Assessing the Efficacy of Chlorophacinone for Mountain Beaver (*Aplodontia rufa*) Control

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ABSTRACT: The mountain beaver is a fossorial rodent species endemic to the Pacific Northwest and portions of California. This herbivore is managed as a pest species because of the impact it has on newly planted Douglas-fir seedlings. Currently, managers are limited to trapping for population control; however, in Washington trapping has been further curtailed by anti-trapping legislation. Presently there are no registered underground toxicants for mountain beaver control. We have documented the efficacy of chlorophacinone, presented in daily doses, as a possible alternative for mountain beaver control. Daily baiting would be unreasonable and costly alternative for timber managers, so we conducted a series of tests to determine if a single or double baiting was efficacious. In addition, we tested the caching behavior of the mountain beaver when offered bags of oats. This behavior may help reduce impacts to non-target species as well reduce environmental exposure and degradation. Mountain beaver readily cached bags of chlorophacinone within their artificial burrows, and efficacy of a one-time and two-time dose was 100%. We determined that even with the highest chlorophacinone residuals (0.354 ppm) that the risk quotient for mink and red-tailed hawk was exactly at the level of concern that EPA recognizes for endangered and threatened species.

KEY WORDS: *Aplodontia rufa*, chlorophacinone, diphacinone, mountain beaver, strychnine, toxicant, zinc phosphide

INTRODUCTION

The mountain beaver (*Aplodontia rufa*) is a primitive rodent species endemic to the Pacific Northwest and California. This small, semi-fossorial rodent is just one of several species in the Pacific Northwest that cause damage to Douglas-fir (*Pseudotsuga menziesii*) trees. Damage can occur both below and above ground. Below ground, mountain beaver can uproot or bury seedlings or undermine the roots of 10- to 20-year-old trees. In addition to clipping seedlings above ground, mountain beaver can climb young trees to clip lateral branches. Basal barking of a wide size of trees also occurs (Cafferata 1992). Successful regeneration of conifer seedlings, therefore, is dependent upon managing mountain beaver populations (Borrecco and Anderson 1980, Black and Lawrence 1992).

Historically, both lethal and non-lethal methods were implemented to control mountain beaver. Most non-lethal methods, such as box traps, individual tree protectors, and fencing, have been only marginally effective and are often cost prohibitive. Lethal methods, therefore, have been the preferred method used to control mountain beaver populations. In Oregon, most managers currently rely on conibear traps, while leg-hold traps are the only feasible method available to Washington managers. Other previous lethal approaches have included strychnine placed on native vegetation or apples (Nelson 1969), a toxic tracking foam incorporating octamethylpyro-phosphoramide (Evans 1987), and strychnine-based Boomer-Rid. Boomer-Rid was registered for use in Oregon (Orco Boomer-Rid mountain beaver bait SLN Reg No OR-840029; Caffereta 1992, Campbell et al. 1992) although efficacy was questionable.

Even though trapping is the preferred control method, some public anti-trapping sentiment and the cost associated with trapping bring to question the future of trapping as a means of controlling mountain beaver. Alternative methods of control are therefore necessary to increase managers' options.

At present there are no toxicants registered for use to control mountain beaver. Four toxicants are currently registered for application in underground rodent burrows to protect agriculture crops: 0.5% strychnine, 2.0% zinc phosphide, 0.005% chlorophacinone, and 0.005% diphacinone. A series of tests was conducted to assess mountain beaver acceptance and subsequent fate when offered bait containing these four toxicants (Arjo and Nolte 2004). Zinc phosphide was not consumed by any of the mountain beaver tested, even with pre-baiting. Efficacy of strychnine was marginal, and some animals became bait shy. The two anticoagulants, diphacinone and chlorophacinone, were readily consumed by the mountain beaver; however, only chlorophacinone was 100% efficacious (Arjo and Nolte 2004).

