. Follow the study protocol for disposal. If the animal is not subject to a test protocol, consult your supervisor.
5. Lighting will be on a 12 hour light-dark cycle, the temperature will be maintained as close to 70 degrees as possible, and the doors to the animal room will be locked when personnel are not engaged in providing care.
6. Between tests, when the room is vacant, the room will be thoroughly cleaned and sanitized.

II. Procedure:
  Daily
1. Check each animal to ensure the animal appears normal. Update daily report, noting abnormalities. Request additional checking from your supervisor if you are uncertain. Report all ill, injured, or dead animals.
2. Water is offered ad libitum. Bottles are refilled daily as required.
3. Follow feeding schedule.
4. Record maximum, minimum and ambient room temperatures.
5. Be certain the animal room and the ante room is clean and neat upon completion of providing daily care.

Weekly
1. Mop floors using a disinfectant.

Every Two weeks
Preferably Tuesday, although the specific day may vary with workloads
1. Replace rack, cages, watering, and feeding utensils. (See WRC-216, on secure identification)

Monthly
1. Inspect the outlet room air filter at the entrance to the room above the door on the last Thursday of each month and replace as needed. If filter replacement is required, dispose of the filter in the glove bag located in the room.

As needed
1. Whenever a cage becomes soiled, move the animals to a clean cage, taking care to maintain identity. (see WRC-216, on secure identification).
2. Wipe down racks and surfaces to maintain cleanliness.
3. Sweep, and or, mop floors to maintain cleanliness.
Emergency Numbers: Al Dale 494-0411
Phyllis Parker 986-0644

or call the Principal Investigator as listed in the testing protocol.

I have read this document and approve of it's contents. The document will be made available to all personnel to which it applies.

A. J. Dale
Unit Chief

7/1/96
Date

H. J. Jolliff
Quality Assurance Officer

7/7/96
Date

W. E. Duenenberg
Animal Care & Use Committee

7/4/96
Date
**To:** Tom Primus  
**Analytical Chemistry Project**  
3350 Eastbrook Dr., Fort Collins, Co 80525

**Subject:** Diphenphione and Chlorophacinone SRO Bait Assay (QA 542)

**Method:** 62A  
**Analysis Date:** July 9, 1998

**AC Notebook Reference:** AC52-pp 134-145

**QC Notebook Reference:** QC 14-pp 62, 64, 67

**Analyst:** C. Furcolow, Margaret J. Goodall

**Sample Description:**

<table>
<thead>
<tr>
<th>Sample ID #</th>
<th>Description on sample bag</th>
<th>Sample ID #</th>
<th>Description on bag</th>
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<td>0.01% Diphenphione Bait</td>
<td>S980709-1</td>
<td>0.005% Chlorophacinone Bait</td>
</tr>
<tr>
<td>S980708-3</td>
<td>SRO Control Bait</td>
<td>S980709-2</td>
<td>0.005% Diphenphione Bait</td>
</tr>
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<td>S980708-4</td>
<td>0.01% Diphenphione SRO Bait</td>
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<td>S980708-5</td>
<td>0.01% Chlorophacinone SRO Bait</td>
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<td></td>
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</tbody>
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**Method Modification(s)/Comments:**

- Bait samples were ground using a coffee mill.
- Three subsamples from each bag were assayed.
- Quality control data are reported. Sample results are not adjusted for recoveries.

**Reviewer:** Margaret J. Goodall

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Page 97 of 109
<table>
<thead>
<tr>
<th>Sample ID #</th>
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<th>Diphacinone (% w/w)</th>
<th>Chlorophacinone (% w/w)</th>
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<td>ND</td>
</tr>
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<td>S980708-3B</td>
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<td>ND</td>
<td></td>
</tr>
<tr>
<td>S980708-3C</td>
<td></td>
<td>ND</td>
<td></td>
</tr>
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<td></td>
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<tr>
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**Diphacinone Bait Assay**

- **Observed % Diphacinone (w/w)**
  - Sample ID: S980630-2A, Date: 7/9/98, %: 0.00948, Mean = 0.00058, SD = 0.00097, CV = 0.12%
  - Sample ID: S980630-2B, Date: 7/9/98, %: 0.00940, Mean = 0.00011, SD = 1.2%
  - Sample ID: S980630-2C, Date: 7/9/98, %: 0.00961, Mean = -0.00052, SD = 1.2%
  - Sample ID: S980708-3A, Date: 7/9/98, %: ND, Mean = -0.00052, SD = 1.2%
  - Sample ID: S980708-3B, Date: 7/9/98, %: ND, Mean = -0.00052, SD = 1.2%
  - Sample ID: S980708-3C, Date: 7/9/98, %: ND, Mean = -0.00052, SD = 1.2%
  - Sample ID: S980708-4A, Date: 7/9/98, %: 0.00967, Mean = 0.00011, SD = 2.0%
  - Sample ID: S980708-4B, Date: 7/9/98, %: 0.00985, Mean = 0.00019, SD = 2.0%
  - Sample ID: S980708-4C, Date: 7/9/98, %: 0.00949, Mean = 0.00011, SD = 2.0%
  - Sample ID: S980709-2A, Date: 7/9/98, %: 0.00514, Mean = 0.00018, SD = 2.0%
  - Sample ID: S980709-2B, Date: 7/9/98, %: 0.00439, Mean = 0.00019, SD = 2.0%
  - Sample ID: S980709-2C, Date: 7/9/98, %: 0.00506, Mean = 0.00018, SD = 2.0%

**Chlorophacinone Bait Assay**

- **Observed % Chlorophacinone (w/w)**
  - Sample ID: S980708-3A, Date: 7/9/98, %: ND, Mean = 0.00019, SD = 1.2%
  - Sample ID: S980708-3B, Date: 7/9/98, %: ND, Mean = 0.00019, SD = 1.2%
  - Sample ID: S980708-3C, Date: 7/9/98, %: ND, Mean = 0.00019, SD = 1.2%
  - Sample ID: S980708-5A, Date: 7/9/98, %: 0.00964, Mean = 0.00018, SD = 1.2%
  - Sample ID: S980708-5B, Date: 7/9/98, %: 0.00969, Mean = 0.00018, SD = 1.2%
  - Sample ID: S980708-5C, Date: 7/9/98, %: 0.0100, Mean = 0.00018, SD = 1.2%
  - Sample ID: S980709-1A, Date: 7/9/98, %: 0.00527, Mean = 0.00019, SD = 2.0%
  - Sample ID: S980709-1B, Date: 7/9/98, %: 0.00542, Mean = 0.00019, SD = 2.0%
  - Sample ID: S980709-1C, Date: 7/9/98, %: 0.00517, Mean = 0.00019, SD = 2.0%
Appendix V. Raw Data Summaries of: Survival, Consumption of 0.01% Chlorophacinone Oat Groats and OPP Rat and Mouse Challenge Diet, and Body Weights.
Appendix V

Raw Data Summary 1. Dates deer mice died during testing (Day 1 = July 19, 1998).

<table>
<thead>
<tr>
<th>Females</th>
<th>Group II</th>
<th>Date Died (1998)</th>
<th>Treatment Day</th>
<th>No. Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>July 22</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July 23</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July 24</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
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<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Date Died (1998)</th>
<th>Treatment Day</th>
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<td>July 23</td>
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<th>Treatment Day</th>
<th>No. Dead</th>
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<td></td>
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<table>
<thead>
<tr>
<th>Group III</th>
<th>Date Died (1998)</th>
<th>Treatment Day</th>
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</table>
Appendix V

Raw Data Summary 2. Bait consumption by 9 female deer mice on the 0.00% diet concentration (Group I) during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Dish O&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dish C&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Total (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.12</td>
<td>13.38</td>
<td>21.50</td>
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<tr>
<td>2</td>
<td>9.91</td>
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<td>24.80</td>
</tr>
<tr>
<td>3</td>
<td>12.39</td>
<td>14.09</td>
<td>26.48</td>
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<tr>
<td>4</td>
<td>9.94</td>
<td>15.90</td>
<td>25.84</td>
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<td>5</td>
<td>10.85</td>
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<td>6</td>
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<td>7</td>
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<td>14.59</td>
<td>23.86</td>
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<td>8</td>
<td>7.62</td>
<td>16.59</td>
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<td>9</td>
<td>14.65</td>
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<td>24.25</td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Total (g)</td>
<td>93.75</td>
<td>129.10</td>
<td>222.85</td>
</tr>
<tr>
<td>Mean (g)</td>
<td>10.42</td>
<td>14.34</td>
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<tr>
<td>SD</td>
<td>2.16</td>
<td>2.23</td>
<td>1.80</td>
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</tbody>
</table>

<sup>a</sup>O = OPP rat and mouse challenge diet.

<sup>b</sup>C = OPP rat and mouse challenge diet.
Appendix V

Raw Data Summary 3. Bait consumption by 10 male deer mice on the 0.00% diet concentration (Group I) during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Dish O\textsuperscript{a}</th>
<th>Dish C\textsuperscript{b}</th>
<th>Total (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.95</td>
<td>9.75</td>
<td>22.70</td>
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<td>2</td>
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<td>25.29</td>
</tr>
<tr>
<td>4</td>
<td>10.55</td>
<td>14.66</td>
<td>25.21</td>
</tr>
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<td>5</td>
<td>17.83</td>
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<td>26.23</td>
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<tr>
<td>6</td>
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<td>7.56</td>
<td>25.93</td>
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<td>11.76</td>
<td>12.86</td>
<td>24.62</td>
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<td>9</td>
<td>14.93</td>
<td>9.74</td>
<td>24.67</td>
</tr>
</tbody>
</table>

\[ \text{N} = \text{9} \]
\[ \text{Total (g)} = 128.03 + 95.75 = 223.78 \]
\[ \text{Mean (g)} = 14.22 + 10.64 = 24.86 \]
\[ \text{SD} = 3.65 + 3.24 = 1.07 \]

\textsuperscript{a}O = OPP rat and mouse challenge diet.
\textsuperscript{b}C = OPP rat and mouse challenge diet.
Raw Data Summary 4. Bait consumption by 10 female deer mice on the 0.01% chlorophacinone grain bait (Group II) during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Dish O&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dish T&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>0.12</td>
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<tr>
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<td>&lt;0.02&lt;sup&gt;c&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup>O = OPP rat and mouse challenge diet.

<sup>b</sup>T = 0.01% chlorophacinone oat groat bait.

<sup>c</sup>Dish weighed more than its original weight; value not used in the statistics.
Appendix V

Raw Data Summary 5. Bait consumption by 10 male deer mice on the 0.01% chlorophacinone grain bait (Group II) during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Bait consumption (g)</th>
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</thead>
<tbody>
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<td></td>
<td>Dish O&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Dish T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
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<td>2</td>
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<td>&lt;0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.00</td>
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<td>7</td>
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<td>Total (g)</td>
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</tbody>
</table>

<sup>a</sup>O = OPP rat and mouse challenge diet.

<sup>b</sup>T = 0.01% chlorophacinone oat groat bait.

<sup>c</sup>Dish weighed more than its original weight; value not used in the statistics.
Appendix V

Raw Data Summary 6. Bait consumption by 10 female deer mice on the 0.01% chlorophacinone grain bait (Group III) during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Dish O&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dish T&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
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<tr>
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<td>0.12</td>
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<tr>
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<td>0.04</td>
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</tbody>
</table>

<sup>a</sup>O = OPP rat and mouse challenge diet.
<sup>b</sup>T = 0.01% chlorophacinone oat groat bait.
<sup>c</sup>Dish weighed more than its original weight; value not used in the statistics.
Appendix V

Raw Data Summary 7. Bait consumption by 10 male deer mice on the 0.01% chlorophacinone grain bait (Group III) during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Bait consumption (g)</th>
<th>Dish O&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dish T&lt;sup&gt;b&lt;/sup&gt;</th>
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</table>

<sup>a</sup>O = OPP rat and mouse challenge diet.

<sup>b</sup>T = 0.01% chlorophacinone oat groat bait.

<sup>c</sup>Dish weighed more than its original weight; value not used in the statistics.
Raw Data Summary 8. Consumption of the OPP rat and mouse challenge diet by surviving deer mice during the 5-day post-testing feeding period.

<table>
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Appendix V

Raw Data Summary 9. Individual deer mice body weights recorded before their placement on the 15-day, 2-choice, feeding trial.

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Appendix V

Raw Data Summary 10. Individual deer mice body weights measured at time of death or termination of study.

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STUDY TITLE:
Chlorophacinone (0.005%): Standard House Mouse Anticoagulant Wax Block Laboratory Test

DATA REQUIREMENT(S):
GDLN 96-12

AUTHOR(S):
Geraldine R. McCann

STUDY COMPLETION DATE:
February 24, 2000

LABORATORY:
USDA/APHIS/WS/National Wildlife Research Center
4101 Laporte Avenue
Fort Collins, CO 80521-2154

LABORATORY PROJECT ID:
QA-506
CDFA No. 94-0620

CITATION:

Page 1 of 132
STATEMENT OF DATA CONFIDENTIALITY

No claim of confidentiality is made for any information contained in this study on the basis of it falling within the scope of FIFRA 10(d)(1)(A), (B), or (C).

Company: California Department of Food and Agriculture

Agent: Nick Condos

Date: 12/27/95

Title: Program Supervisor

Signature: 12/27/95
GOOD LABORATORY PRACTICE/QUALITY ASSURANCE STATEMENT

This study meets the requirements of 40 CFR Part 160. It was maintained on the National Wildlife Research Center (NWRC) Master Schedule. An inspection was performed on March 27, 1997 by Steve Greiner. The results of these inspections were given to the Study Director (Geraldine R. McCann) and to the NWRC Director (Richard D. Curnow) on April 14, 1997.

Agent: Donald J. Elias
Title: Quality Assurance Officer

Submitter: California Department of Food and Agriculture

Agent: Nick Condos
Title: Program Supervisor

Sponsor: U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, NWRC

Agent: Dr. Richard D. Curnow
Title: Director

Study Director: Geraldine R. McCann
Title: Biological Science Technician (Wildlife)
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ABSTRACT

This study was required by the Environmental Protection Agency to partially fulfill data requirements for reregistering the California Department of Food and Agriculture’s 0.005% chlorophacinone wax bait block label SLN CA-890025. This laboratory study determined the efficacy of a 0.005% chlorophacinone oat groat wax bait block for controlling house mice (Mus musculus). Eighty domestic house mice (20 control, 60 treated), equally represented by sex, were placed on a 15-day, 2-choice, feeding trial. Mice were housed in groups of 10, segregated by sex for testing. Each group of control mice received 2 dishes, containing the Office of Pesticide Programs (OPP) rat and mouse challenge diet. Each group of treated mice (Groups II and III) each received one dish of the OPP rat and mouse challenge diet and one 0.005% unweathered chlorophacinone wax bait block. The last group of mice (Group IV), each received one dish of the OPP rat and mouse challenge diet and one 0.005% weathered chlorophacinone wax bait block. By day 5 of the study, the treated mice (Groups II, III, and IV) had consumed 78.1% of the total amount of the wax bait blocks. Forty-three (71.7%) of the 60 treated mice died. Mortality for Groups II, III, and IV was 14, 14, and 15 house mice, respectively. Mouse mortality began on Day 3 and continued until Day 15, with 70% dying between Days 3 and 7. Seventeen treated mice survived the test. The 71.7% mortality meets the 70% minimum mortality standard established by the EPA for verifying efficacy of rodenticides (U.S. EPA 1982).
INTRODUCTION

This data submission was requested by the Environmental Protection Agency (EPA) as partial fulfillment for the reregistration of 0.005% chlorophacinone wax bait blocks (SLN CA 890025) (Appendix I). In 1993, the EPA requested from the California Department of Food and Agriculture (CDFA) efficacy data on the 0.005% chlorophacinone wax bait blocks used for controlling house mice (Mus musculus). To meet this objective, the Vertebrate Pest Control Research Advisory Council (VPCRAC) and the CDFA, in cooperation with the National Wildlife Research Center (NWRC), conducted a laboratory feeding trial that evaluated bait acceptance and mortality among house mice using a wax bait block formulated with 2.0%, technical chlorophacinone. This feeding trial was conducted in compliance with the 1995 Code of Federal Regulations (CFR) 40, Part 160, Good Laboratory Practices, and the appropriate Pesticide Assessment Guidelines (PAG), Subdivision G (U.S. EPA 1982). The test followed the Standard House Mouse Anticoagulant Wax Block and Wax Pellet Laboratory Test Method as outlined in Office of Pesticide Programs (OPP) Designation 1.214 (2-25-74) (Appendix II).

The trial was conducted in March-April 1997 by NWRC and assigned study designation QA-506 (Appendix III). The study tested the following null hypothesis (H₀:) there is no difference in mouse mortality between house mice feeding on the 0.005% chlorophacinone wax bait blocks and the OPP rat and mouse challenge diet, and the control house mice feeding on the OPP rat and mouse challenge diet, alone.
MATERIALS AND METHODS

Formulation

0.01% Chlorophacinone Oat Groat Bait

The 0.01% chlorophacinone oat groat bait was formulated by Rodent Control Outfitters Inc.\textsuperscript{1,2} according to the Confidential Statement of Formula for this CDFA registration. The 0.01% chlorophacinone grain bait was assayed by the Analytical Chemistry Unit of the NWRC using validation ACP Method 62A. The 0.01% chlorophacinone oat groat bait was used to formulate the 0.005% wax bait blocks used in the 15-day feeding trial.

OPP Rat and Mouse Challenge Diet

The OPP rat and mouse challenge diet was prepared at the NWRC as specified by the EPA. The diet was formulated according to the instructions in OPP Designation 1.214 and was composed of the following ingredients (U.S. EPA 1980):

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornmeal (whole yellow ground corn)</td>
<td>65%</td>
</tr>
<tr>
<td>Rolled oat groats (ground)</td>
<td>25%</td>
</tr>
<tr>
<td>Sugar (10X powdered or confectioners, (\geq) 95% purity)</td>
<td>5%</td>
</tr>
<tr>
<td>Corn oil ((\geq) 95% purity)</td>
<td>5%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
</tr>
</tbody>
</table>

After formulation, the OPP rat and mouse challenge diet was packaged in plastic containers, tightly sealed, and maintained at -18°C or colder until used. Before being offered to the test or control mice, the challenge diet and the test bait were brought to room temperature.

\textsuperscript{1}Reference to commercial products or entities does not imply endorsement by U.S. Government.

\textsuperscript{2}Mailing address: P.O. Box 446, Junction City, OR 97448
0.005% Chlorophacinone Wax Bait Block Preparation

The wax bait blocks were prepared at the laboratories of the NWRC as follows:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percent</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01% chlorophacinone oat groat bait</td>
<td>50%</td>
<td>113.5</td>
</tr>
<tr>
<td>Paraffin wax</td>
<td>50%</td>
<td>113.5</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>226.5</td>
</tr>
</tbody>
</table>

After the 0.01% chlorophacinone oat groat bait was assayed, it was warmed to 60°C. The paraffin wax was melted and brought to a temperature of 78°C. In a 10-oz. styrofoam cup 113.5 g (4 oz.) of melted wax was mixed with 113.5 g (4 oz.) of 0.01% chlorophacinone oat groats. Mixing continued until a uniform mixture was achieved. A bent wire was inserted into the wax to be used as a cage fastener. Each block was assigned a unique number. The mixture was allowed to set and cool before the styrofoam cup was peeled away and discarded. Twelve bait blocks were prepared and by random selection, the 12 bait blocks were assigned as follows:

- 6 bait blocks: weathered group.
- 4 bait blocks: unweathered group.
- 2 bait blocks were analyzed to verify the 0.005% concentration by the Analytical Chemistry Unit of the NWRC using validation ACP Method 64B.

The bait blocks were assayed by the Analytical Chemistry Unit of the NWRC using validation ACP Method 64B.

Weathering Wax Bait Blocks

The 6 randomly selected wax bait blocks were weathered in an environmental chamber for 15 days. The thermostat was set at 100°F and 100% humidity.
Test Procedures

Pre-treatment

Animal Procurement

Eighty-six (42 males and 42 females) sexually mature house mice (*Mus musculus*) of the Swiss-Webster genotype weighing between 16.5 g and 25.9 g were purchased from the Taconic Farms, Inc.

Quarantine

After arrival on March 11, 1997, at the NWRC's Animal Research Building, the mice were weighed. They were then separated by gender and placed in triple size cages (63.5 x 24.1 x 17.8 cm). The mice were maintained on a commercial laboratory mouse diet and water provided *ad libitum*. They immediately underwent a 7-day quarantine period. Upon removal from quarantine, the house mice were checked by the resident veterinarian before being released for testing. The test room temperature was maintained between 21°C and 24°C. The light cycle was 12 hours dark and 12 hours light.

Holding Environment Conditions

Following the quarantine period, each animal was again weighed, and separately housed in a uniquely numbered single-size cage. A computer-generated random number program was used to eliminate 2 males and 2 females from the test. Each gender (40M:40F) was then ranked by weight into 10 weight classes, each containing 4 animals.

Each weight class for each gender contributed one animal to each of the 4 groups (I, II, III, and IV). The 4 groups of 10 house mice, separated by gender, were randomly assigned to one of the 4 Groups: 0.0% control (Group I), 0.005% chlorophacinone unweathered wax bait blocks (Groups II and III) or 0.005% chlorophacinone weathered wax bait blocks (Group IV).

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3Reference to commercial products or entities does not imply endorsement by the U.S. Government.

4Mailing Address: 273 Hoyer Avenue, Germantown, NY 12526.
This procedure allocated 10 house mice per gender per group, or a total of 20 animals per treatment. It also evenly distributed the mice by body weight and gender among the 4 groups.

After the mice were assigned to the treatment groups, the members of each group were removed from their individual cages and grouped together in a larger triple size cage (63.5 x 24.1 x 17.8 cm). Each of the 8 cages were previously labeled by sex and treatment group. The animals were then fed their regular ration and water.

3-Day Acclimation Period
After being grouped into their assigned triple cages, the mice were acclimated to the new groups and cages for 3 days before testing. Acclimation and testing took place in the same room where the mice were quarantined under the same temperature and lighting regime as during the quarantine period.

15-Day Feeding Trial
Day 1
On the first day of the 15-day, 2-choice, feeding trial, the regular diet was removed from all cages. Group I received the 0.0% control ration (OPP rat and mouse challenge diet) in 2 rectangular aluminum dishes (15.2 x 3.8 x 3.8 cm) each containing (in excess of 40 g) of the OPP rat and mouse challenge diet. One dish was labeled O and the other dish was labeled C. Groups II and III, received the 0.005% chlorophacinone wax baits, and a rectangular aluminum dish containing (in excess of 40 g) of the OPP rat and mouse challenge diet. Group IV was given the weathered 0.005% chlorophacinone wax bait blocks and one rectangular aluminum dish containing (in excess of 40 g) of the OPP rat and mouse challenge diet. All wax bait blocks were anchored to the bottom of the cage with wire. Trays were placed beneath all baits to catch spillage. The 0.005% chlorophacinone wax bait blocks were positioned on the left and the dishes containing the OPP rat and mouse challenge diet were placed on the right. All dishes were located at the front of the cages. Bottled water was always available to all animals and was positioned at the front of the cages equally distant from the bait dishes. The animal room was closed until Day 2 except for minimal and unobtrusive observation to monitor the animals. The
24-hour period from the time the bait was issued until its removal the next morning was considered Day 1 (March 22 to March 23, 1997).

**Day 2**

The following morning, the bait dishes and any spillage were removed to determine the gross weight to the nearest 0.01 g for both bait types. Sometimes the OPP rat and mouse challenge diet weighed more than it did upon presentation. This may be attributable to either moisture gain from humidity or urine contamination of the dish. In such cases bait weight was reported as a gain but was omitted when summing the total amount of bait consumed. Dishes were replenished with fresh bait to bring them back to their original weight. Any bait that was fouled with urine or feces, it was discarded and replaced. Bait positions were reversed (i.e., chlorophacinone bait blocks on the right, OPP rat and mouse challenge diet dishes on the left). Any mortalities were removed and weighed. The animal room was closed as described for Day 1.

**Days 3-15**

Days 3-15 were similar to Day 2, except the positions of the dishes were alternated daily. Any carcasses were removed and weighed.