Efforts to reduce non-target consumption of toxicants and the ability to minimize labor costs are important characteristics in determining acceptable baits. Previous tests conducted for bait acceptance used daily portions of baits (Arjo and Nolte 2004). This type of baiting regime is not practical for managers who for cost purposes prefer a single-dose baiting. In addition, multiple baiting, even small doses, can increase non-target exposure and risk. Methods that are effective in controlling mountain beaver damage that rely on mountain beaver behavior can be selective means of control (Campbell and Evans 1988). Mountain beaver are highly dependant upon burrow

systems and often cache food items within these systems. Baiting techniques that incorporate these behaviors may reduce non-target impacts while selectively controlling mountain beaver populations.

Complying with data requirements necessary to register a new product with the Environmental Protection Agency (EPA) is an expensive and time-consuming process. However, it may be possible to add mountain beaver to an existing label if a product registered for underground baiting of rodents was determined to be effective. We conducted a study to determine if efficacy was maintained with a single or double baiting of chlorophacinone. In addition, we tested mountain beaver caching behavior to determine if baits would be stored within mountain beaver burrow systems.

METHODS
Baiting Regime Trial

Twenty-four mountain beaver served as subjects. Mountain beaver were penned individually in covered outdoor pens (3 × 3 m) that each contained a simple artificial nest structure. Each nest structure consists of three 76-liter cans with lids connected with perforated plastic pipe (10 cm diameter). Subjects had free access to water, apple, alfalfa, and rodent blox in their pen throughout the study.

Animals were given a minimum of 2 - 4 weeks to adapt to pen and burrow system. After the adaptation period, baits were placed in a trash can (76 L) in each of the pens. A 10-cm diameter hole was cut at the bottom of the trash can to allow access and to mimic the rodent's natural burrow system. We used the recommended dose of 0.005% chlorophacinone (Kings County Agriculture Commission) for rodents of similar size. Eight of the animals received 226.8 g (8 oz) of 0.005% chlorophacinone twice during the study, 8 animals received 226.8 g of chlorophacinone once at the start of the study, while the other 8 animals were controls and received no bait. Bait formulation for each treatment was presented in oats, with control animals receiving plain rolled oats (no bait). Control animals received 226.8 g of oats every 2 weeks for 1 month. The status of animals was monitored at 2-hour intervals for the first 6 hours, then again every 24 hours for the next 28 days. Remaining bait was weighed every day to determine amount consumed. After weighing, the baits were returned to animals' pens.

Chlorophacinone residues, in both whole body and liver, were identified in all bait-treated mountain beaver. Five additional mountain beaver not exposed to chloro-phacinone were used as a standard measure to calibrate the system. For whole-body analyses, samples were first prepared by removing the pelt, head, extremities, and liver, then grinding the carcasses and the liver. The whole-body and liver samples were analyzed with a modified version of the methods used to assay for chlorophacinone residues in Belding ground squirrels (*Spermophilus beldingi*) and pocket gophers (*Thomomys* spp.) (Primus et al. 2001). The method for these samples used an extraction solution containing ammonium hydroxide instead of formic acid. The sample extracts were cleaned up with a 0.50-g Florisil solid phase extraction column for liver samples and 0.5-g silica

columns for whole-body samples, instead of 2-g silica columns. The Keystone high performance liquid chroma-tography analytical column (4.6 mm × 150 mm) was replaced with a Betasil small-bore column (2.0 mm × 150 mm) with a flow rate of 0.25 mL/min.

We used the highest chlorophacinone residues obtained from either the whole-body or liver chlorophaci-none residue analyses to estimate secondary risk. To determine a more average risk, we used an average whole-body residual from the 16 animals analyzed. Mountain beaver are most likely to die below ground and predators such as weasels (*Mustela* spp.), skunks (*Spilogale putorius* and *Mephitis mephitis*), and mink (*M. vison*) are likely scavengers within the mountain beaver burrow system. Above-ground predators include coyotes (*Canis latrans*), bobcats (*Lynx rufus*), and several raptor species. We used food ingestion rates for mink (Bleavins and Aulerich 1981) and red-tailed hawk (*Buteo jamaicensis*) (Craighead and Craighead 1969) in the risk assessment. To calculate worst-case scenario, we assumed that the species' diet for a single day consisted entirely of mountain beaver with the highest and average chlorophacinone concentrations. We used a chlorophaci-none LD_{50} for deer mice (*Peromyscus maniculatus*) at 0.49 mg/kg (Clark 1994) and for a mallard (*Anas platyrhynchos*) at 100 mg/kg (RTECS Ecotoxicity Data Base) to represent the mammal and avian species respectively. For both terrestrial species, the acceptable risk quotients are <0.1 (Sample and Sutter 1997).

Bait Caching

Nine female mountain beaver served as subjects. Mountain beaver were housed in outdoor habitat pens measuring 11 × 16 m. Each pen contained a nest box consisting of a 76-liter trash can buried in the soil with an exit to the surface through a 1.5-m corrugated pipe (10 cm diameter). Opposite the exit pipe was a 0.5-m corrugated pipe buried in the soil to facilitated natural burrowing by the animals. An A-frame roof covered each nesting structure. A feed station and water bowl were located in the middle of the enclosure, and subjects had free access to water and rodent blox in their pen throughout the study. Straw for bedding and alder branches for gnawing were provided weekly.

One small plastic bag (standard sandwich bag) with 25 g of oats and a transmitter (Advanced Telemetry Systems, Istanti, MN) was placed in each mountain beaver's food dish at the start of the study. An additional bag and transmitter was also placed at one feeder hole per female. We monitored the removal and subsequent movement of the bag every 2 hours during the first day and daily for the next 3 weeks. Mountain beaver are likely to cache the bags below ground, so location of the transmitter and bag was marked above ground with flags to assist in future location of the transmitter. After 3 weeks, animals were live captured in Tomahawk traps and placed in indoor holding pens. Transmitters were retrieved to determine location in the mountain beaver's burrow system and bags were inspected to determine if mountain beaver were able to access the oats. Mountain beaver are likely to chew on bags and ingestion of plastic material is likely; however, we did not anticipate any ill

effects since plastic items from other sources are often discovered in wild mountain beaver caches.

Pre-Packaged Baiting Regime

LiphaTech currently holds a chlorophacinone label. Prior to this final study, chlorophacinone on oats was used in all trials to keep the reference substance similar when screening a variety of toxicant. Before conducting a field efficacy trail for subsequent EPA approval, we tested the acceptance of the pre-packaged Lipha-Tech product. Five mountain beaver were presented with one 12-oz package of 0.005% chlorophacinone in test chambers as previously described. An additional 5 mountain beaver received 12 oz of plain oats in similar packages. Mountain beaver were monitored for up to 28 days to determine efficacy of the bait and to record any subsequent caching behavior of the packaged bait.

RESULTS
Baiting Regime Trails

All mountain beaver offered chlorophacinone consumed the toxicant and efficacy was 100% (Figure 1). Animals on the single-dose bait ingested 3.83 ± 0.45 mg/kg of bait. A slightly higher ingestion was seen in the double-dose animals (5.13 ± 1.0 mg/kg) although it was not significantly different from the single dose ($t = -1.46$, $P = 0.17$). In the single-dose bating regime, 7 of the 8 animals died by Day 13. The last mountain beaver did not die until Day 21, even though no additional bait was consumed after Day 14 (Figure 2). All of the mountain beaver on the double dose were dead by Day 18, and daily consumption rates varied between animals (Figure 3). Only 2 animals were alive at Day 15 when the second dose was offered; one of these animals died the following day. Average number of days until death did not differ between the two baiting regimes ($t = -0.68$, $P = 0.51$, single: 11.0 ± 1.57 days, double: 12.38 ± 1.27 days).