**5-Day Post-Testing Feeding Period**

**Day 16**

On the morning of Day 16, after removing the baits and any bait spillage, all survivors were then placed on the OPP rat and mouse challenge diet for 5 days. All control mice and groups of surviving mice were offered one rectangular aluminum dish containing (in excess of 40 g) the OPP rat and mouse challenge diet. The position of the dish (labeled O) was alternated each day. Trays were placed beneath each cage to catch spillage. Bottled water was available at all times and was positioned in the center of the front of the cages. The animal room was closed until Day 17 except for minimal and unobtrusive observations, to monitor the animals.
Days 17-19
In the morning, the bait and any bait spillage were again removed and weighed, following the procedures outlined for Day 16 (5-day post-test feeding period). All dead mice were removed and weighed.

Day 20
In the morning, the bait and any spillage were removed and weighed. The survivors were weighed and then euthanatized with CO₂ and frozen until the carcasses could be incinerated.

Data Recorded During the Study
1. Daily consumption of bait from the bait dishes and bait blocks for each group of house mice.
2. Body weights of the house mice: (1) pre-treatment, (2) after death, or at the end of the study.
3. Dates of mortality, and euthanasia were recorded.

Amount and Proportion of Treated Bait (0.005\% Chlorophacinone Wax Bait Block) Consumed Compared to the OPP Rat and Mouse Challenge Diet
The EPA requires, upon completion of the 15-day study, that calculations be made to determine the proportion of the 0.005\% chlorophacinone wax bait block consumed compared to the consumption of the OPP rat and mouse challenge diet. The treated bait consumption must be 25\% of the total diet to be satisfactory.

Statistics
Bait consumption and body weights were totaled for each group, and a mean and standard deviation were computed. A student’s t-test was used to determined differences in bait consumption between the O and C dishes for male and female control white house mice (Group I).
RESULTS

Bait Analysis

0.01% Chlorophacinone Oat Groat Bait Analysis

Upon receipt at the NWRC, the 0.01% chlorophacinone oat groat bait was assayed by the Analytical Chemistry Unit using validation ACP Method 62A. The bait assayed at a mean of 0.0108% (Appendix IV).

0.005% Chlorophacinone Wax Bait Block Assays

After their manufacture, the 0.005% chlorophacinone oat groat wax baits were assayed by the Analytical Chemistry Unit using validation ACP Method 64B. The bait assayed at a mean (SD) of 0.00585% (0.0004) (Appendix V).

Mortality

No mortality occurred in the control group (Group I) throughout the 20-day study. Forty three of the 60 house mice (71.7%) exposed to the 0.005% chlorophacinone treated baits died during the test. There was little difference in total mortality among the chlorophacinone challenged groups based upon either sex or formulation. Mortality in the unweathered wax bait groups averaged 75% and 65% for the males and females, respectively. Mortality in the weathered wax bait was 80% and 70% for the males and females, respectively. However, it is interesting to note that all of the male mortalities occurred prior to Day 10. Five females or 25% of the total female mortality, occurred after Day 9 (Table 1; Appendix VI, Raw Data Summary 1).

Figure 1 presents the cumulative mortality curves for the three chlorophacinone treatment groups. Mortality in the two unweathered wax bait groups (Groups II and III) followed essentially the same curves, resulting in 70% mortality for Day 15. Exposure to weathered wax bait resulted in more rapid mortality and produced 75% mortality by Day 11.
Bait Consumption - 15-Day Feeding Trial

Control - Group I

Females

The daily bait consumption by the 10 female mice feeding on the OPP rat and mouse challenge averaged 33.30 g (28.91 g to 38.25 g) (Appendix VI, Raw Data Summary 2). From the O dish, total bait consumption for the 10 house mice was 238.29 g during the 15-day feeding period (Table 1). Mean (SD) daily bait consumption was 15.89 g (3.57). Daily bait consumption ranged from 9.28 g to 22.67 g.

The mice consumed slightly more bait from the C dish (t = 0.8430, df = 14, p = 0.4134), a total of 261.26 g during the 15-day feeding period (Table 1; Appendix VI, Raw Data Summary 2). Mean (SD) daily bait consumption was 17.42 g (3.59). Daily bait consumption ranged from 7.41 g to 21.79 g.

Males

The daily bait consumption by the 10 male mice feeding on the OPP rat and mouse challenge averaged 42.34 g (36.19 g - 49.55 g) (Appendix VI, Raw Data Summary 3). From the O dish, total bait consumption for the 10 house mice was 275.01 g during the 15-day feeding period (Table 1). Mean (SD) daily bait consumption was 18.33 g (3.10). Daily consumption ranged from 10.48 g to 22.32 g.

From the C dish, the mice consumed significantly more OPP rat and mouse challenge diet, a total of 360.11 g (t = 5.5244, df = 14, p = 0.000075) (Table 1; Appendix VI, Raw Data Summary 3). Mean (SD) daily bait consumption from the C dishes was 24.01 g (2.10). Daily bait consumption (C dishes) ranged from 19.59 g to 26.35 g.

Treated - Group II

Females

Bait consumption from the O dish on Day 1 was 28.18 g, and increased to 30.18 g on Day 2 (Appendix VI, Raw Data Summary 4). Consumption decreased slightly on Day 3 to 27.04 g, then declined daily until Day 7 when 13.84 g were consumed. From Days 8-15, bait
consumption ranged from 7.94 g to 14.45 g. Total bait consumption for the O dish was 228.06 g (Table 1). Mean (SD) daily bait consumption was 15.20 g (7.21).

From the wax bait block, the mice consumed 4.38 g on Day 1, increased their consumption slightly on Day 2 to 6.56 g, then decreased to 4.94 g on Day 3. Bait consumption dropped sharply on Day 4 to 2.60 g. Bait consumption from Days 5-15 ranged from 0.24 g to 1.49 g. Total bait consumption was 28.14 g (Table 1; Appendix VI, Raw Data Summary 4). Mean (SD) daily bait consumption was 1.88 g (1.97).

**Males**

Bait consumption from the O dish was 31.16 g on Day 1 and increased to 31.66 g on Day 2. Consumption decreased slightly to 31.31 g on Day 3, sharply to 22.06 g on Day 4, and 14.15 g on Day 5. On Days 6-15, bait consumption ranged from 5.35 g to 11.07 g. Total bait consumption for the O dish was 211.72 g (Table 1; Appendix VI, Raw Data Summary 5). The mean (SD) daily bait consumption was 14.11 g (9.85).

Bait consumption from the wax bait block was 5.96 g on Day 1, and increased to 7.66 g on Day 2. Consumption decreased to 7.59 g on Day 3 and to 2.13 g on Day 4. On Day 5, 1.18 g of bait were consumed. From Days 6-15, bait consumption ranged from 0.01 g to 0.55 g. Total bait consumption was 26.15 g (Table 1; Appendix VI, Raw Data Summary 5). Mean (SD) daily bait consumption was 1.74 g (2.84).

**Treated - Group III**

**Females**

Bait consumption from the O dish was 31.62 g on Day 1 and increased to 31.83 g on Day 2. Consumption decreased to 25.48 g on Day 3, and then daily until Day 8, when 7.67 g were consumed. On Days 9-15, bait consumption ranged from 7.67 g to 12.18 g. Total bait consumption for the O dish was 226.77 g (Table 1; Appendix VI, Raw Data Summary 6). Mean (SD) daily bait consumption was 15.12 g (8.42).

From the wax bait block, the mice consumed 3.53 g and 3.21 g on Days 1 and 2, respectively. Bait consumption increased on Day 3 to 4.93 g. The amount of bait consumed declined to 1.62 g on Day 4 and 1.77 on Day 5. Bait consumption from Days 6-15 ranged from
0.60 g to 1.56 g. Total bait consumption was 25.17 g (Table 1; Appendix VI, Raw Data Summary 6). Mean (SD) daily bait consumption was 1.68 g (1.25).

**Males**

Bait consumption from the O dish was 34.40 g on Day 1, and decreased to 32.73 g on Day 2. On Days 3 and 4, consumption decreased to 31.52 g and 25.35 g, respectively. On Day 5, consumption decreased to 18.43 g. From Days 6-15, bait consumption ranged from 8.51 g to 12.81 g. Total bait consumption from the O dish was 250.02 g (Table 1; Appendix VI, Raw Data Summary 7). Mean (SD) daily bait consumption was 16.67 g (9.42).

From the wax bait block, the mice consumed 3.72 g on Day 1, and increased their consumption on Days 2 and 3 to 5.64 g and 7.89 g, respectively. On Day 4, bait consumption decreased to 1.12 g. On Days 5-15, consumption ranged from 0.11 g to 1.26 g. Total bait consumption was 25.24 g (Table 1; Appendix VI, Raw Data Summary 7). Mean (SD) daily bait consumption was 1.68 g (2.28).

**Treated - Group IV**

**Females**

Bait consumption from the O dish was 29.80 g on Day 1, and increased to 27.82 g on Day 2. On Days 3 and 4, consumption further decreased to 23.59 g and 14.88 g, respectively. On Day 5, only 6.92 g were consumed. Bait consumption from Days 6-15 ranged from 4.41 g to 7.54 g. Total bait consumption from the O dish was 164.80 g (Table 1; Appendix VI, Raw Data Summary 8). Mean (SD) daily bait consumption was 10.99 g (8.73).

From the wax bait block, the mice consumed 4.74 g on Day 1, and increased their consumption on Day 2 to 8.07 g. On Days 3 and 4, bait consumption decreased to 6.58 g and 2.28 g on Days 3 and 4, respectively. From Days 5-15, consumption ranged from 0.08 g to 1.25 g. Total bait consumption from the T dish was 28.76 g (Table 1; Appendix VI, Raw Data Summary 8). The mean (SD) daily bait consumption was 1.92 g (2.49).

**Males**

Bait consumption from the O dish was 29.98 g on Day 1, and increased to 31.18 g on Day 2. On Day 3, consumption increased to 32.57 g. On Days 4 and 5, bait consumption decreased
to 21.48 g and 11.64 g, respectively. From Days 6-15, bait consumption ranged from 1.48 g to 9.71 g. Total bait consumption from the O dish was 175.51 g (Table 1; Appendix VI, Raw Data Summary 9). Mean (SD) daily bait consumption was 11.71 g (11.36).

From the wax bait block, the mice consumed 5.00 g on Day 1; bait consumption increased to 6.94 g and 7.64 g on Days 2 and 3, respectively. On Day 4, bait consumption decreased to 0.88 g. On Days 5-15, consumption ranged from 0.02 g to 0.82 g. Total bait consumption was 23.97 g (Table 1; Appendix VI, Raw Data Summary 9). Mean (SD) daily bait consumption was 1.60 g (2.62).

**Mean Bait Consumption Per Mouse Per Day**

Mean consumption of the 0.005% chlorophacinone wait bait block per mouse per day was plotted for the number of house mice alive for Groups II, III, and IV at each feeding day during the 15-day feeding trial (Figure 2).

On Day 1, initial toxic bait consumption per mouse per day for the females ranged from 0.35 g to 0.60 g; whereas, for the males it ranged from 0.37 g to 0.50 g. On Day 2, bait consumption increased for females in Groups II and IV, but declined from 0.35 g to 0.32 g per day per mouse for females in Group III. Bait consumption increased among the 3 groups of males, ranging from 0.56 g to 0.69 g per mouse. Maximum bait consumption per group occurred among females in Groups III and IV, and males Group II. On Day 3, the other 3 groups reached maximum consumption. One Day 4, for all 3 groups bait consumption declined to 0.29 g per mouse or less. On Days 5-15, the highest bait consumption for the 3 groups of females was 0.15 g (Group II), 0.37 g (Group IV), and 0.52 g (Group III), for males 0.16 g (Group IV), 0.27 g (Group II), and 0.42 g (Group III).

**Amount and Proportion of Treated Bait (0.005% Chlorophacinone Wax Bait Block) Consumed Compared to the OPP Rat and Mouse Challenge Diet**

The total amount of OPP rat and mouse challenge diet and 0.005% chlorophacinone wax bait consumed over the 15-day treatment period was used to determine the bait acceptance (Table 2). Dietary consumption of wax bait was calculated for males and females combined by
treatment and bait weathering groups. Unweathered wax bait accounted for approximately 10.3% of the diet in Groups II and III. Weathered wax bait accounted for 13.5% of the total food consumption in Group IV. When all treatment groups were combined, wax bait accounted for 11.1% of the total dietary consumption. The 11.1% total bait consumed does not meet the "amount" of bait acceptance (25%) to be satisfactory for EPA standards.

5-Day Post-treatment Bait Acceptance of the OPP Rat and Mouse Challenge Diet

All 20 house mice that survived in Group I and those 17 mice that survived in Group II (2 males, 4 females), Group III (3 males, 3 females), and Group IV (2 males, 3 females) were tested on the 5-day post-treatment bait acceptance period.

Control - Group I

Females
Total bait consumption of the 10 house mice during the 5-day post-treatment feeding period was 129.49 g, with a mean (SD) daily bait consumption of 25.90 g (10.02) (Table 3). Daily bait consumption ranged from 12.20 g to 35.69 g (Appendix VI, Raw Data Summary 10). All 10 female mice survived the 5-day post-treatment feeding period.

Males
Total bait consumption of the 10 house mice during the 5-day post-treatment feeding period was 168.75 g, with a mean (SD) daily bait consumption of 33.75 g (3.34) (Table 3). Daily bait consumption ranged from 29.81 g to 38.04 g (Appendix VI, Raw Data Summary 10). All 10 male mice survived the 5-day post-treatment feeding period.

Treated - Group II

Females
The total bait consumption of the 4 house mice during the 5-day post-treatment feeding period was 65.55 g, with a mean (SD) daily bait consumption of 13.11 g (2.42) (Table 3). Daily bait consumption ranged from 11.05 g to 16.07 g (Appendix VI, Raw Data Summary 10). All 4 female mice survived the 5-day post-treatment feeding period.
Males
The total bait consumption of the 2 house mice during the 5-day post-treatment feeding period was 48.39 g, with a mean (SD) daily bait consumption of 9.68 g (0.63) (Table 3). Daily bait consumption ranged from 8.85 g to 10.40 g (Appendix VI, Raw Data Summary 10). Both male mice survived the 5-day post-treatment feeding period.

Treated - Group III
Females
The total bait consumption of the 3 house mice during the 5-day post-treatment feeding period was 63.28 g, with a mean (SD) daily bait consumption of 12.66 g (2.59) (Table 3). Daily bait consumption ranged from 8.47 g to 15.11 g (Appendix VI, Raw Data Summary 11). All 3 female mice survived the 5-day post-treatment feeding period.

Males
The total bait consumption of the 3 house mice during the 5-day post-treatment feeding period was 59.60 g, with a mean (SD) daily bait consumption of 11.92 g (2.74) (Table 3). Daily bait consumption ranged from 8.85 g to 15.33 g (Appendix VI, Raw Data Summary 11). All 3 male mice survived the 5-day post-treatment feeding period.

Treated - Group IV
Females
The total bait consumption of the 3 house mice during the 5-day post-treatment feeding period was 37.04 g, with a mean (SD) daily bait consumption of 7.41 g (0.91) (Table 3). Daily bait consumption ranged from 6.18 g to 8.38 g (Appendix VI, Raw Data Summary 11). All 3 female mice survived the 5-day post-treatment feeding period.

Males
The total bait consumption of the 2 house mice during the 5-day post-treatment feeding period was 36.51 g, with a mean (SD) daily bait consumption of 7.30 g (1.87) (Table 3). Daily bait consumption ranged from 4.16 g to 8.68 g (Appendix VI, Raw Data Summary 11). Both male mice survived the 5-day post-treatment feeding period.
Body Weights Pre- and Post-treatment

Mice were also housed together in groups of 10 during the test. Individual mice could not be identified, prohibiting comparison of individual weights pre- and post-treatment. Instead, body weights were compared by group.

The mean pre-treatment body weights for the males in Groups I, II, III, and IV were within 1.9 g of one another, ranging from 211.5 g (Group II) to 213.4 g (Group IV). Whereas, the mean pre-treatment body weights of the females in Groups I, II, III, and IV were within 2.7 g of one another ranging from 213.7 g (Group III) to 216.4 g (Group I).

Control - Group I

Females

Pre-treatment, the 10 house mice weighed 216.4 g, with a mean (SD) of 21.64 g (SD 1.95) (Appendix V, Raw Data Summary 12). Post-treatment, the 10 house mice as a group weighed 238.0 g, with a mean (SD) of 23.80 g (2.04) (Table 4; Raw Data Summary 12). This represented a gain of 21.6 g between pre- and post-treatment group weights. Three of the 10 females post-treatment weighed more than the heaviest pre-treatment female mouse (25.4 g). The other seven females post-treatment weighed more than the lightest female pre-treatment (18.3 g).

Males

Pre-treatment, the 10 house mice weighed 212.6 g, with a mean (SD) of 21.26 g (SD 1.38) (Table 4; Appendix V, Raw Data Summary 12). Post-treatment, the 10 house mice as a group weighed 259.9 g, with a mean (SD) of 25.99 g (1.70) (Table 4; Appendix V, Raw Data Summary 12). This represented a gain of 47.3 g between pre- and post-treatment body weights. Nine of the 10 males post-treatment weighed more than the heaviest male pre-treatment (25.4 g). The tenth male weighed 22.7 g.

Treated - Group II

Females

Pre-treatment, the 10 house mice weighed 213.8 g, with a mean (SD) of 21.38 g (1.74) (Table 4; Appendix V, Raw Data Summary 12). Post-treatment, the 4 surviving house mice
weighed 92.2 g, with a mean (SD) of 23.05 g (1.48) (Table 4; Appendix V, Raw Data Summary 13). This represented a gain of 1.67 g between mean pre- and post-treatment body weights. Only one of the 4 mice weighed more than the heaviest female mouse pre-treatment (24.1 g). The remaining 3 mice weighed more than the lightest female mouse pre-treatment (18.3 g). The post-treatment body weight of those 6 mice that died was 112.54 g, with a mean (SD) of 18.76 g (4.50) (Table 4, Appendix VI, Raw Data Summary 14). This was a loss of 2.62 g between the pre- and post-treatment mean body weights. Pre-treatment, the lightest animal was 18.3 g, and only 2 of the 6 mice that died weighed less than the lightest animal.

**Males**

Pre-treatment, the 10 house mice weighed 211.5 g, with a mean (SD) of 21.15 g (1.93) (Table 4; Appendix VI, Raw Data Summary 12). Post-treatment, the 2 surviving house mice weighed 58.4 g, with a mean (SD) of 29.20 g (1.98) (Table 4; Appendix VI, Raw Data Summary 13). This represented a loss of 8.05 g between mean pre- and post-treatment body weights. The body weights of both animals were greater than the heaviest male pre-treatment (24.7 g). The post-treatment body weight of those 8 mice that died was 123.12 g, with a mean (SD) of 20.54 g (2.61) (Table 4; Appendix VI, Raw Data Summary 14). This was a loss of 0.61 g between pre- and post-treatment mean body weights. Pre-treatment, the lightest animal was 17.6 g, and 2 of the 8 mice that died weighed less than the lightest animal.

**Treated - Group III**

**Females**

Pre-treatment, the 10 house mice weighed 213.7 g, with a mean (SD) of 21.37 g (2.12) (Table 4; Appendix V, Raw Data Summary 12). Post-treatment, the 3 surviving house mice plus a survivor from Group IV weighed 19.5 g, 22.6 g, 21.7 g, and 19.1 g with a mean (SD) of 20.81 g (3.15). The mouse from Group IV was weighed and recorded with the wrong group. No statistics were compiled for this group. The post-treatment body weight of those 7 mice that died was 146.02 g, with a mean (SD) of 20.86 g (3.88) (Table 4; Appendix V, Raw Data Summary 12). This was a loss of 0.51 g between the mean pre- and post-treatment body weights. Pre-
treatment, the lightest animal was 18.3 g, and 3 of the 7 mice that died weighed less than the lightest animal.

**Males**

Pre-treatment, the 10 house mice weighed 213.0 g, with a mean (SD) of 21.30 g (1.59) (Table 4; Appendix V, Raw Data Summary 12). Post-treatment, the 3 surviving house mice weighed 79.9 g, with a mean (SD) of 26.63 g (3.81) (Table 4; Appendix V, Raw Data Summary 13). This represented a gain of 5.32 g between mean pre- and post-treatment body weights. Two of the 3 animals weighed more than the heaviest male pre-treatment. The third animal weighed more than the eighth heaviest animal pre-treatment. The post-treatment body weight of those 7 mice that died was 158.07 g, with a mean (SD) of 22.58 g (3.55) (Table 4; Appendix V, Raw Data Summary 14). This was a gain of 1.28 g between the mean pre- and post-treatment body weights. Pre-treatment, the lightest animal was 19.1 g, and 1 animal weighed less than the lightest animal. Individual body weights pre- and post-treatment are in Summaries 12-14.

**Treated - Group IV**

**Females**

Pre-treatment, the 10 house mice weighed 214.3 g, with a mean (SD) of 21.43 g (1.82) (Table 4; Appendix V, Raw Data Summary 12). Post-treatment, the 2 surviving house mice weighed 47.4 g, with a mean (SD) of 23.70 g (2.26) (Table 4; Appendix V, Raw Data Summary 13). This represented a gain of 2.27 g between mean pre- and post-treatment body weights. One animal weighed more than the heaviest female pre-treatment, and the second animal weighed more than the seventh heaviest female pre-treatment. The post-treatment body weight of those 7 mice that died was 144.28 g, with a mean (SD) of 20.61 g (2.12) (Table 4; Appendix V, Raw Data Summary 14). This was a loss of 0.82 g between the mean pre- and post-treatment body weights. Pre-treatment, the lightest animal was 18.6 g, and 2 animals weighed less than the lightest animal.
Males

Pre-treatment, the 10 house mice weighed 214.3 g, with a mean (SD) of 21.43 g (1.82) (Table 4; Appendix V, Raw Data Summary 12). Post-treatment, the 2 surviving house mice weighed 44.3 g, with a mean (SD) of 22.15 g (1.63) (Table 4; Appendix V, Raw Data Summary 13). This represented a gain of 0.72 g between mean pre- and post-treatment body weights. The post-treatment body weights of those 8 mice that died was 175.10 g, with a mean (SD) of 21.89 g (4.03) (Table 4; Appendix V, Raw Data Summary 14). This was a gain of 0.46 g between the pre- and post-treatment mean body weights. Pre-treatment, the lightest animal was 18.9 g, and 2 animals weighed less than the lightest animal. One animal weighed more than the heaviest male pre-treatment (24.5 g). The remaining 5 mice weighed between the lightest and heaviest mouse pre-treatment.

DISCUSSION

In this study, the house mice preferred the OPP rat and mouse challenge diet over the 0.005% chlorophacinone wax bait block. Among the 90 feeding days (15 days x 3 groups x 2 sexes) the consumption of the 0.005% wax baits on any one day never exceeded that of the OPP rat and mouse challenge diet. By the standard set by the EPA, the consumption of the 0.005% wax bait by house mice would be satisfactory if it exceeds 25% of the total amount of bait consumed. The house mice consumed a total of 1414.31 g of bait (both 0.005% wax baits and OPP rat and mouse challenge diet), of which 157.43 g or 11.1% was the 0.005% wax baits; therefore, consumption of the 0.005% wax baits was not considered satisfactory according to the EPA Standards for the "amount" of bait consumed.

Because 71.7% mortality occurred among the 60 male and female white mice feeding on the 0.005% chlorophacinone baits (Groups II, III, and IV) with no mortality occurring among the 20 control white mice (Group I), we reject the null hypothesis that stated that mortality will be the same for treated and control house mice feeding on the chlorophacinone baits. The 71.7% mortality among the white mice consuming the 0.005% chlorophacinone wax bait meets the 70%
minimum standard for mortality established by the EPA for verifying efficacy of rodenticides (U.S. EPA 1982).