We found no difference in whole-body residue concentration between the baiting regimes ($t = -1.64$, $P = 0.14$). Animals on the double dose had greater chlorophacinone concentrations (0.133 ± 0.046 µg/g) than animals on the single baiting regime (0.055 ± 0.012 µg/g). Individual variation to the toxicant was observed in both baiting regimes. The smallest mountain beaver tested (896.5 g) survived the longest (21 days), after eating an average of 3.76 mg/kg of bait. One animal in the double-dose regime had chlorophacinone levels that were below the baseline threshold and were essentially undetectable. This animal died at Day 11 after consuming 3.16 mg/kg of bait. Analyses of liver residuals were completed for the double-dose animals and were similar to whole-body concentrations (0.14 ± 0.02 µg/g).

The highest chlorophacinone residuals were found in a whole-body sample, 0.354 ppm. Whole-body residuals tended to be greater than liver concentrations, so we used an average (0.094 ppm) whole-body concentration to calculate average risk. Risk quotients using average chlorophacinone concentrations found in whole-body analyses fell below the threshold determined to pose a risk (Table 1). When we considered the worst-case scenario using the highest chlorophacinone concentration,

the calculated risk quotient was right at the level of concern.

Bait Caching

Females readily cached baggies with bags placed at feeder holes slightly preferred ($n = 9$) over baggies placed in food bowls ($n = 6$). On average, females moved baggi-
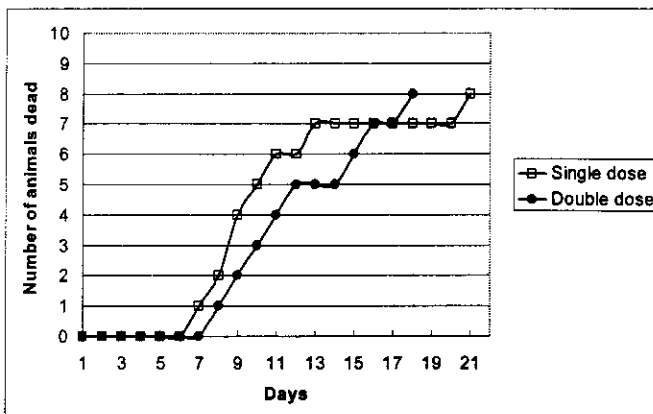

Figure 1. Efficacy of chlorophacinone on mountain beaver when offered single- or double-baiting regime.
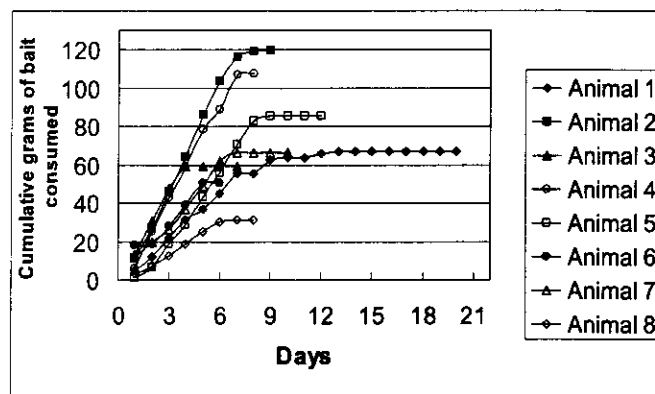