The 17 mice that survived failed to consume a lethal dose of chlorophacinone. Whether or not they consumed any bait early in the study could not be determined as it was impossible to measure individual daily bait consumption in this study. After all mortalities were accounted for, we know the survivors in each Group consumed some toxic bait later in the 15-day feeding trial, but their overall quantity of bait consumption was low. For example, female survivors consumed the following: (1) Group II: 6 mice in 6 days (Days 9-14) consumed a total of 4.07 g or 0.68 g/day. One of these 6 mice died on Day 14, and the remaining 5 mice consumed 1.36 g on Day 15. (2) Group III: 3 mice in 3 days (Days 13-15) consumed a total of 3.95 g or 1.32 g/day. (3) Group IV: 3 mice in 3 days (Days 13-15) consumed a total of 2.76 g or 0.92 g/day. Male survivors consumed the following: (1) Group II - 2 males in 5 days (Days 11-15) consumed a total of 0.73 g or 0.15 g/day. (2) Group II - 3 males in 8 days (Days 8-15) consumed 4.93 g or 0.62 g/day. (3) Group IV - 3 males in 6 days (Days 10-15) consumed a total of 0.49 g in 6 days or 0.08 g/day. These data suggest bait avoidance may have been one factor to consider when explaining why 20 mice survived. Had the experimental design of the Standard House Mouse Anticoagulant Wax Block and Wax Pelle Laboratory Test Method (1.214) specified that the second dish for the control animals contain a wax bait block formulated with sham treated oat groats (oat groats with all ingredients except the toxicant) instead of the OPP rat and mouse challenge diet, the difference in consumption, if any, between the two different wax bait types could have been measured. This data would have given insight into any repellency by the addition of wax to the 0.01% oat groats.

Another factor to consider for the large number of survivors would be the concentration of the bait. In this study, the 0.01% chlorophacinone oat groat bait was diluted with the wax to form the 0.005% chlorophacinone wax bait block. Previous research at this laboratory with a 0.01% chlorophacinone oat groat bait fed to white house mice resulted in killing 37 (94.8%) of the 39 animals tested (McCann and Matschke 1999).

The white mice exhibited the following feeding behavior on the 0.005% chlorophacinone wax bait blocks. Consumption was not uniform throughout the 15-day feeding trial. The major
bait consumption occurred on the first 3 days of exposure to the bait. Bait consumption began to decline on Days 4 and 5 and by Days 6 through 15 the surviving mice were basically non-eaters. On Day 1, mice ingested 17.6% of the total amount of treated bait consumed. On Day 2, bait consumption continued to increase with 67.2% (cumulative) of the total amount of treated bait consumed. On Day 4, bait consumption declined sharply as a total of 16 mice had died. The cumulative total for Day 4 was 73.9%. On Day 5, a total of 21 mice had died, bait consumption further declined as 78.1% (cumulative) of the total amount of treated bait consumed. From Days 6 through 15, the remaining 21.9% (cumulative) of the total amount of treated bait had been consumed.

Our inability to compare individual post-treatment body weights of the house mice with their pre-treatment body weights has made interpretation of post-treatment body weight gain or loss difficult. As a group, both sexes in the control groups gained body weight during the 20-day feeding period. Whether or not all 20 mice gained weight is uncertain. The control female and male groups gained a total of 25.4 g and 43.5 g, respectively. Only 4 female control mice weighed more post-treatment than the heaviest control house mouse pre-treatment, and the 6 remaining house mice weighed less than the heaviest female house mouse pre-treatment. Six control male house mice weighed more post-treatment than the heaviest control mouse weighed pre-treatment. Three male house mice post-treatment weighed less than and one mouse equaled the heaviest male pre-treatment.

Among the mice that died in each group, their mean post-treatment body weights were less than the corresponding mean pre-treatment body weights in Group II (males and females), Group III (females), and Group IV (females). In Groups II, III, and IV females that died, there were 2, 3, and 2 female mice that weighed less than the lightest female mouse pre-treatment, respectively. None of the females that died weighed more than the heaviest female pre-treatment. In Groups II, III, and IV males that died, there were 2, 1, and 2 male mice that weighed less than the lightest mouse pre-treatment, respectively. One male in each of Groups II and III weighed more than the heaviest male pre-treatment.

Among the mice that survived in each group, their mean post-treatment body weights were greater than the corresponding mean pre-treatment body weights in 5 of the 6 groups. The
exception was the females in Group III where no statistics on body weights were available. In Groups II and IV females that survived, there were 1 and 1 female mice that weighed more than the heaviest mouse pre-treatment, respectively. In Groups II, III, and IV males that survived, there were 2, 3, and 0 males that weighed more than the heaviest male mouse pre-treatment, respectively. None of the male and female survivors weighed less than the lightest pre-treatment body weights.

The consumption of the wax bait in this study can be compared with domestic house mice consuming chlorophacinone oat groat bait (McCann and Matschke 1999). The experimental design in their study was similar, i.e., a 15-day, 2-choice, feeding trial, with the choices being the OPP rat and mouse challenge diet and a 0.01% chlorophacinone oat groat bait. The mice in this test also preferred the OPP challenge diet over the toxic oat groat bait, but 37 (95%) of the 39 mice tested consumed a lethal dose of chlorophacinone. The ratio of toxic bait to the OPP challenge diet consumed was 1:3.3%. The proportion of toxic bait acceptance by the white mice was 23.0%, a value that exceeds the proportional value of 11.1% recorded in this study.

Bait consumption and mortality data obtained from the weathered bait blocks does not suggest that this treatment neither increased or decreased overall bait acceptance or mortality among the mice in Group IV. Bait acceptance by the females in Group IV (weathered bait blocks) was the highest among the female treatment Groups. The females in Group IV consumed 28.76 g of the wax bait blocks compared to 28.14 g and 25.7 g by females in Groups II and III, respectively. The males in Group IV (weathered bait blocks) consumed the lowest among the male treatment Groups. The males in Group IV consumed 23.97 g of the bait blocks, compared to 26.15 g and 25.14 g for males in Group II and III, respectively. Fifteen mice (7 females, 8 males) died in Group II and 14 mice (7 females, 7 males) in Group III.

Summary of Conclusions
1. The 71.7% house mouse mortality barely exceeded the minimum 70% standard established by the EPA for verifying efficacy of rodenticides.
2. The percentage of toxic bait consumed (8.16%) compared to total bait consumption did not meet the minimum 25% standard set by the EPA.
REFERENCES


Table 1. Summary of mortality and bait consumption of house mice during the 15-day, 0.005% chlorophacinone wax bait block, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. dead over sample size (% Mortality)</th>
<th>Summary</th>
<th>Bait consumption (g) Days 1-9</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>O^a</td>
</tr>
<tr>
<td>Control Group I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0/10 (0.0%)</td>
<td>Sum</td>
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<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>15.89</td>
</tr>
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<td></td>
<td></td>
<td>SD</td>
<td>3.57</td>
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<td>M</td>
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<td>Sum</td>
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</tr>
<tr>
<td></td>
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<td>Mean</td>
<td>18.33</td>
</tr>
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<td></td>
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<td>SD</td>
<td>3.10</td>
</tr>
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<td>0.005% Chlorophacinone Group II</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>6/10 (60%)</td>
<td>Sum</td>
<td>228.06</td>
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<tr>
<td></td>
<td></td>
<td>Mean</td>
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</tr>
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<td></td>
<td></td>
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</tr>
<tr>
<td>M</td>
<td>8/10 (80%)</td>
<td>Sum</td>
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<tr>
<td></td>
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<td>SD</td>
<td>9.85</td>
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Table 1. Continued.

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<th>Sex</th>
<th>No. dead over sample size (% Mortality)</th>
<th>Summary</th>
<th>Bait consumption (g)</th>
<th>O^a</th>
<th>Wax bait block</th>
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<tr>
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<td>0.005% Chlorophacinone Group III</td>
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<tr>
<td>F</td>
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<td>Sum</td>
<td>226.77</td>
<td>25.17</td>
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<td>SD</td>
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<td>1.25</td>
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<td>M</td>
<td>7/10 (70%)</td>
<td>Sum</td>
<td>250.02</td>
<td>25.24</td>
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<td>Mean</td>
<td>16.67</td>
<td>1.68</td>
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<tr>
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<td></td>
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<td>0.005% Chlorophacinone Group IV</td>
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</tr>
<tr>
<td>F</td>
<td>7/10 (70%)</td>
<td>Sum</td>
<td>164.80</td>
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<td>Mean</td>
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<td>SD</td>
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<tr>
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<td>Sum</td>
<td>175.51</td>
<td>23.97</td>
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<td></td>
<td></td>
<td>Mean</td>
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<td></td>
<td>SD</td>
<td>11.36</td>
<td>2.62</td>
<td></td>
</tr>
</tbody>
</table>

^aDish contained the OPP rat and mouse challenge diet.
^bDish also contained the OPP rat and mouse challenge diet.
^cDish contained the 0.005% chlorophacinone wax bait block.
Table 2. Amount and proportion of treated bait (0.005% chlorophacinone wax block) consumed compared to the OPP rat and mouse challenge diet during the 15-day feeding trial.

<table>
<thead>
<tr>
<th></th>
<th>Total Bait Consumption (g)</th>
<th>Percent of 0.005% Bait in Total Diet</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>OPP Diet</td>
<td>0.005% Bait</td>
</tr>
<tr>
<td>Females</td>
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<td></td>
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<tr>
<td>Group II</td>
<td>228.06</td>
<td>28.14</td>
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<tr>
<td>Group III</td>
<td>226.77</td>
<td>25.17</td>
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<td>Group IV</td>
<td>164.80</td>
<td>28.76</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>211.72</td>
<td>26.15</td>
</tr>
<tr>
<td>Group III</td>
<td>250.02</td>
<td>25.24</td>
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<tr>
<td>Group IV</td>
<td>175.51</td>
<td>23.97</td>
</tr>
<tr>
<td>Total</td>
<td>1,256.88</td>
<td>157.43</td>
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</table>

Proportion of treated bait in diet = \( \frac{157.43 \text{ g}}{1,414.31 \text{ g}} = 0.111 \) (11.1%)
Table 3. Summary of consumption of the OPP rat and mouse challenge diet by surviving house mice during the 5-day post-treatment feeding period.

<table>
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<th>Statistics</th>
<th>Bait consumption (g)</th>
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<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td><strong>Group I</strong></td>
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<tr>
<td>Sum (g)</td>
<td>129.49</td>
<td>168.75</td>
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<tr>
<td>Mean</td>
<td>25.90</td>
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<td>SD</td>
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<tr>
<td><strong>Group II</strong></td>
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<td>Sum (g)</td>
<td>65.55</td>
<td>48.39</td>
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<td>Mean</td>
<td>13.11</td>
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<td>SD</td>
<td>2.42</td>
<td>0.63</td>
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<tr>
<td><strong>Group III</strong></td>
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<tr>
<td>Sum (g)</td>
<td>63.28</td>
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<tr>
<td>Mean</td>
<td>12.66</td>
<td>11.92</td>
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<td>SD</td>
<td>2.59</td>
<td>2.74</td>
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<td><strong>Group IV</strong></td>
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<td>Sum (g)</td>
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<td>Mean</td>
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<td>SD</td>
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Table 4. Summary of pre- and post-treatment body weights of house mice.

<table>
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<tr>
<th>Sex</th>
<th>Summary</th>
<th>Pre-treatment body weight (g)</th>
<th>Post-treatment body weight (g)</th>
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<tr>
<td></td>
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<td>Survivors</td>
<td>Mortality</td>
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<td>F</td>
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<td></td>
<td>SD</td>
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<td>M</td>
<td>Sum</td>
<td>216.4</td>
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<td></td>
<td>Mean</td>
<td>21.64</td>
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<tr>
<td></td>
<td>SD</td>
<td>1.95</td>
<td>1.70</td>
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<td>0.005% Chlorophacinone Group II</td>
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<tr>
<td>F</td>
<td>Sum</td>
<td>211.5</td>
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<tr>
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<td>Mean</td>
<td>21.15</td>
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<td></td>
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<tr>
<td>0.005% Chlorophacinone Group III</td>
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<tr>
<td>F</td>
<td>Sum</td>
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<td>82.9*</td>
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<td>SD</td>
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<td>0.005% Chlorophacinone Group IV</td>
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<td>F</td>
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<tr>
<td>M</td>
<td>Sum</td>
<td>214.3</td>
<td>44.3</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>21.43</td>
<td>22.15</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.82</td>
<td>1.63</td>
</tr>
</tbody>
</table>

* Four female mice were weighed and recorded post-treatment. The extra mouse is from Group IV females.
** Only two female mice were weighed and recorded post-treatment.
FIGURES
Figure 1. Cumulative mortality of house mice (♀ and ♂ combined) during the 15-day 0.005% chlorophacinone concentration feeding trial and 5-day post-testing feeding period. Group I was the control. Groups II and III were exposed to unweathered bait. Group IV was exposed to weathered bait.
Figure 2. Mean daily consumption per mouse per day for house mice by Groups (0.005% chlorophacinone wax bait block) during the 15-day, 2-choice, feeding trial.
Appendix I. 0.005% Chlorophacinone® Wax Bait Block Label
1. Scope

1.1 This test method is designed to determine effectiveness of wax block and wax pellet anticoagulant rodenticide products used for mouse control in wet or damp environments. It is applicable in connection with registration and enforcement procedures under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended.

2. Test Animals

2.1 All mice used in this test shall be house mice (Mus musculus), either wild caught or from a wild mouse colony, or albinos (Swiss-Webster strain preferred). They shall be healthy, active, sexually mature, and shall fall within the following weight classes in grams within seven days prior to start of test:

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Maximum acceptable differences in average weights between sexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory mice</td>
<td>15</td>
<td>.35</td>
<td>5</td>
</tr>
<tr>
<td>House mice</td>
<td>10</td>
<td>.25</td>
<td>3</td>
</tr>
</tbody>
</table>

2.2 Ectoparasite control with appropriate concentrations of carbaryl, malathion or pyrethrum dusts is permissible if applied externally to both test and control animals not less than seven days prior to start of test.

3. Apparatus

3.1 The mice should be placed in solid-bottomed all-metal cages designed to hold laboratory mice and having a bottom surface area of 1500 to 2000 cm² (1.61 to 2.15 ft²).

3.2 Provide shelters in both the test and control cages. Empty soup or beverage cans, with one end removed, slightly flattened to prevent rolling, have been found satisfactory for this purpose. Use one can per five mice.

3.3 Metal or ceramic feeders, designed so that test mice may not nestle or wallow in diet, should be used.

4. Pretest Holding Conditions

4.1 All mice used in this test method must be held, sexes separate, for observation in the laboratory for a period of at least one and not more than four weeks prior to testing, the last seven days of which shall be under laboratory conditions (i.e., temperature, humidity, lighting, etc.) comparable to those of the animal testing room if not actually in the
OPP 1.214

Testing room. The test animals must NOT be fasted prior to testing. Water and a commercial mouse diet must be available to them at all times. Do NOT use the standard OPP rat and mouse challenge diet for pretest feeding.

5. Holding and Test Conditions

5.1 Temperature
20 to 25°C. Strong air currents from heaters or air conditioners shall not blow directly onto test animals.

Relative humidity
50 to 55%

Light
12 h artificial light per day, not to exceed 2153 lx (200 ft candles) at cage location. Total reversing of the natural photoperiods of the test animals by timed lighting is not recommended.

5.2 The standard OPP rat and mouse challenge diet shall be composed of:

- Cornmeal (whole yellow ground corn) 65% by weight
- Rolled oat groats (ground) 25% by weight
- Sugar (10X powdered or confectioners, 95% purity) 5% by weight
- Corn oil (95% purity) 5% by weight

Combine dry ingredients together, add oil, and thoroughly mix. Be certain the mixing utensils are clean of contamination before preparing diet.

5.2.1 The whole (not degeminated) yellow ground corn shall be from the most recently available crop and be reasonably fresh ground. Seventy-five percent (+5%) shall pass through a No. 10 screen (10 meshes to the inch or 2.54 cm) and 50% (+10%) be retained by a No. 20 screen (20 meshes to the inch). The remainder may be either larger or smaller than the screens mentioned.

5.2.2 The oats shall be steam rolled oat groats (oat seed with the hulls removed) coarsely ground after the rolling process. Seventy-five percent (+5%) of the ground oats shall pass through a No. 5 screen (5 meshes to the inch) and 50% (+10%) be retained by a No. 20 screen (20 meshes to the inch). The remainder may be either larger or smaller than the screens mentioned.

5.2.3 The corn oil shall be of the type available as cooking oil, undiluted with other oils, and shall not be rancid.
5.2.4 The standard OPP rat and mouse challenge diet may be stored under refrigeration if it is to be used within three days of preparation. If it is to be held for longer periods the diet shall be packaged in plastic containers (2.2 to 4.5 kg (5 to 10 lb) per container), tightly closed or sealed, and maintained at -18°C or below until it is to be used. It shall be at room temperature when offered to test or control animals. Challenge diets shall not be prepared and stored for longer than six months.

6. Procedure

6.1 A test group consists of a minimum of 20 mice (10 males, 10 females), group caged. Include one untreated control test group of 20 mice (10 males, 10 females), group caged, in each test. If a series of tests is being conducted at the same time on the same species, only one untreated control test group need be included. Acclimate all animals to test conditions for three days prior to exposure to toxicant, immediately following pretest holding period (4.1).

6.2 Water must be available to each animal at all times. Glass water bottles equipped with ball-type watering tubes are recommended. Gravity fed automatic or open-cup type waterers are not recommended.

6.3 The rodenticide and the standard OPP rat and mouse challenge diet are each offered to the test mice in separate containers (3.3) on opposite sides of the cage to provide in excess of the daily food requirement of two grams per animal per day, minimum. The control group is offered only the OPP rat and mouse challenge diet. The gross weight of each container and its contained food are determined daily and either (1) returned to starting weight by addition of the given food or (2) left at the gross weight as long as there is adequate food remaining in excess of the daily food requirement. If food becomes fouled by urine or feces, replace food in each container. Record each day the quantity of each food consumed during the preceding 24 h. Weighing accuracy must be at least to the nearest 0.5 gram. Spilled food should be recovered and weighed to establish exact food consumption data. Where the food spillage is damp it shall be dried to approximately its original moisture content before weighing.

6.4 Reverse the position of the bait and standard OPP rat and mouse challenge diet containers in the cages every 24 h to counter any feeding position preference of the mice. The test mice must have a free choice between treated and untreated food.

6.5 Animals on test should not be subjected to undue or unnecessary stress from noise or human activities (i.e., movement). Human activity within the animal test room shall be minimal.

7. Test Period

7.1 Maintain test period for 15 days, unless a 100% mortality of test mice is recorded prior to that time.

7.2 Remove dead mice daily, or more frequently as observed.
OPP 1.214

7.3 Remove toxicant-treated food at the end of the 15-day test period, leaving and maintaining the untreated food.

7.4 More than a 10% mortality in the control group negates the test, even if a 100% mortality had been achieved in the test group.

7.5 This laboratory efficacy test should be replicated at least once with a diet that has been subjected to 90- to 100% humidity and about 100°F for about 15 days.

8. Test Period Follow-Up

8.1 Maintain observation on surviving mice for a minimum of five days following test period.

8.2 Continue feeding OPP rat and mouse challenge diet.

8.3 Describe unusual activities of test and control mice in report of test and posttest periods.

9. Calculation and Evaluation of Results

9.1 Record date, weight, and sex of each mouse dying during the test and of survivors in both the test and control groups, and amount of treated and untreated food consumed during the test and posttest periods. Retain original laboratory test records for future reference. Grouped data averages should be used for efficacy evaluation.

9.2 The product is considered satisfactory if a minimum of 25% of the food consumed had been treated with the toxicant and if a minimum mortality of 80% is obtained in test animals.
Appendix III. Study Protocol QA-506, Amendments, and SOP’s
NATIONAL WILDLIFE RESEARCH CENTER
Animal Damage Control
Animal and Plant Health Inspection Service
United States Department of Agriculture
1716 Heath Parkway
Fort Collins, CO 80524

STUDY PROTOCOL

I. STUDY PROTOCOL TITLE:
Chlorophacinone and Diphacinone: Standard Mouse Anticoagulant Dry Bait Laboratory Tests

II. SPONSOR:
California Department of Food and Agriculture (CDFA)
USDA/APHIS/ADC/DWRC/NWRC

III. STUDY DIRECTOR AND PARTICIPANTS:
Geraldine R. McCann* and George H. Matschke (Supervisor)

IV. OBJECTIVE/HYPOTHESES:
Our objective is to determine the efficacy of chlorophacinone and diphacinone grain baits for controlling house mice (Mus musculus). We will test the following null hypotheses (Ho): mortality will be the same for treated and control house mice feeding on the diphacinone baits and mortality will be the same for treated and control house mice feeding on the chlorophacinone baits.

* Study Director
V. **JUSTIFICATION AND BACKGROUND:**

The CDFA has entered into an agreement with the USDA/APHIS/ADC/DWRC/NWRC/Ecological Effects Unit to conduct this laboratory study. This study will evaluate the efficacy of chlorophacinone and diphacinone baits against laboratory mice as a partial fulfillment for the reregistration of chlorophacinone and diphacinone commensal rodent labels. The study will be conducted in compliance with Code of Federal Regulations (CFR) 40, Part 160, Good Laboratory Practices (EPA 1993) and the appropriate Pesticide Assessment Guidelines (PAG), Subdivision G (EPA 1982).

VI. **ANIMAL CARE AND USE:**

A. **Where applicable, the number, body weight range, sex, source of supply, species, strain, substrain, and age of the test system.**

Sixty-six sexually mature house mice (*Mus musculus*) of the Swiss-Webster genotype (33M:33F) weighing between 15-35 g will be purchased from Simonson Laboratories, Inc., 1180 Day Road, No. C, Gilroy, CA 95020.

B. **The procedure for identification of the test system:**

The cage of each animal group will be given a unique number (SOP WRC-216).

C. **Identification of chemicals with chemical abstract number (CAS), materials, or devices to be used or tested:**

1. **Chemical:** Chlorophacinone (CAS No. 3691-35-8) and diphacinone (CAS No. 82-66-6)
2. **Material:** Steam rolled crimped oat groats (squirrel type thickness)
3. **Device:** None

D. **Rationale for involving animals, the appropriateness of the species, and the number of animals to be used.**

Animals are required for testing because no alternative system exists that would test the efficacy of the chlorophacinone and diphacinone baits. The species and the number of animals to be tested have been specified by the EPA [Guideline Reference Number (GRN) 96-10].

E. **Source:**

See VI-A.

F. **Trapping:**

NA

G. **Handling/Restraint:**

See SOP WRC-528.
H. **Transport:**
Simonson Laboratories, Inc., will ship the house mice via air to the Denver International Airport then Animal Care will ground transport them to the NWRC/ARB (SOP WRC-294.R2) (SOP WRC-395.R1).

I. **Housing/Maintenance/Diet:**
The house mice will be housed by sex in groups in stainless steel cages (70.8 × 24.1 × 17.8 cm) where they will be fed a rodent laboratory chow diet. Water will be available at all times. They will be maintained per SOP WRC-528.

J. **Quarantine:**
The mice will be held a minimum of 7 days in quarantine (SOP WRC-232.R1).

K. **Euthanasia:**
Survivors at the end of the study will be euthanized with CO₂ gas (SOP WRC-128.R5).

L. **Disposition of Animals:**
All animals will be incinerated upon conclusion of the study (SOP WRC-233.R3 and WRC-436).

M. **Provide written assurance that the activities do not unnecessarily duplicate previous experiments. This must illustrate a good faith on the part of the researcher to find if this experiment duplicates previous experiments.**
In April 1996, the Denver Wildlife Research Center (DWRC) library conducted a literature review, searching the following 17 databases for articles on chlorophacinone or diphacinone on house mice (*Mus musculus*):
- Biosis Previews (R). 1969-1996/Mar W4
- CAB Abstracts. 1972-1996/Feb
- Pascal. 1973-1996/Mar
- Medline (R). 1966-1996/May W4
- CA Search (R). 1967-1996/UD =12414
- Agricola. 1970-1996/Apr
- AGRIS International. 1974-1995/Dec
- World Transl. Index 1979-1996/Feb
- Oceanic Abst. 1964-1996/Apr
- Aquatic Science Abstracts 1979-1996/Apr
- CRIS/USDA 1996/Feb
- Water Resour. Abs. 1967-1996/Feb
- GEOBASE (TM) 1980-1996/Mar
- Pesticide Fact File 1995
A total of 6 published articles on anticoagulants were located; 2 on chlorophacinone, 3 on diphacinone, and 1 article included both compounds. All 6 of the articles were received and reviewed including one in French that was translated but was not applicable to this study.

**Chlorophacinone** - Under laboratory conditions, Lund (1971) fed 0.025% chlorophacinone oat groats bait to groups of 20 mice for each test. His tests consisted of an array of feeding periods varying from 1 day to 21 days. When the feeding period was 1 to 5 days, mouse mortality was 5 to 75%; whereas, when the feeding period was increased to 6, 10 and 21 days, mortality was 80, 90, and 95% respectively.

**Diphacinone** - Marshall (1981), in a laboratory situation, fed 3 differently prepared 0.005% diphacinone wax baits to house mice. Twenty mice individually housed were placed on each of the 3 bait types. The wax baits and a non-toxic alternative diet were fed for 15 days. The percentage of wax baits accepted by the house mice varied greatly, 3.0%, 17.4%, and 38.6%. The highest house mouse mortality, 100%, occurred on the wax bait having 38.6% acceptance. The other 2 wax baits both had 85% mortality.

Advani (1992) evaluated the efficacy of 0.005% diphacinone wax bait blocks on house mice in apartment buildings located in New York City, NY. He monitored the mouse population pretreatment before placing the bait blocks for 4 monthly treatments. Monthly, the mouse population was monitored 7 to 10 days after placement of the baits. The highest percent control (77.7%) occurred after 4 months when comparing the difference between pre-control census (October) and the post-treatment census (December).

Arends et al. (1984) reported that no separate baiting periods of diphacinone pellet bait (concentration not given) were required to achieve greater than 70% mortality among house mice inhabiting chicken houses on a broiler breeder farm. The first baiting period began after determining the number of active mouse burrow systems. Six bait applications occurred (43g/10 active burrow systems), with a bait application every other day. According to a posttreatment census, the number of active burrows declined only 32.7%. A second baiting period was then conducted, with 4 bait applications, one every other day. The second posttreatment census showed the number of active burrows declined 74.8%. Caching of the bait by the mice may explain the low mortality during the first baiting period.

Chlorophacinone - Diphacinone - Rowe and Redfern (1968) measured mortality and bait acceptance among house mice given both chlorophacinone and diphacinone oat baits. Four no-choice tests were conducted, each one lasting either 3, 7, 14, or 18 days. The concentrations tested were as follows: chlorophacinone 0.005% and
0.025% and diphenacinone 0.0125% and 0.025%. Mortality was as follows: for the 3 day tests: less than 50% of the animals died on all four concentrations; for the 7 day test, 100% mortality occurred on the 0.025% chlorophacinone concentration, but 50 to 80% mortality occurred at the 3 other concentrations. For the 14 day test, 100% mortality occurred again on the 0.025% chlorophacinone, and the mortality for the other three concentrations increased to 80-90%. For the 18 day test only the 0.0125% diphenacinone concentration failed to achieve 100% mortality. On this concentration, 2 of the mice survived and consumed a total of 255 and 466 mg/kg of diphenacinone. In the bait acceptance study, the mice were placed on test for 2 days and given a two-choice test. They received untreated pinhead oatmeal in addition to one of the 4 toxic concentrations. For both toxicants, the mice on the 0.025% concentration consumed significantly less (p < 0.001) bait than the untreated bait. No significant difference (p < 0.005) occurred between the consumption of either of the two lower concentrations (0.0125% or 0.05%) or the untreated oat bait.

After reviewing these articles none would support the reregistration requirements established by the EPA for maintaining the CDFA’s chlorophacinone and diphenacinone labels for controlling house mice because none of these publications reported on two-choice feeding tests where either chlorophacinone or diphenacinone were offered at the 0.01% concentration along with the EPA challenge diet to house mice.

N. In regard to potential pain of animals for this experiment, provide written statements addressing each area:

1. That you have considered alternatives to any painful procedures and, if unavailable, you have indicated the principal sources that have been consulted in considering the alternatives (e.g., Biological Abstracts, the Animal Welfare Information Center).

Domestic house mice need to ingest lethal doses of chlorophacinone or diphenacinone to provide the required efficacy data. Analgesics are unacceptable due to the possible distortion of normal bait ingestion and interference with chlorophacinone and diphenacinone’s mode of action and could subsequently affect the toxicity data in this species. With anticoagulants, it would appear reasonable that more than slight pain would be expected in this study.
2. When more than slight pain is reasonably expected, and sedatives or analgesics will not be used, the reasons for this procedure are scientifically justified. 

More than slight pain may be expected, but sedatives or analgesics will not be used because they could alter the toxicity of the chlorophacinone and diphenacine on the test species.

3. That procedures, which cause more than slight or momentary pain, MUST involve during the planning phase a consultation with the attending veterinarian of the Denver Wildlife Research Center. 

A discussion between the Study Director and the DWRC Veterinarian occurred on June 20, 1996, regarding care and welfare of the house mice.

DWRC Veterinarian: [Signature] 
Initials: [Signature] Date: [Signature]

4. If the animals experience severe or chronic pain that cannot be relieved, they will be euthanatized at the end of the procedure or, if appropriate, during the procedure.

If any animals appear to experience severe or chronic pain they will be euthanatized. The decision for euthanasia will be made by the Center veterinarian. All survivors will be euthanatized on Day 20 (SOP WRC-128.R5).

VII. METHODS:

A. Protocol: 

The protocol for this study has been outlined by the EPA in their Standard House Mouse Anticoagulant Dry Bait Laboratory Test Method from the Office of Pesticides Programs (OPP Designation: 1.204) (EPA, 1982).

1. Pretest Holding Conditions

All mice used in this test method must be held, sexes separate, for observation in the laboratory for a period of at least 1 week and not more than 4 weeks prior to testing. They will be under laboratory conditions (i.e., temperature, humidity, lighting, etc.) comparable to those of the animal testing room - if not actually in the testing room. The test animals will NOT be fasted prior to testing. Water and a commercial mouse diet must be available to them at all times. The standard OPP rat and mouse challenge diet will NOT be used during the quarantine period or pretesting period.
2. Pretreatment Procedure

Following the quarantine period, 60 animals (30M:30F) will be randomly selected from the total population using a computer generated random number program (SOP WRC-368.R1). These animals will be weighed and the average body weight of the two sexes will be calculated, using 5 g as the maximum acceptable weight difference between the sexes. If an acceptable difference in body weights exists between the sexes at that time, the mice of each sex will be ranked by weight into 10 weight classes, each containing 3 animals (SOP WRC-59.R3). The groups of ten mice (sexes separate) will be randomly assigned to one of the two concentrations (0.01% or 0.00%) for a total of 10 animals per sex per each concentration, or a total of 20 animals per each concentration. The chlorophacinone and diphacinone will not be tested simultaneously.

Day 1 of Testing

On the first day of testing, the 15-day two-choice feeding study will begin by removing the regular diet. Each group of 10 mice will then be offered two dishes, which have been placed on opposite sides of the cage. Every group receiving the toxicant diet will be given one dish containing in excess of 20 g of the 0.01% chlorophacinone or diphacinone grain bait and a second dish containing an excess of 20 g of the standard OPP rat and mouse challenge diet. The two dishes will be of equal weight. Each group of mice receiving the control diet will be given two 20 g dishes of the standard OPP rat and mouse challenge diet. On Day 1, the toxic dishes will be positioned on the left, and the challenge dishes will be placed on the right in the front of the cage. EPA does not require that separate test dishes measuring moisture gain or loss be used. Bottles of water will be available to all animals. The bottle will be positioned at equal distances from the feed dishes. The animal room will be closed until Day-2 except for minimal and unobtrusive observations of the animals as conditions permit to monitor for animals experiencing pain.

Day 2

The dishes will be removed to determine the gross weight to the nearest 0.5 g of each container and its contents. At that time fresh bait will be added to the dishes and the dish weighed. If food becomes fouled by urine or feces, it will be discarded and replaced. The position of the dishes will be reversed when replaced in the cages; i.e., toxic dishes will be placed on the right and the challenge dishes will be set on the left in the front of the cage. The animal rooms will be closed and not reentered until Day 3.
Days 3-15

Days 3-15 will be a repeat of Day 2, except the position of dishes will be reversed daily. Animals will be checked for mortality and dead animals will be removed and weighed.

3. Posttesting Period

Day 16

Animals will be checked for mortality and the dishes will be removed and the remaining bait will be weighed. Each survivor on the chloropacnione or diphacinone treatment will be given one 20-g dish of the challenge diet.

Days 17-19

Animals will be checked daily for mortality, the dishes will be removed, and the remaining bait will be weighed, and fresh bait added.

Day 20

All dishes will be removed, and the remaining bait will be weighed. The survivors will be euthanized with CO₂ (SOP WRC-128.R5).

B. Analytical Chemistry: Briefly summarize the analytical chemistry portion of the experimental design.

The chloropacnione and diphacinone grain baits will be assayed by DWRC's Analytical Chemistry Unit. Chemists will use validated Method 62A for both chloropacnione and diphacinone.

Analytical Chemistry Project Leader: __________________________ 7/2/96

C. Bait Formulation:

The bait will be formulated by the Rodent Control Outfitters of Junction City, OR. The baits will be prepared according to the Confidential Statement of Formula for the 0.01% chloropacnione and 0.01% diphacinone oat baits.

The standard OPP rat and mouse challenge diet shall have the following composition: % (by weight)

- Cornmeal (whole yellow ground corn) 65
- Rolled oat groats (ground) 25
- Sugar (10X powdered or confectioners, 95% + purity) 5
- Corn oil (95% + purity) 5

Combine dry ingredients, add oil, and mix thoroughly.
After formulation, the OPP rat and mouse challenge diet will be packaged in plastic containers, tightly sealed, and maintained at -18°C or below until it is to be used.

When offered to the test or control animals the challenge diet and the test bait will be at room temperature.

D. Location of Work:
All research will be conducted at the NWRC/ARB facilities, Colorado State University Campus, Fort Collins, CO 80524-2719

E. Cooperators and Consultants:
USDA/APHIS/ADC/DWRC/NWRC/BEU
California Department of Food and Agriculture

F. Related Study Protocols:
None

G. Justification for selection of the test systems:
See VI-D.

H. A description and/or identification of the diet used in the study as well as solvents, emulsifiers, and/or other materials used to dissolve or suspend the test or control substances before mixing with the carrier. The description shall include specifications for acceptable levels of contaminants that are reasonably expected to be present in the dietary materials and are known to be capable of interfering with the purpose or conduct of the study if present at levels greater than established by the specifications. An oil will be added to the oats as an adhesive for the technical chlorophaeinone and diphaeinone. The technical chlorophaeinone or diphaeinone will not be dissolved or suspended in any substance before they are applied directly to the oats treated with the oil adhesive.

I. The route of administration and the reason for its choice:
The route of administration will be orally by an oat groat bait. Grain baits treated with chlorophaeinone or diphaeinone will be used as if applied in an operational control program.

J. Each dosage level, expressed in milligrams per kilogram of body weight or other appropriate units of the test or control substances to be administered, and the method and frequency of administration.
The 0.01% chlorophaeinone and diphaeinone concentrations will be assayed. The mg/kg intake will depend upon the quantity of bait consumed by each mouse. The bait will be placed in metal dishes, from which the mice will be fed for 15 days.
K. A description of the experimental design including the method for the control of bias: Animals will be randomly selected for testing. Bias will be controlled by randomly assigning each group of mice to one of the 3 different treatments (0.0%, 0.01% chlorophaeinone or diphacinone, and 0.01% chlorophaeinone or diphacinone) (SOP WRC-59.R3).

L. Statistical Analyses:

1) A 3-factor repeated measures ANOVA would compare treatment, sex, and days on test with days being the repeated factor in the analysis.

2) A 3-factor repeated measures ANOVA would compare consumption of control bait for treatment and sex groups, until the first mortality occurs.

3) A 3-factor repeated measures ANOVA would be conducted on the proportion of total consumption that was represented by the treated bait.

4) Contingency table methods will be used to compare overall survival between the treated groups and control groups for both sexes.

5) Kaplan-Meier survival curves will be used to compare treatment (diphacinone or chlorophaeinone) sex groups if appropriate.

M. Environmental Conditions of the Study:
Temperature of the animal rooms will be recorded daily
Light (12 h light:12 h darkness cycle)

N. Accountability of the Test Substance:
All bait concentrations will be under control of personnel from the Biological and Chemical Effects Unit of NWRC's Product Development Section. Just before testing, these baits will be forwarded to the Ecological Effects Unit, which will maintain the chlorophaeinone or diphacinone bait in a locked storage cabinet at ambient temperature until disposal. The quantity of the bait types used each day will be recorded on end use product chain of custody forms (SOP A19R.2). The EPA Challenge diet will be maintained under refrigeration at -18°C.
O. The Records to be Maintained:
- Purchase of mice
- Shipping of mice
- Quarantine animal care records
- Postquarantine animal care records
- Formulation of 0.01% chlorophacinone or diphacinone grain baits
- Assay of the 0.01% chlorophacinone or diphacinone grain baits
- Weighing and ranking records
- Random allocation of the groups of 10 mice to the treatments
- Bait formulation records
- Daily bait consumption
- Date of death, weight, and sex
- Descriptions of unusual activities displayed by test and control animals in report on test and posttest periods.

P. Authority and Permits:
- NA

Q. Standard Operating Procedures (SOP's):
- SOP A-19.R.2: Chain of Custody
- SOP A-31.R.1: Personal Protective Equipment
- SOP WRC-59.R.3: Small Mammal Ranking for Testing
- SOP WRC-128.R.5: Animal Euthanasia with CO₂ Gas
- SOP WRC-216: Animal Handling Procedure to Maintain Identifications
- SOP WRC-232.R.1: Quarantine Procedures for All the Animals at DWRC
- SOP WRC-233.R.3: Incinerator Use and Maintenance
- SOP WRC-294.R.2: Transporting Small Mammals by Air
- SOP WRC-368.R.1: Computer Generated Random Numbers
- SOP WRC-395.R.1: Ground Transport of Pocket Gophers and other Small Mammals
- SOP WRC-436: Incineration of Animal Carcasses and Tissue
- SOP WRC-465.R.1: Hazard Communication
- SOP WRC-528: Laboratory mouse handling and Maintenance

VIII. COMPLIANCE WITH ENDANGERED SPECIES ACT (SECTION 7):
- NA

IX. COMPLIANCE WITH THE NATIONAL ENVIRONMENTAL POLICY ACT:
- Does the study, as proposed, have the potential for significant impact on the environment? Yes __ No _X_
X. EMPLOYEE SAFETY:
USDA/APHIS/ADC/DWRC/NWRC safety regulations will be followed. Routine safety procedures will be followed, and appropriate gloves will be worn by employees while handling house mice. Similarly, appropriate protective clothing and equipment will be used while preparing and handling the grain baits (SOP A-31.R1 and WRC-465.R1).

XI. SCHEDULE:

Proposed experiment starting date: Chlorophacinone: October 1996
Proposed experiment completion date: Chlorophacinone: November 1996
Study completion date: Chlorophacinone: March 1997

Diphacinone: August 1996
Diphacinone: September 1996
Diphacinone: March 1997

XII. STAFFING:

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XIII. COST ESTIMATE FOR EACH FISCAL YEAR*:

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<tr>
<td>Bait analysis</td>
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<td>182</td>
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<tr>
<td>Supplies</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Animal care</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Baits (Grain Bait)</td>
<td></td>
<td>(Grain Bait)</td>
</tr>
<tr>
<td>1. 0.01% Chlorophacinone</td>
<td>63</td>
<td>0.01% Diphacinone</td>
</tr>
<tr>
<td>2. EPA Challenge Diet</td>
<td>42</td>
<td>42</td>
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<tr>
<td>Direct costs</td>
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<td>$6,757</td>
</tr>
<tr>
<td>Indirect costs</td>
<td>1,093</td>
<td>1,091</td>
</tr>
<tr>
<td>Total</td>
<td>$7,861</td>
<td>$7,848</td>
</tr>
</tbody>
</table>

* All expenses are paid by CDFA Accounting Code 67374-03124

XIV. QUALIFICATIONS OF STAFF:
Study participants have documentation supporting education, experience, and special classes which qualify them for the work they will be performing in this study.

XV. ARCHIVING:
All raw data, documentation, protocol, and final report will be transferred to the archives at the close of the study (which is the day that the final report is signed).
XVI. REFERENCES:


Title: Chlorophacinone and Diphacinone: Standard House Mouse Acute Dry Bait Laboratory Test

SUBMITTED BY:

[Signature]
(Study Director)
8/19/96
Study Initiation Date

REVIEWED BY:

[Signature]
(Peer Reviewer)
7/31/96
Date

[Signature]
(Statistician)
7/24/96
Date

CONCURRENCE:

[Signature]
(Director of Quality Assurance Office)
8/6/96
Date

[Signature]
(Animal Care and Use Committee)
7/31/96
Date

APPROVED BY:

[Signature]
(Section Chief)
8/2/96
Date

[Signature]
(Director's Office)
8/4/96
Date

Any changes in, or deviations from, this protocol will be documented on the Study Protocol Amendment Form, signed and dated by the Study Director, Section Chief, Animal Care and Use Committee (if applicable), and Quality Assurance Officer. This amendment will be distributed to all participants.
Study Protocol Amendment/Revision
USDA/APHIS/ADC/NWRC/PDS

Chlorophacinone and Diphacinone: Standard Mouse
Study Title: Anticoagulant Dry Bait Laboratory Tests     Date: March 5, 1997
Study Director: Geraldine R. McCann        Study Protocol No.: QA-506
California Department of Food & Agriculture (CDFA) and
Study Sponsor: the USDA/APHIS/ADC/NWRC        Amendment/Revision No.: _1_

Protocol Item(s) to be Changed:
Page I. II: Sponsor: Add California Vertebrate Pest Advisory Council

Page 2. IV: Objective/Hypothesis: Change the grain bait to wax bait.

Page 2. VI: Justification and Background: Add California Vertebrate Pest Advisory Council right after CDFA
and change the bait to wax.

Page 2. VI: Animal Care and Use: A.: Change the number of animals to be used to eighty-six and 43M:43F.

Page 2. VI: Animal Care and Use: C.3.: Change the device to wax.

Page 3. VI: Animal Care and Use: H.: Change the first sentence to read that the animals will be shipped by
ground transport to Animal Care at the Animal Research Building at the NWRC.

Page 3. VI: Animal Care and Use: L.: Add to the first sentence: unless designated to be used for further research
and their destination/disposition will be documented.


Page 7. VII: Methods: A. 2.: In the first sentence: change the number of animals being used to 80 animals
(40M:40F). In the third sentence change the weight class numbers from 3 to 4, and in the fourth sentence, change
0.01% to 0.005%.

Page 7. VII: Methods: A. 2.: Day 1 of Testing: Change the toxicant bait to wax bait block of 0.005% and a
second dish containing the standard OPP rat and mouse challenge diet will be in excess of 40 g.


Page 8. VII: Methods: A. 3. Posttesting Period: Day 16: Change the second sentence to read in excess of 40
 g of OPP standard rat and mouse challenge diet.

Page 8. VII: Methods: B.: Change the grain baits to wax bait blocks.

Page 8. VII: Methods: H.: Change the ingredients of the grain baits to wax bait blocks.

Page 8. VII: Methods: L.: Change the ingredients of the grain baits to wax bait blocks.

Page 8. VII: Methods: J.: Change the ingredients of the grain baits to 0.005% wax bait blocks.
Page 8. VII: Methods: K.: Change the ingredients of the grain baits to 0.005% wax bait blocks.

Page 8. VII: Methods: O.: Change the records to be kept from grain baits to 0.005% wax bait blocks.

Proposed Protocol Revision:
Page 1. II: Sponsor: California Vertebrate Pest Advisory Council
California Department of Food and Agriculture (CDFA)
USDA/APHIS/ADC/DWR/NEWR

Page 1. IV: Objective/Hypothesis: Our objective is to determine the efficacy of chlorophacinone and diphacinone wax baits for controlling house mice (Mus musculus). We will test the following null hypotheses (Ho): mortality will be the same for treated and control house mice feeding on the diphacinone wax baits and mortality will be the same for treated and control house mice feeding on the chlorophacinone wax baits.

Page 2. V: Justification and Background: The CDFA and the California Vertebrate Pest Advisory Council have entered into an agreement with the USDA/APHIS/ADC/DWR/NEWR/Ecological Effects Unit to conduct this laboratory study. This study will evaluate the efficacy of chlorophacinone and diphacinone wax baits against laboratory mice as a partial fulfillment for the reregistration of chlorophacinone and diphacinone commensal rodent labels.


Page 6. VII: Methods: A. Protocol: The protocol for this study has been outlined by the EPA in their Standard House Mouse Anticoagulant Dry Bait Laboratory Test Method from the Office of Pesticides Programs (OPP Designation: 1.214) (EPA, 1982).

Page 7. VII: Methods: A. 2.: Following the quarantine period, 80 animals (40M:40F) will be randomly selected from the total population using a computer generated random number program (SOP WRC-368.R1). If an acceptable difference in body weights exists between the sexes at that time, the mice of each sex will be ranked by weight into 10 weight classes, each containing 4 animals (SOP WRC-395.R1). The groups of ten mice (sexes separate) will be randomly assigned to one of the two concentrations (0.00% or 0.005%) for a total of 10 animals per sex per each concentration, or a total of 20 animals per each concentration.

Page 7. VII: Methods: A. 2.: On the first day of testing, the 15-day two-choice feeding study will begin by removing the regular diet. Each group of 10 mice will then be offered one dish and a wax bait block, which have been placed on opposite sides of the cage. Every group receiving the toxicant diet will be given one wax bait block of 0.005% chlorophacinone or diphacinone bait and a second dish containing an excess of 40 g of the standard OPP
rat and mouse challenge diet. Each group of mice receiving the control diet will be given two 20 g dishes of the standard OPP rat and mouse challenge diet. On Day 1, the wax bait block will be positioned on the left, and the challenge dishes will be placed on the right in the front of the cage. EPA does not require that separate test dishes measuring moisture gain or loss be used. Bottles of water will be available to all animals. The bottle will be positioned at equal distances from the OPP diet and the wax bait block.

Page 7. VII: Methods: A. 2.: Day 2: The dishes and wax bait block will be removed to determine the gross weight to the nearest 0.5 g of each container and its contents. At that time fresh bait will be added to the dishes and the dish weighed. If food becomes fouled by urine or feces, it will be discarded and replaced. The position of the dish and wax bait block will be reversed when replaced in the cages; i.e., wax bait blocks will be placed on the right and the challenge dishes will be set on the left in the front of the cage. Change the toxicant bait to wax bait block.

Page 8. VII: Methods: A. 3. Posttesting Period: Day 16: Animals will be checked for mortality and the dishes and wax bait blocks will be removed and the remaining bait will be weighed. Each survivor on the chlorophasocine or diphacinone treatment will be given one dish in excess of 40 g of the challenge diet.

Page 8. VII: Methods: B.: The chlorophascine and diphacinone wax bait blocks will be assayed by the DWRC/NWRC Analytical Chemistry Unit.

Page 8. VII: Methods: C.: The 0.01% chlorophasocine or diphacinone grain baits will be suspended in paraffin wax before they are fed to the mice.

Page 8. VII: Methods: D.: Grain baits treated with chlorophasocine or diphacinone suspended in wax will be used as if applied in an operational control program.

Page 8. VII: Methods: E.: The 0.005% chlorophasocine and diphacinone wax baits will be assayed. The mg/kg intake will depend upon the quantity of bait consumed by each mouse. The bait will be placed on plastic plates, from which the mice will be fed for 15 days.

Page 8. VII: Methods: F.: Bias will be controlled by randomly assigning each group of mice to one of the 4 different treatments (0.0%, 0.005% chlorophasocine or diphacinone wax bait blocks, and 0.005% chlorophasocine or diphacinone wax bait blocks) (SOP WRC-59.R3).

Page 8. VII: Methods: O.: Formulation of 0.005% chlorophasocine or diphacinone wax baits
Assay of the 0.005% chlorophasocine or diphacinone wax baits

Signatures:
Study Director
Date March 5, 1997
QA Officer
Date
Section Chief Kathleen A. Fagerstone
Date 3/4/97
Animal Care & Use Committee
Date 3/5/97
Study Protocol Amendment/Revision
USDA/APHIS/ADC/NWRC/PDS

Chlorophacinone and Diphacinone: Standard Mouse

Study Title: Anticoagulant Dry Bait Laboratory Tests
Study Director: Geraldine R. McCann
Study Sponsor: California Department of Food & Agriculture (CDFA) and the USDA/APHIS/ADC/NWRC

Date: March 5, 1997
Study Protocol No.: QA-506
Amendment/Revision No.: 2

Protocol Item(s) to be Changed:


Proposed Protocol Revision:


Reason(s):

The protocol is being amended to clarify finishing the statistical analysis, final report writing, and archiving all records for each segment of each test. The completion process was delayed because of the many different species being tested in the same protocol and the 2 bait types, which add additional facets to each test in the same protocol.