Figure 2. Cumulative amount of bait consumed (g) by mountain beaver when offered a single dosage of chlorophacinone.
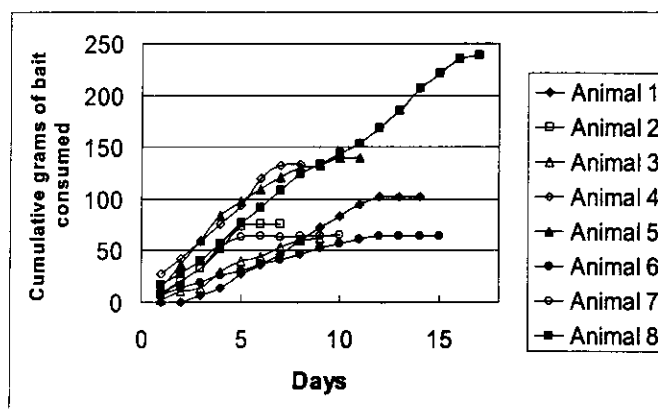

Figure 3. Cumulative amount of bait consumed (g) by mountain beaver when offered chlorophacinone on Day 1 and Day 15.

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Table 1. Chlorophacinone risk assessment for a mammal and avian species based on mountain beaver whole-body residue analyses.

Common name	Ingestion Rate (g food/g bodyweight/day)	Chlorophacinone residue (ppm)	LD$_{50}$ (mg/kg)	Risk quotient
Mink[1]	0.14	0.094	0.49	0.03
Mink[2]	0.14	0.354	0.49	0.1
Red-tailed hawk[1]	0.1	0.094	100	0.0001
Red-tailed hawk[2]	0.1	0.354	100	0.003

[1] calculated using average whole-body chlorophacinone residues
[2] calculated using highest whole-body chlorophacinone residues

ies by Day 5 (range 2 - 8 days). Cached bags were opened and the oats consumed. Two females did not consume any food during the study, nor were they retrapped in their respective pens. These females likely either escaped the outdoor enclosure or died underground. Another female gained access to these 2 abandoned pens and cached 3 bags from those pens. Four of the 5 transmitters she cached were located together.

Pre-Packaged Bait Test

Four of the treated animals removed the bags of bait from the test chamber, with 3 of the animals caching the bags elsewhere. Four of the animals opened bags on the first day, and the fifth animal by Day 10. Efficacy occurred after 20 days, although 3 of the animals succumbed by Day 10 ($\bar{x} = 14 \pm 3.56$ days). The amount of bait consumed varied between the subjects but averaged 4.09 ± 1.22 mg of bait. Two control animals also cached and opened their packaged oat bags.

DISCUSSION

Mountain beaver cause more serious damage to Douglas-fir plantations that any other species in the Pacific Northwest (Cafferata 1992). Attempts to reduce damage by decreasing mountain beaver populations through lethal and non-lethal methods have met with varied success. Vexar tubing to protect seedlings is a timely and costly process, with limited results. Even with tubing, roots of seedlings are barked, plants are undermined, and the tops of seedlings clipped above tubes (Cafferata 1992). Live-trapping and relocating mountain beaver is not effective method, because it only moves the problem to a different area. In the Pacific Northwest, trapping is the most common method of control; however, it is costly. Trapping at the Weyerhaeuser Twin Harbor Tree Farm averages $40/acre with traps checked every 24 hours (J. Todd, pers. commun.). Average trapping time for each unit varies but on average is 5 - 14 days. With public perception of trapping unfavorable, there is a possibility in the near future that all trapping may be banned. If this occurs, managers are left with few options to reduce mountain beaver damage.

Toxicants previously tested for mountain beaver control included zinc phosphide, strychnine alkaloids, dipahcinone on bait blocks and oats, bromadiolone, Vacor® (RH-787; DRC- 6091; N-3-pyridlymethyl N'- p-nitrophenyl urea), DCR-4575 (TAR-1688; benzenesulfonic acid hydrazide) (Campbell and Evans 1988, Arjo and Nolte 2004). Vacor® was taken off the market, and

diphacinone bait blocks and bromadiolone were not found effective (Campbell and Evans 1988). Strychnine products also met with limited success even with long periods of pre-baiting (Arjo and Nolte 2004), and the only registered strychnine product for mountain beaver control, Boomer-Rid, no longer is available. Only recently has the efficacy of chlorophacinone been examined (Arjo and Nolte 2004). Registration of new toxicants is timely and costly; therefore, if a toxicant already approved for underground baiting could be found effective for mountain beaver, this species could potentially be added to the label at a minimal cost. Of the 4 toxicants previously tested, only chlorophacinone showed both high efficacy and bait acceptance (Arjo and Nolte 2004).