Signatures:

Study Director
Date 2/27/97

Section Chief
Date 3/27/97

FDA Officer
Date 4/1/97

Animal Care & Use Committee
Date 4/1/97
I. PURPOSE:

A. All Test and Control Substance (TCS) materials ordered for DWRC and its field entities will be handled in the manner specified by this SOP.

B. Definition - TCS Materials

All technical grade chemicals, manufacturing-use products (e.g. concentrates), dyes and markers, purchased end-use products (e.g. spray concentrates and formulations), and reference standards involved in registration activities will be referred to as TCS materials in this SOP.

C. Analytical, bioassay, field, and other data gathered using TCS materials have the potential of being used as evidence in legal proceedings and/or in interagency communications involving APHIS registration activities. Thus, it is important that all DWRC personnel follow strict custody criteria for such materials from the time received until final disposal. Deficiencies in TCS custody will jeopardize the validity of data gathered using these materials.

2. Chain of custody procedures are necessary for identification, tracking, inventory, and troubleshooting purposes, as well as serving to document material integrity and physical security.

D. This SOP does not include storage of materials formulated by DWRC or others (such as baits) or for storage of samples collected in the field. In addition, candidate compounds (pilot studies, screening materials) do not go through the chain-of-custody until they are past the laboratory and preliminary field testing stage and are being pursued for potential registration.
II. PROCEDURE:

A. Sample Custodian/Alternate - Duties and Responsibilities

1. The DWRC Sample Custodian/Alternate will be supervised by the Chemical Development/Registration Section and will be responsible for overseeing the handling of all TCS materials ordered by DWRC personnel. Both will be fully aware of the custodian requirements and potential hazards of chemicals and pesticide formulations.

2. The Sample Custodian/Alternate will have an assigned room with good ventilation and temperature control as well as vented cabinets (for ambient storage), freezers, and refrigerators. This room and its contents will be locked and accessible only to the Sample Custodian/Alternate. An emergency key will be retained by the Quality Assurance Officer.

3. The Sample Custodian/Alternate is responsible for officially receiving all TCS materials, assigning a unique reference number to each (custodian number), proper storage, submitting a sample to DWRC Analytical Chemistry Section for initial assay if applicable, and preparation and archiving of documentation as long as the TCS materials are under DWRC custody. The Sample Custodian/Alternate is also responsible for other related duties such as the preparation, numbering, documentation, and archiving of sub-samples, the maintenance of records, and TCS disposal according to SOP No. A-17 (every 5 years or upon expiration date).

4. TCS related documentation will be stored in a file as well as backed up on a computer disc, all in a secure area.

5. The Sample Custodian/Alternate will provide the library with yearly inventory/status reports of all TCS materials.

B. Ordering TCS Materials

TCS materials will be ordered or procured by the Study Director. The Sample Custodian/Alternate is not responsible for ordering the TCS. All Study Directors will check with the Sample Custodian/Alternate for existing supplies BEFORE ordering any TCS material.
C. Receiving TCS Materials

1. In most cases (including field stations), newly ordered TCS materials will be delivered directly to the Sample Custodian/Alternate for logging-in on the TCS Worksheet (Figure 1), weighing, sampling, assay, etc. Exceptions to this procedure will be approved in writing by the Sample Custodian/Alternate. The only exceptions at this time are the receipt of 55-gallon drums at field stations and reference standards specifically for the Analytical Chemistry Section (ACS; see Section E-1, page 4).

2. The TCS Worksheet will be completed in permanent ink by the DWRC Sample Custodian/Alternate only, and will be retained in a secure area with copies provided to the study director when the material is checked out.

3. Upon receipt of TCS materials, the containers will be inspected by the Sample Custodian/Alternate for their overall condition. Any leakage or other evidence of damage will be noted, and a photograph will be taken. In cases of damage, the Sample Custodian/Alternate will inform the Study Director, who will contact the appropriate sources and make a decision regarding analysis or sample disposition.

4. Copies of freight bills or other documentation related to the incoming shipment will be initialed, dated, assigned the unique reference number (custodian number) associated with the TCS, and retained by the Sample Custodian/Alternate.

5. Each storage container shall be labeled by name, chemical abstract service number (CAS) or code number, batch number, date of receipt, expiration date (if any), custodian number, initialed, and storage conditions given (if appropriate).

6. Except for ACS reference standards, a 5-gram aliquot of the TCS will be removed by the Sample Custodian/Alternate for archiving, assigned the custodian reference number, and retained by the Sample Custodian/Alternate throughout the life of the registration. The TCS Worksheet will be completed.

7. An aliquot will be removed for assay (except for dyes, markers, and ACS materials), numbered, and sent to the ACS, Analytical Services Project, with a request for analysis. Except for those chemicals actually received in the field or by the ACS, materials will not be checked out before the assay is completed. The Study Director will
provide the Sample Custodian/Alternate with the Confidential Statement of Formula or Certificate of Analysis for the TCS when this information is available. If the assay result is more than 10% below the nominal value, the TCS material will not be checked out to users until the discrepancy is investigated by the Sample Custodian/Alternate. If the ACS does not have an analytical method for a TCS, the assay value(s) stated on the manufacturers statement or certificate will be used.

8. The TCS may then be checked out for use, and the Study Director will receive copies of the TCS Worksheet and a Chemical Tracking Form (Figure II) from the Sample Custodian/Alternate.

9. The Sample Custodian/Alternate is solely responsible for preparing sub-samples from TCS materials. When a sub-sample is prepared from an existing supply, it will be placed in a new container, labeled with the unique reference number (custodian number), weighed, etc., and a Chemical Tracking Form will accompany the sub-sample.

E. Exceptions

1. EPA Primary Reference Standards and other reference standards for the Analytical Chemistry Section (ACS) are the exception. No aliquots will be removed from EPA Primary Reference Standards because of the limited quantities available. When these materials are ordered by or for the ACS, they will be on permanent check-out to an ACS-designated alternate custodian until disposal. ACS will maintain their own records.

2. For 55-gallon drums, a Field Alternate Sample Custodian (the Study Director) may be designated to allow receipt of TCS materials at field stations. In this case, arrangement will be made before ordering between the DWRC Sample Custodian/Alternate and the respective Study Director. The Field Alternate Sample Custodian must be aware of the rules for shipping hazardous materials, obtain shipping materials, and follow the Sample Custodian's SOP for removing an archive aliquot and an assay aliquot from the TCS. The Field Alternate Sample Custodian will keep records (documentation) including the TCS Worksheet and Chemical Tracking Form, all of which must be returned to the Sample Custodian/Alternate at the DWRC as soon as possible.
F. TCS Material Use

1. Only the Sample Custodian/Alternate will aliquot and number any sub-samples of TCS materials. The Sample Custodian/Alternate will distribute the TCS materials to the user. The unique identification number (custodian number) assigned to the TCS material by the Sample Custodian/Alternate will be used to reference the TCS at all times. The TCS material received from the Sample Custodian/Alternate will not be renumbered, renamed, or sub-sampled.

2. The user is responsible for tracking TCS material use in analyses, tests, or studies, in a manner designed to preclude the possibility of contamination, deterioration, damage, or loss. The Chemical Tracking Form will be used for this purpose. The Chemical Tracking Form will accompany the TCS material, will be maintained throughout the use of the material, and the completed Chemical Tracking Form will be returned to the Sample Custodian/Alternate with the remainder of material or the empty container for archiving of paperwork and disposal of the chemical or container. The amount of chemical used will be accounted for, and the "purpose of use" entry must be specific enough to identify how the material was used, when, by whom, etc. The sample must be trackable from this description.

3. The user will have the TCS material re-assayed before use in all cases unless the initial Sample Custodian/Alternate assay has been performed within the past 90 days. This re-assay can be performed by the ACS or a contract laboratory. If the material is known to be unstable or visually appears contaminated, the material will be assayed regardless of the date of the most recent assay. The Sample Custodian/Alternate will be notified immediately if any material has deteriorated or has been contaminated. Material that is determined to be unusable must be returned with a completed Chemical Tracking Form to the DWRC Sample Custodian/Alternate for documentation and disposal.
I have read this document and approve of its contents. I certify that it will be made available for all personnel to whom it applies.

[Signature]
Director

[Signature]
Date
<table>
<thead>
<tr>
<th>Chemical Name:</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Appearance:</td>
<td>Nominal Amt. Received:</td>
</tr>
<tr>
<td>Certified Assay (from ACS or Certificate):</td>
<td>(Circle One)</td>
</tr>
<tr>
<td>Storage Location: Room No.</td>
<td>Cabinet No.</td>
</tr>
<tr>
<td>Storage Conditions: Ambient</td>
<td>Refrigerator</td>
</tr>
<tr>
<td>Gross Weight of Container on Arrival:</td>
<td>Balance Serial No.:</td>
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<tr>
<td>Stability/Expiration Date:</td>
<td></td>
</tr>
<tr>
<td>Other Comments:</td>
<td></td>
</tr>
</tbody>
</table>

Quantitative - Analytical Tests:

**ORIGINAL ASSAY:**

Date: ____  Initials: ____  Method No.: ____  Invoice No.: ____

Weight of Container at Disposal: ____  Balance Serial No.: ____

Sample Disposal: ____________________________

Initials: ____  Date: ____

**FIGURE I**
EXAMPLE

Date Received: 9/1/89  Initials: HYG  Custodian No.: CSW-9

TEST AND CONTROL SUBSTANCES (TCS) WORKSHEET

Chemical Name: Sodium Mono-fluoracetate

DRC No. ______________________ Company ________________ Batch/Lot No. 41230  CAS No. 62-74-8

Appearance: White Powder  Nominal Amt. Received: 116 g

Source: Tull Chemical Company  Nominal Conc.: 90.0%

Certified Assay (from Assay Certificate): 92.32 ± 3.13% (n = 5)

Storage Location: Room No. 1109/Bldg. 20 Cabinet No. 2

Storage Conditions: Ambient X  Refrigerator X  Freezer X  Hood

Gross Weight of Container on Arrival: 290.0 g  Balance Serial No.: 27845

Stability/Expiration Date: 9/92

Other Comments:

Quantitative - Analytical Tests:

ORIGINAL ASSAY:

Date: 9/6/89  Initials: JG  Method No.: 13.8  Invoice No.: 89-013

Weight of Container at Disposal: Balance Serial No.: 

Sample Disposal: 

Initials: ___________________________ Date: __________

00923

Page 74 of 132
<table>
<thead>
<tr>
<th>Date</th>
<th>Weight (Container &amp; Contents)</th>
<th>Amount Used</th>
<th>Appearance</th>
<th>Purpose of Use</th>
<th>Pesticide Storage Location</th>
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</tr>
</thead>
<tbody>
<tr>
<td>9/2/89</td>
<td>290.0g</td>
<td>288.9g</td>
<td>1.1 g</td>
<td>White Powder</td>
<td>Chemical Analyses</td>
<td>HVG</td>
</tr>
<tr>
<td>9/2/89</td>
<td>288.9</td>
<td>283.9g</td>
<td>5.0</td>
<td>White Powder</td>
<td>Archive Sample</td>
<td>HVG</td>
</tr>
<tr>
<td>9/8/89</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/10/89</td>
<td>283.9</td>
<td>273.9g</td>
<td>10.0</td>
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<td>Checked out to Peter Savarie</td>
<td>PJ5</td>
</tr>
<tr>
<td>9/11/89</td>
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<td>PJ5</td>
</tr>
<tr>
<td>11/15/90</td>
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<td>102.0g</td>
<td>White Powder</td>
<td>QA-79, Book No. 6, Pg. 5</td>
<td>PJ5</td>
</tr>
</tbody>
</table>

*This amount includes 2 oz. that was collected and disposed of in a labeled hazardous waste container.*

**Note:** Form, container, and remaining contents **MUST** be returned to the custodian when anticipated use is completed. All material must be accounted for.

**ASSAY RESULTS:**

<table>
<thead>
<tr>
<th>Invoice No</th>
<th>Date</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>80-013</td>
<td>9/7/89</td>
<td>92.3% ± 2.4% (n = 5)</td>
</tr>
<tr>
<td>79-077</td>
<td>7/17/90</td>
<td>91.7% ± 2.9% (n = 6)</td>
</tr>
</tbody>
</table>
STANDARD OPERATING PROCEDURE

PERSONAL PROTECTIVE EQUIPMENT

I. PURPOSE:

To ensure proper personal protective equipment (PPE) is provided, appropriately used, and maintained in a reliable condition to effectively protect employees from hazards present in their work environment as required by the Occupational Safety and Health Administration (OSHA).

II. PROCEDURE:

A. General Requirements

1. In accordance with OSHA’s "General requirements" for PPE (29 CFR 1910.132); adequate and safe protective equipment for eyes, face, head, extremities, clothing, and respiration shall be provided, used, and maintained in a sanitary and reliable condition wherever it is necessary to prevent injury or impairment from absorption, inhalation, or physical contact. To ensure maximum protection, the materials used in protective equipment shall be specific for the type, intensity, and duration of potential hazards. Defective or damaged equipment shall not be used. Employees shall notify their supervisor of any specific concerns or personal needs regarding protective equipment.

B. Workplace Hazard Assessment

1. Supervisors shall be responsible to ensure work areas are assessed (documentation required) to determine the actual and potential hazards which may reasonably exist, along with the proper PPE to be used in that area to protect employees from those hazards. Workplace hazard assessment documentation shall include the identity of the work area, the date the assessment was performed, and a certification by the evaluator to verify the workplace was assessed (see attachment #1). Contact the DWRC Chemical Hygiene Officer for guidance concerning the assessment of hazards or the selection and use of PPE.
C. Training

1. Supervisors shall be responsible to ensure each employee who wears PPE is trained (documentation required) and demonstrates an understanding of the following:
   a. When PPE is necessary.
   b. What PPE is necessary.
   c. How to properly put-on, take-off, adjust, and wear PPE.
   d. The limitations of the PPE.
   e. The proper care, use, maintenance, useful life, and disposal of the PPE.

2. Training documentation shall include the names of the employees trained, the date of the training, a list of the subjects taught, and a certification by the instructor to verify that those employees have received and understood the required training (see attachment #2). Re-training shall occur whenever new forms of PPE or hazards are brought into the work area. Contact the DWRC Chemical Hygiene Officer for training materials or guidance.

D. Requirements for Specific Types of PPE:

1. Appropriate eye and face protection shall be selected to protect against the specific hazard(s) which may be encountered in the work area (e.g., chemical splashes, vapors, flying particles, dust, sparks, or intense light). See 29 CFR 1910.133(a)(5) for the minimum requirements for filter lenses to be worn during different types of welding operations. The employer must accommodate those employees who wear corrective lenses with either prescription eye protection or appropriate protection to cover prescription lenses. All protective equipment for the eyes and face must comply with American National Standard Institute (ANSI) minimum specifications.

2. Protective helmets shall be worn in the working area whenever there exists a reasonable potential for falling objects, or where the head may be exposed to overhead electrical hazards. All protective equipment for the head must comply with ANSI minimum specifications.

3. Protective footwear shall be worn in work areas where there exists a reasonable potential for falling or rolling objects, objects capable of piercing the sole, or where the feet may be exposed to ground level electrical hazards. All protective equipment for the feet must comply with ANSI minimum specifications.

4. Protective equipment for the hands or skin shall be worn in work areas where there exists a reasonable potential for skin absorption of harmful substances,
severe cuts, lacerations, abrasions, punctures, chemical burns, or temperature extremes. Employees shall use only those protective gloves and clothing which provide the maximum protection against the specific hazard(s) being handled.

5. Employees who work in atmospheres which contain chemical concentrations above the "Permissible Exposure Limits" (see 29 CFR 1910.1000), shall be equipped with respiratory protection. Respirator cartridges are specific for individual or groups of chemicals. Employees should read all manufacture instructions and warnings before use. See DWRC Standard Operating Procedure A-28 "Use of Air-Purifying Respirators" and 29 CFR 1910.134 "Respiratory Protection" for specific requirements concerning respirators.

6. Employees who work in areas where there exists a sustained noise at 85 decibels or above for 8 hours a day or at a time weighted average equivalent (see 29 CFR 1910.95 "Occupational Noise Exposure"), shall use appropriate hearing protection and participate in periodic hearing exams. Hearing protection is rated on the ability to reduce noise going into the ear (the NRR value). Dividing the NRR value in half will give the estimated number of decibels which are reduced.

E. Maintaining PPE

1. All PPE shall be maintained for maximum effectiveness against the hazard(s) for which it is designed. Periodic inspections and preventative maintenance should be performed to ensure the equipment is in proper working condition (see instruction book or owners manual for specific procedures). Protective equipment which is defective, worn, or damaged to the extent by which it is no longer effective will be taken out of service for repair or replacement. All protective equipment to be used for emergency situations will be kept in a highly visible and readily accessible location.

I have read this document and approve of its contents. I certify that it will be made available for all personnel to whom it applies.

[Signature]
Director

[Signature]
Date
<table>
<thead>
<tr>
<th>HAZARDS</th>
<th>SOURCE:</th>
<th>RECOMMENDED PROTECTIVE EQUIPMENT:</th>
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PERSONAL PROTECTIVE EQUIPMENT - TRAINING DOCUMENTATION

Instructor/Source: ________________________________

Subjects Taught:  
OSHA Regulations and APHIS/ADC/DWRC Policies Concerning PPE
Workplace Hazard Assessment Results
When PPE is Necessary
What PPE is Necessary
How to Properly Put On, Take Off, Adjust, and Wear PPE
Limitations of the PPE
Proper Care, Maintenance, Useful Life, and Disposal of PPE

I certify that I have received training in the subjects listed above, and that I understand the information as presented by the instructor/source.

PRINT NAME  SIGNATURE  DATE

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DWRC SOP A-31 RL Attachment #2

Page 81 of 132  00930
Attached are three DWRC Standard Operating Procedures (SOP’S). These SOP’s have been written or revised to reflect current regulatory requirements from the Occupational Safety and Health Administration (OSHA). The draft SOP’s were circulated to DWRC Section Chiefs, and comments received have been incorporated into the final versions:

1) SOP# A-33 "Control of Hazardous Energy" is specific for certain situations within DWRC which might require compliance with the OSHA "Lockout/Tagout" program of 29 CFR 1910.147 (e.g. performing service or maintenance on machinery or equipment). Supervisors are responsible to ensure employees who perform service or maintenance on equipment are aware of and perform de-energizing procedures or processes to release or contain stored energy.

2) SOP# VRC-465.R1 "Hazard Communication" includes revisions by OSHA to the "Hazard Communication Program" of 29 CFR 1910.1200 which now requires chemical shipments from laboratories be properly labeled and an Material Safety Data Sheet (MSDS) sent to the recipient. Supervisors are still responsible to ensure employees who work with chemicals know how to read and understand labels and MSDS’s, are able to select and use proper protective equipment, and know what to do in case of an emergency.

3) SOP# A-31.R1 "Personal Protective Equipment" includes revisions by OSHA of 29 CFR 1910 Subpart I which now specifies minimum filter lenses for welding operations, expands the definition when protective head and footwear are required, and most importantly, now requires documented hazard assessments be performed for work areas where reasonably potential hazards exist along with documentation of training for the selection and use of protective equipment by the employees who work in those areas. Supervisors will still be responsible to ensure these requirements are met.

The new OSHA and DWRC SOP requirements for the "Control of Hazardous Energy" and "Hazard Communication" are common sense procedures and compliance should not involve any changes to current DWRC operations other than the additional level of awareness. However, the new
requirements for "Personal Protective Equipment" is a substantial change and will involve a fair amount of initial effort. Although, as soon as the documented assessment and training requirements are met, efforts to maintain the program will be minimal.

Hazard assessments are only required for those areas where a reasonable potential for a hazard exists (e.g. laboratories, chemical storage areas, animal pens, tool/machine shops, etc.). I will assist with the assessment of those work areas at Denver, and I am available for guidance to the field stations for assessments at their locations.

Training is only required for those employees who may be exposed to the potential hazards or wear protective equipment in those work areas. I will continue to present an annual training seminar on personal protective equipment for the employees at Denver, and I will also continue to provide the field stations with information or materials for their own training programs.

Special note to the field stations: As soon as possible after the start of the fiscal year, I will organize a packet of training materials which will be sent to each field station to cover all training subjects as specified in SOP A-31.K1. Employees will document they have read/watched the material for compliance with the requirement. Please send me a copy of your workplace assessments and training documentation as soon as they are completed.

HAZARD ASSESSMENTS AND TRAINING DOCUMENTATION AT ALL DWRC LOCATIONS MUST BE COMPLETED BY JANUARY 1, 1995.

Please call me if you have any questions or problems with these new requirements.

Steve Greiner
DWRC Safety and Health Specialist
(303) 236-0553
Subject: DWRC Standard Operating Procedures

Date: August 3, 1994

To: DWRC Section Chiefs and Field Station Leaders

Listed below are four different DWRC Standard Operating Procedures (SOP'S). These SOP's have been created, revised, or deleted to reflect new requirements which have recently taken effect. The attached SOP's are being circulated for comments or correction concerning the following actions which have been taken:

1) SOP# WRC-465 "Hazard Communication" has been revised to become WRC-465.R1, which incorporates additional requirements by OSHA to 29 CFR 1910.1200.

2) SOP# A-30 "Vehicle Emergency Equipment" has been deleted due to the implementation of the APHIS Motor Vehicle Fleet Management Manual which now specifies the emergency equipment to be kept in Government Owned Vehicles (see chapter 4.4 - attached).

3) SOP# A-31 "Personal Protective Equipment" has been revised to become A-31.R1, which incorporates additional requirements by OSHA to 29 CFR 1910 Subpart I.

4) SOP# A-33 "Control of Hazardous Energy" has been created to cover any potential situations within DWRC which might require compliance with the OSHA "Lockout/Tagout" program of 29 CFR 1910.147.

Please review these SOP's and forward any comments, corrections, or concerns to me by Friday, August 26, 1994. Thank you.

Steve Greiner
DWRC Chemical Hygiene Officer
(303) 236-0553
II. Purpose:

A. To standardize the ranking of small mammals by weight for laboratory testing.

B. To assure the closest possible mean weight distribution of each animal to a treatment group.

C. To insure random assignment of each animal to a treatment group.

II. Procedure:

A. Randomly select a sample population for testing from the entire population available using the computer generated random number program (per SOP WRC-368.R1).

B. Weigh the test sample population using the Mettler PE 3600 (procedure found in WRC SOP-138.R1). The weight for the individual animals should be recorded in a permanent log (WRC SOP-52.R2).

C. All persons handling small mammals should wear leather gloves, a respiratory filter mask, a laboratory coat, and the doors should be closed to the area while working with the animals.

D. Select enough weight classes to include all animals with the number of treatment groups equalling the number of animals in each class. For example:

<table>
<thead>
<tr>
<th>Weight Class</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>96g-102g</td>
<td>1. 98.4g</td>
</tr>
<tr>
<td>106g-115g</td>
<td>1. 106.5g, 1. 116.0g</td>
</tr>
<tr>
<td>116g-125g</td>
<td>2. 116.5g, 2. 116.5g</td>
</tr>
<tr>
<td>126g-135g...</td>
<td>2. 130.6g</td>
</tr>
</tbody>
</table>

The weight classes selected depend on the species being tested and 2 treatment groups would equal a total of 20 animals being tested.
E. In a container, place enough numbers (1 and 2 for this example) to represent the animals in the weight classes. In another container, place numbers to represent the treatment groups (also 2 for this test example).

F. Randomly select one number from each container to assign treatment groups to each animal in the weight class.

G. Assign the treatment group selected to the animal selected from each class, taking care to note other identification numbers to prevent mix-ups.

III. Equipment List:
- Pair of leather gloves for those handling small mammals
- Lab coat
- Net for capture of loose animal
- Closed weighing container of adequate size
- Respiratory filter mask
- Mettler PE 3600 electronic balance

IV. Specifications:
A. Closed containers used for weighing should be adequate to temporarily hold a small mammal as per SOP-MRC 297.R2.

B. All persons handling small mammals should wear leather gloves, a respiratory filter mask, and a laboratory coat.

C. Close all doors while working with small mammals.
I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

Kathleen A. Ferguson  
(Section Chief)  
4/13/94  
(Date)

D. May Mitchell  
(Quality Assurance Officer)  
4/19/94  
(Date)

Donald J. Chao  
(Animal Care Committee)  
April 14, 1994  
(Date)
Title: Animal Euthanasia With CO₂ Gas

I. Purpose:

A. To standardize animal euthanasia procedure.

II. Procedure:

A. Operation, if CO₂ is used from a cart held cylinder

1. Insure that CO₂ cylinder is properly secured to cart.

2. Turn the secondary valve (located on the regulator) counterclockwise until it is loose. This indicates the valve is shut.

3. Turn the primary valve on top of the tank counterclockwise. This will open flow of gas to the regulator. The right gauge (closest to primary valve) will indicate the remaining cylinder pressure.

4. Open the exhaust valve on the box and position the tubing from the exhaust valve next to a window or exhaust vent. The exhaust valve on the box is located on the front.

5. To start the gas flow, slowly turn the secondary valve clockwise. The left gauge indicates the line pressure out of the regulator. When the gas starts to flow, slowly adjust the secondary valve for the desired flow rate which is 20 lbs. for 60 seconds, this should be sufficient to fill the box. To measure this flow, use the red, inside indicator circle. It is labeled CO₂.

6. To stop the flow, turn the secondary valve counterclockwise.

7. Close the exhaust valve on the box.
8. To release the regulator pressure, make sure the primary valve is shut (turn clockwise), then open the secondary valve (turn clockwise) until both gauges indicate zero pressure.

9. The primary and secondary valves are to be shut, and pressure released from the system when the tank is not in use. This minimizes the chance for inadvertent loss of cylinder pressure.

B. Operation. If CO₂ is used from the tank room utilizing the medical gas system.

1. Ensure the CO₂ metering device is securely connected to the wall mounted CO₂ outlet. Remove the protective cover on the CO₂ outlet, press the metering device firmly into the receptacle and finger tighten the DISS connector.

2. Attach the long connector of the black hose to the chamber first. The reason this must be done as the first step is because the long connector does not swivel and the hose must be free to turn as the connection is made.

3. Attach the opposite end (with the DISS connector which does swivel) to the base of the CO₂ metering device.

4. Adjust the flow of CO₂ by turning the knurled knob of the wall mounted metering device. Clockwise to reduce the flow, counter clockwise to increase the flow. Arrows on the knurled knob indicate the proper direction.

C. Introduction of animals to CO₂ atmosphere.

1. Open the exhaust valve on the box and position the tubing from the exhaust valve next to a exhaust vent. The exhaust valve on the box is located on the front.

2. Using proper animal handling techniques, place the animals, one at a time, into the CO₂ rich atmosphere of the already filled container. Follow the specific instructions for the euthanasia chamber being used. For example, if the unit has two compartments, animals can be added to first one compartment and then the other to speed the task. Add CO₂ as needed by adjustment of flow of CO₂ from the regulator and open the exhaust valve on the box as needed to ensure death is as painless as possible. When more than one animal is to be euthanized, maintain a continuous flow of CO₂ and leave the exhaust valve open.

3. The time required for death is dependent upon the species. Visually observe the animal for movement or respiration. CO₂ is heavier than air and most will remain in the box even when opened. Nonetheless, USE CAUTION WHEN REMOVING ANIMAL TO AVOID BREATHING CO₂.
4. Death by euthanasia is confirmed by loss of heartbeat and/or no reaction when the area about the eye is touched.

5. After all animals have been processed, secure the cylinder, or the wall mounted equipment and clean the container.

6. Properly dispose of animal carcass according to intended use: incineration, residue analysis, freezer storage, etc.

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

[Signatures and dates]

Unit Chief

Date

Quality Assurance Officer

Date

Animal Care & Use Committee

Date
Title: Animal Handling (General) so that secure and accurate identification is maintained.

I. Purpose: To standardize animal handling methods so that cage identification may serve as an accurate means of individual animal identification

A. Wild caught animals must be securely identified for research purposes, and yet remain free of artifacts which traditional identification methods may introduce.

II. Procedure:

A. Removal of animals from individual cages.

1. Never undertake to remove any animal or change any cage unless you have an exact plan on how to proceed.

2. Always bear in mind, the cage number is your only means of identification. Each animal must be matched to their cage at all times.

3. Never involve more than one animal and one cage in any procedure.

4. Complete procedure, (record, examine, treat and etc.) and replace animal before moving to next animal and next cage.

5. At any time, for whatever reason, if any confusion exists, consult your supervisor immediately.

6. Be certain identifying number is securely attached to cage.
B. Replacement of cages:

This is where the greatest possibility of error lies. All of the above rules apply, especially #3 and #6.

1. Do not allow yourself to be distracted by conversation.

2. If for any reason, you are distracted. Halt what you are doing, note exactly where you are in your procedure, and attend to the distraction.

3. Do not hurry during this period. Be precise and methodical.

4. At any time, for whatever reason, if any confusion exists, consult your supervisor immediately.

C. Transport of cages:

1. As each cage is removed from the rack, be certain the cage is securely identified and closed.

2. If the cage is of the suspended type, without a top, slide a transport top over the cage as the cage is removed from the rack.

3. Do not stack loose cages.

4. Always bear in mind, the cage number is your only means of identification. Each animal must be matched to their cage at all times.

Emergency Numbers:  
Al Dale 494-0411  
Phyllis Harris 986-0644  
or Call principal investigator as listed on the testing protocol.

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

Al Dale  
Chief Animal Care  
Section Chief  

May 20, 1989  

May Mitchell  
Quality Assurance Officer  

July 21, 1989
STANDARD OPERATING PROCEDURE

Title: Quarantine procedures for Animals at the National Wildlife Research Center

I. Purpose:

A. To standardize Quarantine methods.

II. General:

1. All incoming animals, except those collected for immediate euthanasia will be placed in quarantine for an observation and stabilization period.

2. Normally, the quarantine period for wild caught animals will be 14 days. This period may be extended as conditions dictate.

3. Normally, the quarantine period for laboratory purchased animals will be 7 days. This period may be extended as conditions dictate.

4. Prior to arrival of the animals, the room and cages will be prepared to receive the animals.

5. Only animals of the same specie will be quarantined together, and as well as can be determined, only animals trapped in the same area or received from the same source will be quarantined together.

6. Cages will be labeled with index cards or tape with the animal number. Unless instructed by a supervisor to house in a different manner, the animals will be housed individually. (See SOP WRC-216)
7. Animal records shall be stored in designated area at the close of each day.

8. Except those animals kept under natural lighting conditions, the lighting cycle will be a 12 hour light, dark cycle.

9. Feeding, watering, and care will conform to the appropriate WRC-SOP for the specie involved.

III. Procedure

1. Complete entry Form

2. Complete Pre-Quarantine Forms if required. (Such as waiting to assemble a group)

3. Complete Quarantine Form. (Entry and Release)

4. Release to Study Protocol or Post-Quarantine.

5. Ensure you have obtained all necessary signatures.

6. Complete records each day.

Emergency Numbers: Al Dale 494-0411
Phyllis Harris 986-0644
or call Principal investigator as listed on the testing protocol.

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

[Signatures]

Unit Chief
Date

Quality Assurance Officer
Date

Animal Care and Use Committee
Date
STANDARD OPERATING PROCEDURE

Title: Incinerator Use and Maintenance

I. Purpose: To standardize incinerator operation and disposal of material from the incinerator:

A. Standard Procedures

1. Keep door to incinerator room locked when not in use.
2. Do not add additional material to the unit while the burn is taking place.
3. If equipment malfunction is suspected advise your supervisor.

B. Standard Incinerator Operation

1) Record time from counter, and enter the time, date, and the weight of the amount you intend to burn in the Incinerator Record Book. This record is required to comply with our permit to operate this incinerator. Forms are provided for this purpose. The form in use (the present month) will be maintained in the room with the incinerator. After completion, the monthly summaries are to maintained in the section office.

2) Wear a mask, and be certain the ashes are cool, before removing the ashes. Take care that the ash pull tool does not make heavy contact with the incinerator interior. Store the removed ash in a metal trash container which has been lined with a plastic bag.

3) Clean out all air ports.

4) Turn system to on. A Red light appears on the panel and the air activated louvers will open.

5) Turn control timer past stop. This will initiate the preheat cycle. A Green light appears on the panel when the control burner fires. The Red light will stay on until the
pre-set temperature (1400 degrees F, 760 degrees centigrade) has been reached. The red light will go off, and a blue light will appear on the panel when the pre-set temperature listed above is reached. When this temperature is reached, and the blue light appears, the combustion blower will start.

To summarize:
- The green light on the panel should stay on throughout the cycle. This indicates the control burner is firing.
- The red light indicates the control burner is below 1400 degrees.
- The blue light indicates the control burner is above 1400 degrees.

6) During the above pre-heat cycle (step 4), and while waiting for the pre-heat temperature to be reached, load incinerator, taking care the opening for the burner at the distal end of the incinerator chamber is not blocked. When closing door, simply snug the latch. If the door is tightly closed the heat will make the door nearly impossible to open.

7) When the pre-set, pre-heat temperature is reached (the red light turns off, and the blue light is on) the refuse chamber burner is ready to ignite and to begin the burn. To initiate the firing of the refuse chamber, set the timer of the refuse chamber (lower chamber) past stop. Generally it should be set to at 5 and 1/2 hours. (A yellow light will appear to indicate the refuse burner has fired)

8) The timer of the control chamber (upper chamber) should always be set for 30 minutes longer than the refuse chamber. Therefore, generally this will be 6 hours.

9) The following morning the incinerator will have automatically shut off at the time chosen, but the control fan will still be operating. Turn the system to off, and this will turn off the fan.

C. Ash Disposal
1) Dispose of the ashes when the metal container is one half full.
2) Wearing a mask, remove the plastic bag containing the ashes and place into a cardboard box of suitable size.
3) Tape the cardboard box shut and place the box in the dumpster.
Additional Information:

Even if the refuse (lower) chamber timer is turned to on, the refuse burner will not ignite until the control (upper) chamber has reached its pre-set temperature.

The magahelic gauge should always show a negative draft.

The fan (built into the North wall) is controlled and activated by the thermometer on the South wall (next to the louvers). For instance, if this thermometer is set at 70 degrees, the fan will turn on when the temperature in the room reaches 70 degrees, and turn off when the temperature drops below 70 degrees.

If the system fails to operate, the electronic controls may require manual reset. To do this, open the main front control panel to find the reset switch.

The temperature of the upper and lower chambers are pre-set.

The door latch operates a mechanical-electrical switch located on the lower left side of the door. Opening and closing of the door engages this switch. The purpose of the switch is to provide operator safety by disengaging the primary burner and the fan if the door is opened.

Emergency Numbers: Al Dale 494-0411
Phyllis Parker 986-0644

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

[Signatures and dates]
Title: Transporting Small Mammals by Air.

I. Purpose:
To provide guidelines for the preparation and air transport of small mammals.

II. Procedures:
A. Plan ahead. Anticipate a full shipment while trapping and make reservations for a flight which is the most direct to the animal's destination.

B. Notify the Animal Care personnel/receiving personnel of a shipment of small mammals to arrive for quarantine, provide a QA number or PS title, the name of the airlines, the flight number, and estimated time of arrival.

C. Obtain the completed veterinary inspection report, the interstate shipping permit (if required) and a copy of the air waybill must accompany the shipping containers.

D. Arrive at the shipping airport at least 1 hour before the animal's scheduled reservations; (note any changes that have occurred in the flight schedule and notify receiving personnel of any changes).

III. Specifications:
A. The shipping containers should be appropriately sized for the species being shipped and meet the requirements of 9 CFR Ch. 1 Subpart F.

B. Adequate absorbent materials and food stuffs must be provided for the specific species (ie.: rodent chow and carrots for pocket gophers, or rodent chow and apple pieces for mice).

C. Sufficient air space in the shipping containers and/or the transport crates must meet the requirements specified in 9 CFR Ch. 1 Subpart F and the containers/crates must be clearly and prominently identified with the works: LIVE ANIMALS.
D. The amount of time the small mammals spend in the shipping containers/transport crates should be reduced as much as possible.

E. When the animals are placed in the shipping containers/transport crates they must be protected at all times from direct sunlight, temperature extremes, and inclement weather, and must be transported in a covered vehicle to and from the trapping site and the shipping airlines.

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

Kathleen A. Ferguson
(Section Chief)
3/13/98
(Date)

J. Edward Mitchell
(Quality Assurance Officer)
3/16/95
(Date)

Donald C. King
(Animal Care Committee)
6/20/95
(Date)
STANDARD OPERATING PROCEDURE

Title: Computer generated random numbers.

I. Purpose:
   A. To provide guidelines for computer generated random numbers.

II. Procedure:
   A. Enter the Epistat program by Tracy L. Gustafson, M.D., and specify the random number generator program called "Randomiz".
      1. Select a survey sample from a population to generate a random number set.
      2. Enter the print mode: either screen viewing or printed.
   B. Enter the smallest number, the largest number and how many random numbers between the smallest and largest numbers are needed.
   C. This program will allow multiple randomizations by asking "Do you want to perform another randomization?". Enter y for yes and n for no.

III. Specifications:
   A. The random sample generator in the statistical program called "Epistat" aids in the selection of random samples for several purposes.
   B. It can provide a random subset of a larger population or it can assign cases randomly to independent or paired groups.
I have read this document and approve of its contents. I certify that it will be made available to all appropriate personnel.

Kathleen A. Fagerstone  
Section Chief  
4/14/94  Date

V. Clay Mitchell  
Quality Assurance Officer  
4/15/94  Date
STANDARD OPERATING PROCEDURE

Title: Ground Transport of Pocket Gophers and Other Small Mammals

I. Purpose:

A. To provide guidelines and standardize a method for shipping/transporting pocket gophers or other small mammals humanely from the airport or the field to the laboratory.

II. Specifications:

A. The container size should be appropriate for the size of the pocket gopher or small mammal and meet the requirements of 9 CFR Ch. 1 Subpart F.

B. Safe handling procedures should be followed for the small mammals such as mice (see WRC SOP-548)

C. Pocket gophers or other wild small mammals should be dusted with flea powder upon removal from the trap and then placed in individual shipping containers.

D. Absorbent materials, appropriate foodstuffs, and a water source should be provided the pocket gopher or small mammals when placed in the shipping container.
E. Any traps or containers with pocket gophers or other small mammals in them must be protected from direct sunlight, temperature extremes, inclement weather at all times, and must be transported in a covered vehicle. Small mammals, such as mice, may be transported in a covered pickup bed with more than adequate bedding for cold temperatures, keep the vehicle out of direct sunlight, and the area must be well ventilated.

III. Procedure:

A. Notify the animal care personnel that a shipment of pocket gophers or other small mammals is expected to arrive for quarantine and their estimated time of arrival.

B. Prepare shipping containers. If it is safer, and the time period the animals will be held in transport less than 1 hour, pocket gophers or other small mammals may be temporarily transported in live traps while in the field.

1. Shipping containers to hold pocket gophers or other small mammals should contain absorbent bedding materials, appropriate foodstuffs, and a water source.

2. Remove the pocket gopher or small mammal from the live trap and dust with flea powder before placing the animal in the shipping container.

3. Pocket gophers and other small mammals must be protected from direct sunlight, temperature extremes, and inclement weather at all times.

C. All small mammals should be transported in a covered vehicle directly to their assigned quarantine space; again, mice should be handled according to WRC SOP-548 in case of the possible existence of an infection.

D. All small mammals shipped in commerce must meet the requirements specified in 9 CFR Ch. 1 Subpart F, which requires health certificates and appropriate shipping containers. Animal care personnel should be notified if arrangements are needed for prompt pickup of a shipment from an airport terminal or other drop off point.

E. After each shipment, all bedding, fecal material, and feed should be removed from the shipping containers or live traps and properly disposed before cleaning and disinfecting (steam cleaning or dishwasher cleaning) shipping containers or live traps for the next shipment (see WRC SOP-548).
I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

_____________________________  8/28/96
Kathleen A. Fegerston  
(Section Chief)  

_____________________________  9/18/96
Lydia J. Rand  
(Quality Assurance Officer)  

_____________________________  9/18/96
William E. Rowland  
(Animal Care Committee)  

(Dates)
STANDARD OPERATING PROCEDURE

Title: Incineration of Animal Carcass and Tissue

I. Purpose:
   A. To provide guidelines and standardize a method of disposal for animal carcasses from field and laboratory testing and spent tissue samples.

II. Specifications:
   A. Tissues will be inventoried and recorded in a permanent log for archiving. Specimens will then be placed in plastic bags adequate for transport and sealed.
   B. The package to be incinerated should be double bagged before leaving the storage area to avoid any leakage and contamination in transport.
   C. Disposable latex gloves and laboratory coat are recommended apparel for safety.
   D. See WRC-233.R2 for other safety tips and incinerator operation.

III. Procedure:
   A. Coordinate with Animal Care Section as to when incinerator will be available.
   B. See WRC-233.R2 for incinerator use and operation.
   C. Transport packages to be incinerated as soon as possible after double bagging and preparation for transport.
I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

[Signature]
Section Chief

3-19-92
(Date)

[Signature]
[Name]
Quality Assurance Officer

3/26/92
(Date)

[Signature]
[Name]
Animal Care Committee

3/17/92
(Date)
STANDARD OPERATING PROCEDURE

HAZARD COMMUNICATION

I. PURPOSE:

To ensure the chemical hazards present within the laboratories of the Denver Wildlife Research Center are evaluated and the information concerning those hazards is transmitted to the employee as specified by the Occupational Safety and Health Administration (OSHA) in 29 CFR 1910.1200 (b)(3).

II. PROCEDURE:

A. Labels

1. All containers of incoming chemicals shall be checked for a visible and legible label which should contain the following information in English:
   a. The chemical name (and common name if available).
   b. The appropriate hazard warnings.
   c. The name and address of the manufacturer or distributor.

2. Chemicals received without a label shall not be used and must be returned to the distributor or manufacturer as soon as possible.

3. The label or the information contained on the label will not be removed, covered, obscured, altered, or defaced.

B. Material Safety Data Sheet (MSDS)

1. A MSDS must exist for all chemicals on hand. If an MSDS does not exist, then one must be requested from the distributor or manufacturer, or created according to the criteria in 29 CFR 1910.1200(g).
2. No chemical shall be dispensed or used without an MSDS on hand.

3. A collection of MSDS's for every chemical on hand must be kept in a readily accessible location.

C. Employee Awareness

1. Supervisors are responsible to ensure employees are informed of any chemical hazards which may exist in the workplace and are trained on how to work safely with those chemicals. Contact the DWRC Chemical Hygiene Officer for training materials or guidance. Training shall include:
   a. How to read and understand a chemical label.
   b. How to read and understand an MSDS.
   c. How to select and use protective equipment.
   d. What to do in case of an emergency.
   e. The location of the chemicals, MSDS's, fire extinguishers, fire alarms, spill kits, safety equipment, personal protective equipment, emergency eye washes and showers, and emergency escape routes.

D. Shipping Chemicals

1. The shipment of chemicals from a laboratory shall be in compliance with the same regulations which govern chemical shipments from manufacturers or distributors:
   a. Containers shall be labeled with the same information listed in part II.A.1 of this SOP (in accordance with 29 CFR 1910.1200(t)(f)).
   b. Recipients of the shipment shall be provided an MSDS which has been prepared in accordance with 29 CFR 1910.1200(g). The MSDS shall be either placed in the shipment container or sent directly to the recipient at the time of shipment.

I have read this document and approve of its contents. I certify that it will be made available for all personnel to whom it applies.

[Signature]
DWRC Quality Assurance Officer

[Signature] 9/1/94
Date

Page 108 of 132
STANDARD OPERATING PROCEDURE

Title: Mouse Maintenance

I. Purpose:
   A. To standardize mouse maintenance.
      1. Records on animals shall be stored in designated area at the close of each day.
      2. Feeding schedules, light schedules, and room temperatures will be maintained without change throughout testing periods. To improve health status of the animals, adjustments to these schedules may occur at other times. Ensure the proper schedules are in place.
      3. Cages will be labeled with index cards or tape with the ID# of the animal.
      4. If an animal is found dead, securely attach the ID# to the animal, or to the bag in which the animal is placed. Follow the study protocol for disposal. If the animal is not subject to a test protocol, consult your supervisor.
      5. Lighting will be on a 12 hour light-dark cycle, the temperature will be maintained as close to 70 degrees as possible, and the doors to the animal room will be locked when personnel are not engaged in providing care.
      6. Between tests, when the room is vacant, the room will be thoroughly cleaned and sanitized.

II. Procedure:
   Daily
   1. Check each animal to ensure the animal appears normal. Update daily report, noting abnormalities. Request additional checking from your supervisor if you are uncertain. Report all ill, injured, or dead animals.
   2. Water is offered ad libitum. Bottles are refilled
daily as required.
3. Follow feeding schedule.
4. Record maximum, minimum and ambient room temperatures.
5. Be certain the animal room and the ante room is clean and neat upon completion of providing daily care.

**Tuesday**
1. Replace rack and cages, and replace watering and feeding utensils. (See WRC-216, on secure identification)
2. Mop floors using a disinfectant.

**Mondays, Wednesdays and Friday**
1. Change papers under cages.

**Monthly**
1. Inspect the outlet room air filter at the entrance to the room above the door on the last Thursday of each month and replace as needed.

**As needed**
1. Whenever a cage becomes soiled, move the animals to a clean cage, taking care to maintain identity. (see WRC-216, on secure identification).
2. Wipe down racks and surfaces to maintain cleanliness.
3. Sweep, and or, mop floors to maintain cleanliness.
Emergency Numbers:  Al Dale  494-0411
Phyllis Parker  986-0644

or call the Principal Investigator as listed in the testing protocol.

I have read this document and approve of its contents. The document will be made available to all personnel to which it applies.

[Signatures]

Unit Chief  4/10/96

Quality Assurance Officer  4/17/96

Animal Care & Use Committee  4/17/96
Appendix IV. Bait Analysis - 0.01% Chlorophacinone Oat Groat Bait
To: George Matschke  
Research Wildlife Biologist  
Product Development Section  
Subject: 0.01% Chlorophacinone S. R. O. Bait Assay (QA-506)  
Method: 62A  
Analysis Date(s): 12-24-96  
AC Notebook Reference: AC 56:pages 8-12  
QC Notebook Reference: QC 11:pages 138-139  
Analyst: Stephanie A. Volz  

Sample Description:  

S960320-1 One sample of 0.01% Chlorophacinone S. R. O. Bait received on March 20, 1996. Previously assayed (invoice #96-023) with a reported observed concentration of 0.0114% chlorophacinone.  

S961219-1 One sample of 0.01% Chlorophacinone S. R. O. Bait was received on December 19, 1996.  