Anticoagulants such as chlorophacinone work by blocking the epoxide reductase enzyme inhibiting recycling of Vitamin K (Silverman 1980). Without sufficient incoming vitamin K, the ability to produce clotting factors is inhibited and hemorrhaging begins (Hadler and Buckle 1992). Although single dose anticoagulants are available, first-generation anticoagulants like diphacinone and chlorophacinone are more effective if administered in small doses over a period of time (Hadler and Buckle 1992). Even though both baiting regimes offered large quantities of bait to mountain beaver, animals consumed small quantities of the bait over a series of days and effects were noticed after one week of baiting. Both single- and double-dose baiting was efficacious, with the majority of the mountain beaver dying in less than 2 weeks. Results from the double-dose baiting actually took longer than the single dose, although this may be due to individual differences in toxicant tolerance. We conclude, therefore, that a single dose offered to mountain beaver may be sufficient to induce death as long as animals have continued access to the bait and there is little environmental degradation.

Several species other than mountain beaver frequent mountain beaver burrow systems (Feldhamer and Rochelle 1994), and species, such as rabbits (*Sylvilagus* spp.), weasels, and skunks may likely consume baits. Non-target species must be exposed to baits and the baits must be palatable for primary poisoning to occur (Kaukeinen et al. 2000). A delivery system that reduces the amount of bait in the environment as well as limiting access by other species is desirable. Pre-packaged paraffinized chlorophacinone pellets may reduce environmental exposure problems especially in the Pacific Northwest. In both the habitat pen trails and the pre-packaged bait trails, mountain beaver readily cached

bags. In addition, these bags were opened usually within the burrow system and the contents consumed. The unique caching behavior of mountain beaver allows for bait placement within a burrow system, that likely will be incorporated into the nest or food cache of the target species and reduce primary non-target exposure.

Predation on mountain beaver can occur both above ground and below ground. Mustelid predation accounted for 63% of the total predation mortality on one study site in western Washington (Arjo, unpubl. data). On 2 other study sites in western Washington, mustelid predation accounted for only 14% and 9%, but carnivore predation (coyote or bobcat) caused 43% and 63% of total predation mortality, respectively. Using the worst-case scenario for chlorophacinone concentration, we determined that the mammalian risk quotient was exactly at the level of concern (0.1) that EPA recognizes for endangered and threatened species. Even using the highest concentrations in calculating risks to raptors, the risk quotient fell well below the level of concerned risk.

In Oregon and Washington, there are a few endangered and threatened species of concern: lynx, bald eagle (*Haliaeetus leucocephalius*), Northern spotted owl (*Strix occidentalis caurina*), fisher (*Martes pennanti*), wolverine (*Gulo gulo*), and ferruginous hawk (*Buteo regalis*). The first 3 species are federally threatened species and the remaining 3 are state species of concern. All of the species listed, with the exception of the spotted owl, are carrion eaters and may potentially consume mountain beaver carcasses. However, mountain beaver that consume chlorophacinone are most likely to die underground; therefore, predators exposed to baited carcasses are limited to semi-fossorial species such as those found among the mustelids. Current knowledge on carnivore distribution suggests that only the wolverine and fisher may overlap mountain beaver distributions in southern Oregon. Although more data need to be analyzed on the distributional overlap and potential that mountain beaver are a food source for the spotted owl, we feel that it is unlikely that spotted owls are able to prey on mountain beaver above ground, and that access to animals below ground is limited.

ACKNOWLEDGEMENTS

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