Method Modification(s)/Comments:  

Each sample was ground prior to analysis using a blender. Five replicate weighings of each sample were assayed. The data were not corrected for QC recovery.
# Results:

**Chlorophacinone Bait Assay**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Date of Analysis</th>
<th>% Chlorophacinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>S960320-1A</td>
<td>12-24-96</td>
<td>0.0118</td>
</tr>
<tr>
<td>-1B</td>
<td></td>
<td>0.0114</td>
</tr>
<tr>
<td>-1C</td>
<td></td>
<td>0.0118</td>
</tr>
<tr>
<td>-1D</td>
<td></td>
<td>0.0122</td>
</tr>
<tr>
<td>-1E</td>
<td></td>
<td>0.0115</td>
</tr>
<tr>
<td>S961219-1A</td>
<td>12-24-96</td>
<td>0.00992</td>
</tr>
<tr>
<td>-1B</td>
<td></td>
<td>0.00948</td>
</tr>
<tr>
<td>-1C</td>
<td></td>
<td>0.00924</td>
</tr>
<tr>
<td>-1D</td>
<td></td>
<td>0.00959</td>
</tr>
<tr>
<td>-1E</td>
<td></td>
<td>0.00938</td>
</tr>
</tbody>
</table>

Mean = 0.0117%

\[ SD = 0.00031\% \]

\[ CV = 2.6\% \]

**Quality Control Results**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Observed</th>
<th>% Chlorophacinone</th>
<th>Target %</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC-1</td>
<td>Not Detected</td>
<td></td>
<td>Control</td>
<td>N/A</td>
</tr>
<tr>
<td>QC-2</td>
<td>0.00858</td>
<td>0.00915</td>
<td>93.8</td>
<td></td>
</tr>
<tr>
<td>QC-3</td>
<td>0.00825</td>
<td>0.00923</td>
<td>89.4</td>
<td></td>
</tr>
<tr>
<td>QC-4</td>
<td>0.00909</td>
<td>0.00977</td>
<td>93.0</td>
<td></td>
</tr>
</tbody>
</table>

Mean = 92.1%

\[ SD = 2.3\% \]

\[ CV = 2.5\% \]

cc: M. Goodall, J. Johnston, G. McCann, E. Petty, S. Volz
Appendix V. Bait Analysis - 0.005% Chlorophacinone Wax Bait Blocks
To: George Matschke  
Research Wildlife Biologist  
Product Development Section  

Subject: 0.005% Chlorophacinone S.R. O. Grain/Paraffin Assay (QA-506)  

Method: 64B  
Analysis Date(s):  1-9-97  
AC Notebook Reference: AC 56:pages 13-16  
QC Notebook Reference: QC 11:pages 142  
Analyst: Stephanie A. Volz  

Sample Description:  

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Cross Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>S970108-1</td>
<td>#18</td>
<td>One sample of 0.005% Chlorophacinone Grain/Paraffin Bait received on January 8, 1997.</td>
</tr>
<tr>
<td>S970108-2</td>
<td>#7</td>
<td>One sample of 0.005% Chlorophacinone Grain/Paraffin Bait received on January 8, 1997.</td>
</tr>
</tbody>
</table>

Method Modification(s)/Comments:  

Each sample was ground prior to analysis using a hand operated meat grinder. A subsample was then ground again using a Black & Decker coffee mill. Three replicate weighings of each sample were assayed in duplicate. The data were not corrected for QC recovery.
Results:

Chlorophacinone Grain/Paraffin Assay

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Date of Analysis</th>
<th>% Chlorophacinone</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>S970108-1A</td>
<td>1-9-97</td>
<td>0.00629</td>
<td></td>
</tr>
<tr>
<td>-1B</td>
<td>&quot;</td>
<td>0.00613</td>
<td></td>
</tr>
<tr>
<td>-1C</td>
<td>&quot;</td>
<td>0.00596</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean = 0.0061%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 0.00017%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CV = 2.8%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Date of Analysis</th>
<th>% Chlorophacinone</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>S970108-2A</td>
<td>1-9-97</td>
<td>0.00572</td>
<td></td>
</tr>
<tr>
<td>-2B</td>
<td>&quot;</td>
<td>0.00556</td>
<td></td>
</tr>
<tr>
<td>-2C</td>
<td>&quot;</td>
<td>0.00545</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean = 0.0056%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 0.00014%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CV = 2.5%</td>
<td></td>
</tr>
</tbody>
</table>

Quality Control Results

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Observed Average</th>
<th>% Chlorophacinone</th>
<th>Target %</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC-1</td>
<td>0.00461</td>
<td>0.00484</td>
<td>0.00489</td>
<td>95.2</td>
</tr>
<tr>
<td>QC-2</td>
<td>0.00478</td>
<td>0.00489</td>
<td>0.00494</td>
<td>97.8</td>
</tr>
<tr>
<td>QC-3</td>
<td>0.00491</td>
<td>0.00494</td>
<td>Control</td>
<td>99.4</td>
</tr>
<tr>
<td>QC-4</td>
<td>Not Detected</td>
<td>Control</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

Mean = 97.5%
SD = 2.1%
CV = 2.2%

cc: M. Goodall
J. Johnston
E. Petty
S. Volz
Appendix VI. Raw Data Summaries: Survival, Consumption of 0.005% Chlorophacinone Wax Bait Blocks, OPP Rat and Mouse Challenge Diet, and Body Weights.
Appendix VI

Raw Data Summary 1. Dates house mice died during testing (Day 1 = March 23, 1997).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>Mar 26</td>
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<td>2</td>
<td>Mar 26</td>
<td>4</td>
<td>2</td>
<td>Mar 25</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mar 27</td>
<td>5</td>
<td>1</td>
<td>Mar 27</td>
<td>5</td>
<td>1</td>
<td>Mar 26</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Mar 29</td>
<td>7</td>
<td>1</td>
<td>Mar 28</td>
<td>6</td>
<td>2</td>
<td>Mar 28</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Apr 4</td>
<td>13</td>
<td>1</td>
<td>Apr 2</td>
<td>11</td>
<td>1</td>
<td>Mar 31</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Apr 6</td>
<td>15</td>
<td>1</td>
<td>Apr 5</td>
<td>14</td>
<td>1</td>
<td>Apr 2</td>
<td>11</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar 26</td>
<td>4</td>
<td>1</td>
<td>Mar 25</td>
<td>3</td>
<td>1</td>
<td>Mar 26</td>
<td>4</td>
<td>5</td>
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<tr>
<td>Mar 27</td>
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<td>2</td>
<td>Mar 26</td>
<td>4</td>
<td>2</td>
<td>Mar 30</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Mar 28</td>
<td>6</td>
<td>1</td>
<td>Mar 27</td>
<td>5</td>
<td>2</td>
<td>Apr 6</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Mar 30</td>
<td>8</td>
<td>3</td>
<td>Mar 28</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar 31</td>
<td>9</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix VI

Raw Data Summary 2. Bait consumption by 10 female house mice (Group I) on the OPP rat and mouse challenge diet during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Consumption (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dish O&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>14.39</td>
</tr>
<tr>
<td>2</td>
<td>12.85</td>
</tr>
<tr>
<td>3</td>
<td>16.67</td>
</tr>
<tr>
<td>4</td>
<td>9.28</td>
</tr>
<tr>
<td>5</td>
<td>10.63</td>
</tr>
<tr>
<td>6</td>
<td>18.76</td>
</tr>
<tr>
<td>7</td>
<td>22.67</td>
</tr>
<tr>
<td>8</td>
<td>18.59</td>
</tr>
<tr>
<td>9</td>
<td>18.56</td>
</tr>
<tr>
<td>10</td>
<td>12.84</td>
</tr>
<tr>
<td>11</td>
<td>17.15</td>
</tr>
<tr>
<td>12</td>
<td>14.47</td>
</tr>
<tr>
<td>13</td>
<td>14.89</td>
</tr>
<tr>
<td>14</td>
<td>17.65</td>
</tr>
<tr>
<td>15</td>
<td>18.89</td>
</tr>
<tr>
<td>N</td>
<td>15</td>
</tr>
<tr>
<td>Total (g)</td>
<td>238.29</td>
</tr>
<tr>
<td>Mean (g)</td>
<td>15.89</td>
</tr>
<tr>
<td>SD</td>
<td>3.57</td>
</tr>
</tbody>
</table>

<sup>a</sup>O = OPP rat and mouse challenge diet.
<sup>b</sup>C = OPP rat and mouse challenge diet.
Appendix VI

Raw Data Summary 3. Bait consumption by 10 male house mice (Group I) on the OPP rat and mouse challenge diet during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Dish O(^a) (g)</th>
<th>Dish C(^b) (g)</th>
<th>Total (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.48</td>
<td>25.71</td>
<td>36.19</td>
</tr>
<tr>
<td>2</td>
<td>15.14</td>
<td>25.10</td>
<td>40.24</td>
</tr>
<tr>
<td>3</td>
<td>17.45</td>
<td>26.35</td>
<td>43.80</td>
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<tr>
<td>4</td>
<td>17.52</td>
<td>21.59</td>
<td>39.11</td>
</tr>
<tr>
<td>5</td>
<td>16.82</td>
<td>22.14</td>
<td>38.96</td>
</tr>
<tr>
<td>6</td>
<td>21.79</td>
<td>19.59</td>
<td>41.38</td>
</tr>
<tr>
<td>7</td>
<td>19.20</td>
<td>21.26</td>
<td>40.46</td>
</tr>
<tr>
<td>8</td>
<td>22.32</td>
<td>27.23</td>
<td>49.55</td>
</tr>
<tr>
<td>9</td>
<td>17.02</td>
<td>24.07</td>
<td>41.09</td>
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<tr>
<td>10</td>
<td>20.25</td>
<td>24.04</td>
<td>44.29</td>
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<tr>
<td>11</td>
<td>16.63</td>
<td>24.10</td>
<td>40.73</td>
</tr>
<tr>
<td>12</td>
<td>19.51</td>
<td>25.53</td>
<td>45.04</td>
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<td>13</td>
<td>17.86</td>
<td>25.73</td>
<td>43.59</td>
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<tr>
<td>14</td>
<td>21.32</td>
<td>23.64</td>
<td>44.96</td>
</tr>
<tr>
<td>15</td>
<td>21.70</td>
<td>24.03</td>
<td>45.73</td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Total (g)</td>
<td>275.01</td>
<td>360.11</td>
<td>635.12</td>
</tr>
<tr>
<td>Mean (g)</td>
<td>18.33</td>
<td>24.01</td>
<td>42.34</td>
</tr>
<tr>
<td>SD</td>
<td>3.10</td>
<td>2.10</td>
<td>3.36</td>
</tr>
</tbody>
</table>

\(^a\)O = OPP rat and mouse challenge diet.
\(^b\)C = OPP rat and mouse challenge diet.
Appendix VI

Raw Data Summary 4. Bait consumption by 10 female house mice (Group II) on the OPP rat and mouse challenge diet and 0.005% chlorophacinone wax bait blocks during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Dish O(^a)</th>
<th>Wax bait block</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28.18</td>
<td>4.83</td>
</tr>
<tr>
<td>2</td>
<td>30.18</td>
<td>6.56</td>
</tr>
<tr>
<td>3</td>
<td>27.04</td>
<td>4.94</td>
</tr>
<tr>
<td>4</td>
<td>15.36</td>
<td>2.60</td>
</tr>
<tr>
<td>5</td>
<td>12.17</td>
<td>1.08</td>
</tr>
<tr>
<td>6</td>
<td>9.71</td>
<td>0.49</td>
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<tr>
<td>7</td>
<td>13.84</td>
<td>1.49</td>
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<td>8</td>
<td>14.45</td>
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<td>8.30</td>
<td>0.26</td>
</tr>
<tr>
<td>11</td>
<td>14.00</td>
<td>0.47</td>
</tr>
<tr>
<td>12</td>
<td>12.97</td>
<td>0.90</td>
</tr>
<tr>
<td>13</td>
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<td>1.11</td>
</tr>
<tr>
<td>15</td>
<td>11.02</td>
<td>1.36</td>
</tr>
</tbody>
</table>

\(N\) | 15 | 15 |

Total (g) | 228.06 | 28.14 |
Mean (g) | 15.20 | 1.88 |
SD | 7.21 | 1.97 |

\(^a\)O = OPP rat and mouse challenge diet.
Appendix VI

Raw Data Summary 5. Bait consumption by 10 male house mice (Group II) on the OPP rat and mouse challenge diet and 0.005% chlorophacinone wax bait blocks during the 15-day, 2-choice, feeding trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Consumption (g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dish O&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wax bait block</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>31.16</td>
<td>5.96</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>31.66</td>
<td>7.66</td>
<td></td>
</tr>
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<sup>a</sup>O = OPP rat and mouse challenge diet.
Appendix VI

Raw Data Summary 6. Bait consumption by 10 female house mice (Group III) on the OPP rat and mouse challenge diet and 0.005% chlorophacinone wax bait blocks during the 15-day, 2-choice, feeding trial.

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<td>0.65</td>
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<td>8</td>
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<td>0.81</td>
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<tr>
<td>9</td>
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<tr>
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</table>

*O = OPP rat and mouse challenge diet.
Appendix VI

Raw Data Summary 7. Bait consumption by 10 male house mice (Group III) on the OPP rat and mouse challenge diet and 0.005% chlorophacinone wax bait blocks during the 15-day, 2-choice, feeding trial.

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<td>0.72</td>
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N = 15
Total (g) = 250.02
Mean (g) = 16.67
SD = 9.42

*O = OPP rat and mouse challenge diet.
Appendix VI

Raw Data Summary 8. Bait consumption by 10 female house mice (Group IV) on the OPP rat and mouse challenge diet and 0.005% chlorophacinone wax bait blocks during the 15-day, 2-choice, feeding trial.

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<td>0.86</td>
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<td>8</td>
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<td>14</td>
<td>5.97</td>
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</table>

*O = OPP rat and mouse challenge diet.
Appendix VI

Raw Data Summary 9. Bait consumption by 10 male house mice (Group IV) on the OPP rat and mouse challenge diet and 0.005% chlorophacinone wax bait blocks during the 15-day, 2-choice, feeding trial.

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aO = OPP rat and mouse challenge diet.
Appendix VI

Raw Data Summary 10. Bait consumption of the OPP rat and mouse challenge diet by surviving house mice during the 5-day post-treatment feeding period (Groups I and II).

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<th>Males</th>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
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<td>4</td>
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Group II

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</tr>
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<td></td>
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<tr>
<td>5</td>
<td>16.07</td>
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<td>48.39</td>
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Appendix VI

Raw Data Summary 11. Bait consumption of the OPP rat and mouse challenge diet by surviving house mice during the 5-day post-treatment feeding period (Groups III and IV).

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<td>Males</td>
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</table>

| Group IV |                      |          |
| 1        | 6.74                 | 4.16     |
| 2        | 6.18                 | 7.02     |
| 3        | 7.87                 | 8.25     |
| 4        | 7.87                 | 8.40     |
| 5        | 8.38                 | 8.68     |
| Total    | 37.04                | 36.51    |
| Mean     | 7.41                 | 7.30     |
| SD       | 0.91                 | 1.87     |
Appendix VI

Raw Data Summary 12. Individual house mice body weights recorded before their placement on the 15-day, 2-choice, feeding trial.

<table>
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<th>Group II (g)</th>
<th>Group III (g)</th>
<th>Group IV (g)</th>
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<td>18.3</td>
<td>18.3</td>
<td>18.2</td>
<td>18.6</td>
</tr>
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<td>213.7</td>
<td>214.3</td>
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</tr>
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<td>1.74</td>
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<td>1.82</td>
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</table>

|                |             |              |               |              |
| **Males**      |             |              |               |              |
|                | 23.7        | 24.7         | 23.9          | 24.5         |
|                | 22.4        | 22.8         | 23.4          | 23.1         |
|                | 22.2        | 21.8         | 22.2          | 22.4         |
|                | 21.8        | 21.8         | 21.6          | 21.6         |
|                | 21.4        | 21.3         | 21.2          | 21.5         |
|                | 21.1        | 21.2         | 21.3          | 21.0         |
|                | 20.9        | 20.7         | 20.7          | 20.6         |
|                | 20.4        | 20.4         | 20.5          | 20.0         |
|                | 19.8        | 19.2         | 19.1          | 19.8         |
|                | 18.9        | 17.6         | 19.1          | 18.9         |
| **Total**      | 212.6       | 211.5        | 213.0         | 213.4        |
| **Mean**       | 21.26       | 21.15        | 21.30         | 21.34        |
| **SD**         | 1.38        | 1.93         | 1.59          | 1.67         |
### Appendix V

**Raw Data Summary 13.** Individual house mice body weights of survivors recorded at the termination of the study.

<table>
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<td>26.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>238.0</td>
<td>92.2</td>
<td>82.90</td>
<td>47.4</td>
</tr>
<tr>
<td>Mean</td>
<td>23.80</td>
<td>23.05</td>
<td>20.72</td>
<td>23.70</td>
</tr>
<tr>
<td>SD</td>
<td>2.04</td>
<td>1.48</td>
<td>1.69</td>
<td>2.26</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>259.9</td>
<td>58.4</td>
<td>79.9</td>
<td>44.3</td>
</tr>
<tr>
<td>Mean</td>
<td>25.99</td>
<td>29.20</td>
<td>26.63</td>
<td>22.15</td>
</tr>
<tr>
<td>SD</td>
<td>1.70</td>
<td>1.98</td>
<td>3.81</td>
<td>1.63</td>
</tr>
</tbody>
</table>

*One of these 4 body weights in Group III belongs in Group IV.*
Appendix V

Raw Data Summary 14. Individual house mice body weights recorded at the time of death.

<table>
<thead>
<tr>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body wt. (g)</td>
<td>Date</td>
<td>Body wt. (g)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>112.54</td>
<td></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>18.76</td>
<td></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>4.50</td>
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</table>

<table>
<thead>
<tr>
<th>Males</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. (g)</td>
<td>Date</td>
<td>Body wt. (g)</td>
<td>Date</td>
</tr>
<tr>
<td>22.68</td>
<td>3/27/97</td>
<td>23.01</td>
<td>3/26/97</td>
</tr>
<tr>
<td>18.94</td>
<td>3/30/97</td>
<td>27.29</td>
<td>3/28/97</td>
</tr>
<tr>
<td>16.99</td>
<td>3/31/97</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>154.79</td>
<td></td>
<td>158.07</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>19.35</td>
<td></td>
<td>22.58</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>3.15</td>
<td></td>
<td>3.55</td>
</tr>
</tbody>
</table>

*Animals were fed upon by surviving mice; animals were not used in the statistics.*
RESTRICTED USE PESTICIDE
Due to Hazards to Nontarget Organisms
For retail sale to and use only by Certified Applicators or persons under their direct supervision and only for those uses covered by the Certified Applicator's certification.

RODENT BAIT
CHLOROPHACINONE
TREATED GRAIN (0.01%)

Active Ingredient:
Chlorophacinone (2-[(p-Chlorophenyl)phenyl acetyl]-1,3-indandione)...........0.01%

Preparation Formulation:
Granular

Environmentally Hazardous
This product is toxic to fish, birds, and other wildlife. Exposure to soils may be hazardous to birds and other wildlife. Dogs and other predatory and scavenging animals might be poisoned if they feed upon animals that have eaten this bait. Do not apply directly to water. In areas where ground squirrels consume forage such as alfalfa and high-water mark. Do not contaminate water when disposing of equipment wash water or runoff.

ENDANGERED SPECIES CONSIDERATIONS
Notice: Killing of an endangered species may result in fine and/or imprisonment under the Endangered Species Act. Use of this product may pose a hazard to a Federally designated endangered/threatened species. For protection of federally listed species, users shall consult the U.S. EPA Endangered Species Office for the county in which the application will occur. A copy of the bulletin may be obtained from the county agricultural commissioner or downloaded from the internet at the following website: http://www.cdpr.ca.gov/docs/endspec/prescint.htm

CAUTION
KEEP OUT OF REACH OF CHILDREN

FIRST AID
Anticoagulant (Bis-hydroxycoumarin class)
Have the product container or label with you when calling a poison control center or doctor, or going for treatment.

If swallowed:
è Call a poison control center or doctor immediately for treatment advice.
è Have person up a glass of water if able to swallow.
è Do not induce vomiting unless told to do so by the poison control center or doctor.
è Do not give anything by mouth to an unconscious person.

If on Skin:
è Take off contaminated clothing.
è Wash skin immediately with plenty of water for 15-20 minutes.
è Call a poison control center or doctor for further treatment advice.

If Inhaled:
è Move person to fresh air.
è If breathing is not normal or is stopped, give artificial respiration immediately.
è Call a poison control center or doctor for further treatment advice.

If In Eyes:
è Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.
è Call a poison control center or doctor for further treatment advice.

STORAGE AND DISPOSAL
Do not contaminate water, food, or feed by storage and disposal.
Nonrefillable container. Do not reuse or refill this container. Offer for recycling if available.

Pesticide Storage: Store in a dry place. Do not store near the home. Store in a locked enclosure or other location not accessible to children, pets, domestic animals or wildlife.

Pesticide Disposal: Waste resulting from the use of this product may be disposed of on site or at an approved waste disposal facility. Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, sprayer mixtures, or rinsates is a violation of Federal Law. If these wastes cannot be disposed of by the user according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Water representatives at the nearest EPA Regional Office for guidance.

Container Handling: Completely empty bag into application equipment. Thoroughly clean any supply bag in a normal hayfield, by irrigation, or release it into the field after use for control of weeds. Do not use in or near wells, lakes, ponds, fish-bearing streams, aquifers, irrigation return flows, or irrigation reservoirs. Do not contaminate water when disposing of equipment wash water or runoff.

Endangered Species Considerations
This product contains chlorophacinone, an anticoagulant that is toxic to fish, birds, and other wildlife. This is a dangerous toxin that is not easily removed from the environment. This product is toxic to birds and other wildlife. Dogs and other predatory and scavenging animals might be poisoned if they feed upon animals that have eaten this bait. Do not apply directly to water. In areas where ground squirrels consume forage such as alfalfa and high-water mark. Do not contaminate water when disposing of equipment wash water or runoff.

Note to Physician
Contains chlorophacinone, an anticoagulant that is toxic to fish, birds, and other wildlife. For dogs that have ingested or that are suspected of having ingested chlorophacinone, and/or have observed poisoning symptoms, such as bleeding or have elevated prothrombin times, give Vitamin K1 to follow: subcutaneous injection. For anticoagulants with long half-lives, if known, it might be necessary to check prothrombin times every 3-7 days until values return to normal. See ‘Note to Physician’ for additional information.

Note to Veterinarian
Contains chlorophacinone, an anticoagulant that is toxic to fish, birds, and other wildlife. For dogs that have ingested or that are suspected of having ingested chlorophacinone, and/or have observed poisoning symptoms, such as bleeding or have elevated prothrombin times, give Vitamin K1 to follow: subcutaneous injection. For anticoagulants with long half-lives, if known, it might be necessary to check prothrombin times every 3-7 days until values return to normal. See ‘Note to Physician’ for additional information.

DIRECTIONS FOR USE
It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

This PRODUCT MAY ONLY BE DISTRIBUTED AND USED BY CALIFORNIA
AGRICULTURAL COMMISSIONERS.

READ THIS LABEL: Read this entire label and follow all use directions and precautions.

IMPORTANT: Store product not in use in a location out of reach of children, pets, domestic animals, and wildlife. Dispose of product container, unused, spoiled, or un consumed bait as specified in the "STORAGE AND DISPOSAL" section of this label.

Use Restrictions – for All Uses
This product may only be used to control CALIFORNIA GROUND SQUIRRELS [Spermophilus beecheyi], MEADOW MICE [Callosperomys volans (voles californianus)] and MONTANE VOLES [O. montanus], and POCKET Gophers [ Thomomys spp.] at the use sites and using the application methods identified in the "USE RESTRICTIONS" paragraphs indicated below for the target species groupings. Contact your local County Agricultural Commissioner's office if you need help in identifying the target species you intend to control or are not sure about the limits of the site designations.

Do not apply this product in or around homes or other human residences. Do not apply this bait or sites to control pests not indicated on this label. Do not apply this product by aircraft application methods not specified in this label. Do not bait piles.

Do not apply this product in occupied habitat of endangered species that may be harmed by exposure to this bait or to animals that have consumed it. Contact your local County Agricultural Commissioner's office or http://www.cdpr.ca.gov/docs/endspec/prescint.htm for information on endangered species that used this product in your area might impact.

Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protect handlers may be in the area during application. Keep all other persons out of the treatment area during application.

Do not apply bait in or near actively growing food or food crops or in areas where contaminating food or surfaces that may contact food is likely. Do not apply bait by aircraft at sites where bait may contaminate bodies of water. When applying bait along canals and other waterways, ensure that equipment that directs bait away from the water.

Follow-Up Operations – for All Uses
Collect dead rodents and dispose of them by deep burying, burning (if permitted in your County or community), or by double-plastic bagging or wrapping in newspaper and discarding them with trash. Do not apply plastic gloves or other suitable hand protection if you must pick up carcasses by hand.

VINEARDS, ORCHARDS, GROVES, FORESTRY PLANTATIONS, PASTURES, RANGELAND AND SELECTED NICHOPRODUCTS

Use Restrictions: This product may be used to control California Ground Squirrels [Spermophilus beecheyi] in broadcast bait applications in vineyards, orchards and groves (non-bearing season only), vine-brood crops, and fallow lands, along sides of fences rows and rights-of-way adjacent to canal banks, ditch banks, highways, levees, railroad lines, and utilities, and inannels, and in recreational areas, interior pastures, plantations of forest trees, pastures and rangeland.

DO NOT APPLY TO ENDANGERED SPECIES

California Ground Squirrels

Use Restrictions: This product may be used to control California Ground Squirrels [Spermophilus beecheyi] in broadcast bait applications in vineyards, orchards and groves (non-bearing season only), vine-brood crops, and fallow lands, along sides of fences rows and rights-of-way adjacent to canal banks, ditch banks, highways, levees, railroad lines, and utilities, and inannels, and in recreational areas, interior pastures, plantations of forest trees, pastures and rangeland.

Do not graze livestock, or plant food or feed crops in spot-treated areas while bait is present. Applications in orchards, groves, and vineyards may only be made after harvest and during the dormant period and may not be made after true and vine growth resumes in the spring.

Acceptable Treatments: Before applying toxic bait, test for target species' readiness to accept treatment using untreated crimped oat grains to portions of the inland water. Using flaging or other similar items, mark areas where oats were scattered and return on the following day to observe where oats are being consumed. Monitor sites that appear to have consumed oats and site that appear to have consumed oats and areas that appear to have consumed oats. Do not apply toxic bait if it appears that nontarget species are primarily responsible for consuming the oats.

Apply toxic bait only if ground squirrels appear to have accepted the oats readily. Untreated crimped oat grains may be obtained from the County Agricultural Commissioner’s office.

Broadcast baiting: Using a ground-based mechanical spreader or aircraft, apply bait at 10 pounds per acre swath, depending upon the density of ground squirrels. Make a second application 4 days after the first treatment.
MEADOW MICE (VOLES)

Broadcast Baiting
(Mechanical Spreader / Aircraft)

USE RESTRICTIONS: This product may be used to control California voles and montane voles in dormant season applications only in orchards, groves, and vineyards; in noncrop borders and fallow lands; along the outside of fence rows and rights-of-way adjacent to canal banks, ditch banks, highways, levees, railroad lines, and utilities; in campgrounds, recreational areas, horticultural nurseries, plantations of forest trees, pastures and rangeland.

Do Not: graze livestock or plant food or feed crops in spot-treated areas while bait is present. Applications in orchards, groves, and vineyards may only be made after harvest or during the dormant period and may not be made after tree and vine growth resumes in the spring.

BROADCAST BAITING: Using a ground-based mechanical spreader or aircraft, apply bait at 6-10 pounds per swath acre, depending upon the density of voles. Make a second application 4 days after the first treatment.

VINEYARDS, ORCHARDS, GROVES, AGRICULTURAL CROPS, RANGELAND, FORESTRY PLANTATIONS AND SELECTED NONCROP AREAS

POCKET GOPHERS

Underground Baiting

USE RESTRICTIONS: This product may be used only in manual, subterranean applications to control pocket gophers (Thomomys spp.) in orchards, groves, vineyards, agricultural crops (forage crops, grain and edible seed crops, oil crops, fiber crops, fruits, and vegetable crops), rangeland, and non-crop areas (fallow lands, campgrounds, recreational areas, horticultural nurseries, rights-of-way adjacent to canal banks, ditch banks, highways, levees, railroad lines, and utilities).

Bait must be applied directly into pocket gophers underground tunnels. Do not apply bait above the ground surface. Do not apply this product by use of a burrow-builder machine.

MANUAL BAITING: Using a burrow probe or other suitable implement, probe ground around fresh pocket gopher mounds to locate the main underground tunnel. Begin probing on the flat side of the fan-shaped mound, 6 to 12 inches away from the plug. When resistance against the probe drops suddenly, the main tunnel has been located. Carefully rotate probe to enlarge opening. Using a funnel, place ½ cup of bait into the main tunnel. Remove the funnel and close the hole using a clod of soil or a rock, taking care not to allow dirt to fall in and cover the bait. Make two bait sets per active burrow system. Four days after the first treatment, make a second round of treatments to all burrow systems that remain active (as determined by appearance of fresh mounds, new plug formations, or other signs of pocket gopher activity). Revised 3/8/11
Montane Vole Control with Rozol® Paraffinized Pellets in Orchards of the Pacific Northwest

David T. Bryson
LiphaTech, Inc., Gridley, CA

ABSTRACT: Rozol® Paraffinized Pellets (0.005% chlorophacinone) were effective in reducing populations of montane voles greater than the EPA standard of 70%. This test substance was applied using a Vicon spreader at the approximate rate of 10 lbs/acre to the vegetation of the orchard floor. An intensive mark-recapture census was used as a direct census to determine population change as a result of the test substance application. An apple slice index was used as an indirect method to confirm population change. Few carcasses were found during the carcass search practiced in this study. Determination of active ingredient residues was done on the vole carcasses found during the carcass search events.

KEYWORDS: apple orchard, chlorophacinone, deer mouse, efficacy, mark-recapture census, Microtus montanus, montane vole, Peromyscus maniculatus, rodent control, rodenticide, whole-body residue

INTRODUCTION

The montane vole (Microtus montanus) is the predominant vole species that causes damage to orchards of the Pacific Northwest. In one study, it was reported that montane vole damage resulted in losses in production of 21% from trees of a red delicious apple orchard and a 51% loss of production from trees in a golden delicious apple orchard (Askham 1988). Little published information is available about the biology of montane voles that reside in orchards of the Pacific Northwest. Vole populations tend to be cyclical in nature, and the amount of damage realized within an orchard can be dependent upon vole population numbers, amount and duration of snow, as well as age and planting density of trees. LiphaTech, Inc. is applying for a §3 Federal EPA registration of Rozol® Paraffinized Pellets (0.005% chlorophacinone) for use as a broadcast application at the rate of 10 lbs/acre to control voles in orchards. Rozol® Paraffinized Pellets have been used for years in orchards to control voles as allowed by 24(c) labels acquired at the state level.

During October 2002, a field efficacy study was performed to show that Rozol® Paraffinized Pellets could reduce vole populations in orchards by 70% or greater as required by EPA. This study was performed following Good Laboratory Practices (GLP) requirements.

METHODS AND MATERIALS

Study Site

The test and control plots were located in an orchard 7.5 miles south of the town of Ephrata in Grant County, Washington. Elevation at this site is approximately 1,280 feet above sea level. The tree variety of the orchard is red delicious, with crab apple trees and banana apple trees planted throughout the orchard to serve as pollinators. Trees were approximately 22 years old, planted with 10-foot spacing between trees within a row, and at a density of 242 trees per acre. The orchard is watered using sprinkler irrigation. Tall fescue is the predominant plant species growing on the orchard floor, with clover, dandelion, mallow, and orchard grass making up a small percentage of the vegetation.

Four treatment plots, in a block configuration, were used in this study. The block of 4 plots covered an area of 4.36 acres with each treatment repetition being 1.09 acres. Each of the 4 plot repetitions was broken up into 66 subplots of 720 square feet each. This grid pattern was established to help better document vole movement and home ranges by identifying where voles are trapped during the direct census event and found during the carcass search. Twenty-one of the subplots of each plot repetition served as a buffer zone between other plot repetitions within the block of 4 plot repetitions. The remaining 45 were used to calculate the efficacy of the trial through the direct census method used in this study. A buffer zone of approximately 23 feet around the perimeter of the block of 4 plot repetitions was treated with the test substance. The untreated control plots were set up in the same configuration except that a treated buffer was not established around the perimeter of the plots.

Population Census

Two methods were used to monitor for change in vole population within each treatment and untreated control plot repetition. A mark-recapture census (MRC) was used as a direct measure of the vole population and to obtain information on vole movement within the orchard. An apple slice index (ASI) was used as an indirect census method.

The MRC was performed 2 times, with each being a 2-consecutive-day event, in both the plots repetitions of the treatment and of the untreated control. A Sherman live-catch trap was placed in each of the subplots of a plot repetition for acclimation 3 days before the actual census event began. An acclimation period was performed on the 3 consecutive days preceding the pre-treatment MRC as well as during the 3 consecutive days preceding the post-treatment MRC. During the acclimation period, each trap door was clipped in the open position and oats...
were placed on the trap trigger pan and in front of the trap. A dime-sized portion of peanut butter was added to the inside trap surface on the last day of acclimation. At this time, the air temperature was low enough to preclude slugs and ants from being attracted to the peanut butter in the trap.

Trapping began immediately following the acclimation period. Trapping was only performed during the day since it was determined prior to the study, by snap-trapping areas of the orchard that were not included in the study, that the voles were mainly active during the day. Not trapping overnight avoided catching deer mice (*Peromyscus maniculatus*) and helped to avoid trap mortality that might have been realized during an overnight trapping period. Each trap was set in the morning and baited with oats, a dime-sized portion of peanut butter, and a small slice of potato to supply trapped animals with a source of moisture. Traps were checked and captured voles ear-tagged (or ear-tag numbers were recorded in the case of repeat catches), the afternoon of the same day they were set. All trap doors were left in the closed position after they were checked.

The ASI was performed 2 times. The first (pretreatment) ASI was done the day following the last day of the first (pretreatment) MRC event. A second ASI was performed 7 days after the test substance was broadcast in the orchard. ASI events helped in the determination of when the second (post-treatment) MRC could be done and served as a general indicator of population trend. To perform the ASI, 1 apple slice was placed in each of 10 randomly chosen subplots of a plot repetition. The slices were placed in the subplots during the morning and graded for activity the afternoon of that same day.

**Bait Application**

A Vicon Granular Applicator Model PS203, mounted on a John Deere 5400N orchard tractor, was used to apply the test substance. A low-flow kit, provided by the manufacturer of the Vicon Spreader, was used to plug 2 of the 3 discharge holes in the applicator hopper. The test substance was applied at approximately 10 lbs/acre (actual application was 10.16 lbs/acre) to the vegetation of the orchard floor. An applicator uniformity test at this rate showed that the test substance was broadcast at an average density of 5.5 pellets per square yard.

**Efficacy Determination**

Change between pretreatment MRC and post-treatment MRC events was determined for the treatment plot repetitions using the equation in Table 1. The change for the untreated control plot repetitions was calculated using the equation found in Table 2. Another equation was used to determine the recapture percentage. The recapture percentage is the percentage of animals captured, ear-tagged, and released during the first 2-day MRC event that were recaptured during the second 2-day MRC event. The equation used to calculate this percentage is found in Table 3.

The ASI gives more of a general measurement of population trend by measuring feeding activity. The grading of apple slices is shown in Table 4. Each score is then multiplied by the number of occurrences of that particular grading score, and the product of all grading categories are summed. This sum is divided by the number of apple slices placed in the plot repetition being indexed to give the ASI score for that repetition. The two scores obtained from the first ASI and the second ASI are then used in the equation presented in Table 5 to obtain the percentage change realized between the two ASI events in that particular plot repetition.

**Carcass Search / Wildlife Observations**

Both the treatment and untreated control plot repetitions were searched for carcasses by walking each area on a daily basis starting on the second day after application of test substance to the treatment plots. During the daily carcass search, the person performing the search walked all subplots at a pace that allowed visual scanning of the area of each subplot. Dead animals were collected, assigned unique identification numbers, and their locations noted by plot and subplot number.

Carcasses of *M. montanus* were frozen and shipped to the National Wildlife Research Center for whole-body analysis to determine chlorophacinone residues. The only other carcass found were those of *P. maniculatus*. These were frozen for subsequent necropsy to determine if signs of anticoagulant poisoning were present. During the study, observations regarding wildlife in the orchard were noted.

**RESULTS AND DISCUSSION**

**Efficacy**

The calculations of the MRC data used in determining the efficacy of the test substance in controlling *M. montanus* are found in Table 6. This intensive direct census technique showed that the test substance exceeded the required EPA standard of 70% population reduction required.

The results from the percentage recapture equation (Table 7) also show that within the treatment plots, very few individuals ear-tagged during the pre-treatment MRC were present during the post-treatment MRC. Table 8 shows the calculations used to determine the change in population between the pre-treatment MRC and the post-treatment MRC for the untreated control plot repetitions and the average change of all 4 repetitions.

The results from the percentage recapture equation in Table 9 show that within the untreated control, over one-third of the individuals ear-tagged during the pre-treatment MRC are present during the post-treatment MRC. This is a large percentage of recaptures, considering the population growth that the untreated control experienced between the pre-treatment MRC and the post-treatment MRC. When the average percentage recapture for the treatment plots is compared to the untreated control plots, there were 94.2% fewer recaptures in the treatment.

The ASI is more sensitive to environmental differences between plot repetitions. Two plot repetitions of the untreated control and two plot repetitions of the treatment were not used, due to such influences. Plot repetitions 1 and 2 of the untreated control were not
Table 1. Equation used to calculate the efficacy of test substance in controlling voles in the plot repetitions of the treatment.

\[
\begin{align*}
\text{Treated Block population change} \% & = \left( \frac{T29_{\text{surv}} + T30_{\text{surv}} - (T12 + T13_{\text{nt}})}{T12 + T13_{\text{nt}}} \right) \times 100 \\
\text{Where:} & \\
T12 & = \text{number of voles trapped on Oct 12.} \\
T13_{\text{nt}} & = \text{number of voles trapped on Oct 13 that did not have ear-tags from Oct 12.} \\
T29_{\text{surv}} & = \text{total number of voles captured on Oct 29, both with and without eartags.} \\
T30_{\text{surv}} & = \text{total number of voles captured on Oct 30 that were not captured on October 29.}
\end{align*}
\]

Table 2. Equation used to calculate the change in population numbers between mark-recapture census (MRC) events performed in the repetitions of the untreated control.

\[
\begin{align*}
\text{Control Block population change} \% & = \left( \frac{C29_{\text{surv}} + C30_{\text{surv}} - (C12 + C13_{\text{nt}})}{C12 + C13_{\text{nt}}} \right) \times 100 \\
\text{Where:} & \\
C12 & = \text{number of voles trapped on Oct 12.} \\
C13_{\text{nt}} & = \text{number of voles trapped on Oct 13 that did not have ear-tags from Oct 12.} \\
C29_{\text{surv}} & = \text{total number of voles trapped on Oct 29, both with and without eartags.} \\
C30_{\text{surv}} & = \text{total number of voles captured on Oct 30 that were not captured on Oct 29.}
\end{align*}
\]

Table 3. Equation used to calculate the recapture percentage or number of voles tagged in each plot repetition during the first mark-recapture census (MRC) that were recaptured in that plot repetition during the second MRC.

\[
\text{Number of voles ear-tagged during first MRC that were recaptured during second MRC} \cdot \frac{100}{\text{Number of voles captured, tagged and released during first MRC event}}
\]

Table 4. Scale used in grading apple slices of apple slice Index event for feeding activity.

- 0 = no biting on apples
- 1 = one small area chewed on apple slice
- 2 = two or more areas chewed on apple slice (less than half the apple slice eaten)
- 3 = more than half the apple slice eaten
- 4 = the apple slice completely eaten

Table 5. Equation for calculating the percentage difference between the first and second apple slice Index event for each plot repetition.

\[
\text{Percentage change between Apple Slice Index (ASI) for given plot repetition} = \left( \frac{\text{Score of second ASI} - \text{Score of first ASI}}{\text{Score of first ASI}} \right) \times 100
\]

Table 6. Efficacy calculation for the plot repetitions of the treatment and the average control for all plot repetitions of the treatment plot.

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Number of voles captured on Oct 12</th>
<th>Number of voles captured on Oct 13</th>
<th>Number of voles captured on Oct 29</th>
<th>Number of voles captured on Oct 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT Plot 1</td>
<td>10</td>
<td>13 untagged (of 17 total)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>TRT Plot 2</td>
<td>13</td>
<td>9 untagged (of 13 total)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>TRT Plot 3</td>
<td>17</td>
<td>10 untagged (of 18 total)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>TRT Plot 4</td>
<td>14</td>
<td>12 untagged (of 18 total)</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

The corresponding efficacy calculations are:

- TRT Plot 1: \( \left( \frac{(2+3) - (10+13)}{10+13} \right) \times 100 = \frac{-18 \times 100}{23} = -78.3\% \)
- TRT Plot 2: \( \left( \frac{(3+3) - (13+9)}{13+9} \right) \times 100 = \frac{-16 \times 100}{22} = -72.7\% \)
- TRT Plot 3: \( \left( \frac{(2+2) - (17+10)}{17+10} \right) \times 100 = \frac{-23 \times 100}{27} = -85.2\% \)
- TRT Plot 4: \( \left( \frac{(3+0) - (14+12)}{14+12} \right) \times 100 = \frac{-23 \times 100}{26} = -88.5\% \)

Average population change of the treatment plot repetitions = \(-81.2\%\)
Table 7. Percentage of voles ear-tagged and released in the plot repetitions of the treatment during the pre-treatment mark-recapture census (MRC) that were recaptured during the post-treatment MRC.

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Number of voles captured, tagged &amp; released on Oct 12/13</th>
<th>Number of voles recaptured Oct 29/30 with tags from Oct 12/13</th>
<th>Recapture percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT Plot 1</td>
<td>23</td>
<td>1</td>
<td>1/22 = 4.3%</td>
</tr>
<tr>
<td>TRT Plot 2</td>
<td>22</td>
<td>0</td>
<td>0/22 = 0.0%</td>
</tr>
<tr>
<td>TRT Plot 3</td>
<td>27</td>
<td>0</td>
<td>0/26 = 0.0%</td>
</tr>
<tr>
<td>TRT Plot 4</td>
<td>26</td>
<td>1</td>
<td>1/26 = 3.8%</td>
</tr>
</tbody>
</table>

Average recapture percentage for the treated plot repetitions = 2.0%

Table 8. Calculations used to determine the change of population between the pre-treatment mark-recapture census (MRC) and the post-treatment MRC for the untreated control plot repetitions and the average change of all 4 repetitions.

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Number of voles captured on Oct 12 C12</th>
<th>Number of voles captured on Oct 13 C13mt</th>
<th>Number of voles captured on Oct 29 C29surv</th>
<th>O Number of voles captured on Oct 30 C30surv</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTC Plot 1</td>
<td>12</td>
<td>17 untagged (of 20 total captured)</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>UTC Plot 2</td>
<td>20</td>
<td>9 untagged (of 15 total captured)</td>
<td>29</td>
<td>20</td>
</tr>
<tr>
<td>UTC Plot 3</td>
<td>10</td>
<td>5 untagged (of 9 total captured)</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>UTC Plot 4</td>
<td>15</td>
<td>18 untagged (of 20 total captured)</td>
<td>31</td>
<td>18</td>
</tr>
</tbody>
</table>

The corresponding population change calculations are

UTC Plot 1: \((21+14)-(12+17)\times \frac{100}{100}=+20.7\%\)

UTC Plot 2: \((29+20)-(20+9)\times \frac{100}{100}=+68.9\%\)

UTC Plot 3: \((18+8)-(10+5)\times \frac{100}{100}=+73.3\%\)

UTC Plot 4: \((31+18)-(15+18)\times \frac{100}{100}=+48.5\%\)

Average population change in the control block = +52.9%

Table 9. Percentage of voles ear-tagged and released in the plot repetitions of the untreated control during the pre-treatment mark-recapture census (MRC) that were recaptured during the post-treatment MRC.

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Number of voles captured, tagged &amp; released on Oct 12/13</th>
<th>Number of voles recaptured Oct 29/30 with tags from Oct 12/13</th>
<th>Recapture percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTC Plot 1</td>
<td>29</td>
<td>5</td>
<td>5/29 = 17.2%</td>
</tr>
<tr>
<td>UTC Plot 2</td>
<td>29</td>
<td>8</td>
<td>8/29 = 27.6%</td>
</tr>
<tr>
<td>UTC Plot 3</td>
<td>15</td>
<td>9</td>
<td>9/15 = 60.0%</td>
</tr>
<tr>
<td>UTC Plot 4</td>
<td>33</td>
<td>11</td>
<td>11/33 = 33.3%</td>
</tr>
</tbody>
</table>

Average recapture percentage of the control plot repetitions = 34.5%

Table 10. Average active ingredient (chlorophacinone) observed in each of four Microtus montanus carcasses found in the treatment plots.

<table>
<thead>
<tr>
<th>Specimen #</th>
<th>Weight (g) of skinned animal</th>
<th>Average A.I. (ppm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>12.5</td>
<td>0.864</td>
</tr>
<tr>
<td>102</td>
<td>8.1</td>
<td>0.851</td>
</tr>
<tr>
<td>104</td>
<td>6.6</td>
<td>2.210</td>
</tr>
<tr>
<td>108</td>
<td>16.5</td>
<td>1.700</td>
</tr>
</tbody>
</table>

*observed concentrations not adjusted for QC recoveries
Table 11. Average A.I. (chlorophacinone) observed in each of two Microtus montanus carcass found in the untreated control plots.

<table>
<thead>
<tr>
<th>Specimen #</th>
<th>Weight (g) of skinned animal</th>
<th>Average A.I. (ppm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>13.0</td>
<td>less than method level of detection (MLOD**)</td>
</tr>
<tr>
<td>3</td>
<td>12.2</td>
<td>less than method level of detection (MLOD**)</td>
</tr>
</tbody>
</table>

* observed concentrations not adjusted for QC recoveries
** MLOD is reported to be 0.14 ppm

Table 12. Wildlife observations during the course of the study.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific name</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red-tailed hawk (dark phase)</td>
<td>Buteo jamaicensis</td>
<td>Sitting on wind machine in treated block</td>
</tr>
<tr>
<td>American kestrel</td>
<td>Falco sparverius</td>
<td>Hunting open fields next to orchard</td>
</tr>
<tr>
<td>Ring-necked pheasant</td>
<td>Phasianus colchicus</td>
<td>Feeding on apples on ground</td>
</tr>
<tr>
<td>Morning dove</td>
<td>Zenaidura macroura</td>
<td>Seen flying through orchard and on dirt roads between orchard blocks</td>
</tr>
<tr>
<td>Common flicker (Northern flicker)</td>
<td>Colaptes auratus</td>
<td>In poplars at edge of orchard</td>
</tr>
<tr>
<td>Black billed magpie</td>
<td>Pica pica</td>
<td>In orchard prior to harvest</td>
</tr>
<tr>
<td>Common Raven</td>
<td>Corvus corax</td>
<td>Flying high over orchard</td>
</tr>
<tr>
<td>American Robin</td>
<td>Turdus migratorius</td>
<td>Migrating through during early part of study – seen feeding on crab apples</td>
</tr>
<tr>
<td>Audubon’s warbler (Yellow-rumped warbler)</td>
<td>Dendroica coronata</td>
<td>Migrating through</td>
</tr>
<tr>
<td>Slate-colored junco</td>
<td>Junco hyemalis</td>
<td>Migrating through</td>
</tr>
</tbody>
</table>

harvested, and apples were still available in these plots during the second ASI event. Plot repetitions 1 and 2 of the treatment had banana apple trees for pollinators. These large, moist apples were present during the first ASI and seemed to be attractive to the voles. During the second ASI, these apples had all deteriorated, thus not competing with the ASI. By averaging the ASI results from the two remaining plots of the untreated control, a 31.5% increase in feeding is obtained. An average of the percentage change between ASI events for plot repetitions 3 and 4 of the treatment shows that there was a 78.8% decrease in feeding. These results support a conclusion that the test substance gave acceptable control of M. montanus.

Carass Search, Analysis and Necropsy, Wildlife Observations

The carcasses of four M. montanus and of three P. maniculatus were found in the treatment plots during the carcass search. Two M. montanus carcasses and one P. maniculatus carcass were found in the untreated control plots. One of the two vole carcasses from the untreated control was found the day after the final carcass search, but it was also submitted for carcass analysis.

Table 10 shows the amount of chlorophacinone that was determined to be in each of the vole carcasses found in the treatment plot. The small number of carcasses found did not allow for statistical analysis of the results. All three of the P. maniculatus carcass necropsies showed signs of anticoagulant poisoning.

Analysis of the two M. montanus carcasses from the untreated control is presented in Table 11. As would be expected, these animals were not found to have chlorophacinone residues. Necropsy of the P. maniculatus found in the untreated control showed no signs of anticoagulant poisoning.

In frequent visits to the orchard during the study, no wildlife were observed feeding on the test substance. Avian predators that were observed resting in the orchard were only seen hunting in the open fields surrounding the orchard. Wildlife observations are summarized in Table 12. Most of the birds were migrants and were not seen by the study midpoint. No mammalian predators or scavengers were seen in the orchard.

LITERATURE CITED
