

**National Wildlife Research Center
Wildlife Services
Animal and Plant Health Inspection Service
United States Department of Agriculture**

Title:

Efficacy of rodenticide baits for the control of black rats, Polynesian rats, and mice.

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Executive Summary:

- 1.** We tested the efficacy and palatability of 9 commercial rodenticide formulations on 165 Polynesian rats (Rattus exulans), 160 black rats (R. rattus), and 130 house mice (Mus musculus).
- 2.** Efficacy varied by rodenticide tested and rodent species of interest. On average, rodenticides were more effective against mice than either of the rat species and mice tended to eat more rodenticide bait than laboratory chow.
- 3.** Efficacy was highest for the second generation anticoagulants tested (Havoc® (brodifacoum), Maki® (bromadiolone), and Generation® (difethialone)). However, this varied across products and a first generation rodenticide, Rozol® (chlorophacinone), had similar effectiveness to the second generation anticoagulants.
- 4.** Palatability varied by rodenticide tested and rodent species of interest. Palatability was the lowest for the acute rodenticides.
- 5.** Palatability affected the efficacy of rodenticides. Rodenticides that were not preferred by rodents had lower mortality rates. Rodenticides that were preferred had high mortality rates.
- 6.** Rodenticide products currently registered for use in Hawaii were not as effective as other products on rodents found in Hawaii. Although some of the other products have higher toxicity, some products were highly effective and are considered similar in toxicity to products currently registered.

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INTRODUCTION

Invasive rodents have decimated native flora and fauna, reduced agriculture production, and threatened human health in Hawaii. Although many rodenticides are commercially available nationally, few are available for use in Hawaii or have been tested with wild rodents commonly found in the Pacific (Jacobs 1994). We proposed a comprehensive study to test the efficacy and palatability of commercially available rodenticides to identify the most effective rodenticides for use in Hawaii. This research can be used to support the registration of additional rodenticides for use in conservation areas, agricultural crops, and to protect human health.

The use of any rodenticide is limited by what is labeled for a particular use. However, pursuit of a pesticide label is often limited because most products have not been tested using wild rodents and managers are hesitant to attempt projects with other chemicals with limited data (G. Howald, Island Conservation, pers. comm.). The efficacy of many commercially available products on wild black rats (Rattus rattus), Polynesian rats (R. exulans), and mice (Mus musculus) is unknown. Further, the few products that have been used successfully have not been systematically tested in a common laboratory environment or compared to other available products (Swift 1998). However, products vary according to their toxicity to non-target animals, speed of action, method of action, resistance to toxicity, and bioaccumulation and biomagnification potential (Park 1982, Hadler and Buckle 1992, Howald et al. 1999, Stone et al. 1999, Linder and Joermann 2001). Thus, a product appropriate for one application may not be appropriate for all and limiting the pool of potential products restricts a manager's ability to mitigate the effects of an eradication effort because the managers are initially limited to a few

products. A major data gap is the effectiveness of these products on feral rats typically found on Pacific islands and a comparison of the effectiveness of the products available.

Resource protection

Currently, the only rodenticide product registered in Hawaii to protect native species from rodent predation is the diphacinone-based Ramik® Mini-Bars (SLN# HI-980005). Two other products were voluntarily canceled by another manufacturer in September 2005. The availability of multiple products for resource protection is desired for multiple reasons. Continued reliance on one product (or active ingredient) may result in reduced palatability and/or the development of resistance in rodent populations, both of which can lead to lower efficacy. In addition, resource managers may be subject to fluctuations in product availability, given only one product is available. Furthermore, the company currently producing this product could stop making it, decrease production, or fail to renew their label. For all of these reasons, additional products were tested and evaluated.

During the last 15 years, efforts to eradicate rodents from islands have increased and numerous eradications have been completed using commercially available rodenticides (Veitch and Clout 2002, Howald et al. 2007). Few projects have been completed in the United States, but several projects are now in the initial stages on Palmyra Atoll, Lehua (Hawaii), and Cocos Island (Guam). Successful eradications have been completed for roof rats using diphacinone from Buck Island in the U.S. Virgin Islands (Witmer et al. 1998) and using brodifacoum on the Anacapa Islands (3 islands) off the California coast (Howald et al. 2005) and Midway Atoll and for Polynesian rats on Rose and Kure Atolls (J. Murphy, USDA APHIS, pers. comm.). Several

other efforts may have been successful eradications but have not been well documented (Green Cay, and Low Cay). Projects are now in the planning stages for Palmyra Atoll, Lehua (Hawaii), and Cocos Island (Guam). Most of the international efforts have also used brodifacoum or diphacinone. Only a few laboratory studies have been conducted with the goal of generating efficacy data for conservation uses of rodenticides on species other than white laboratory rats (Swift 1998, Witmer et al. 1998). Unsuccessful eradication efforts have been documented for brodifacoum applied in bait stations (Palmyra Atoll, J. Murphy, USDA, APHIS, pers. comm.) and by hand broadcast (Congo Cay, J. Eisemann, USDA APHIS, pers. comm.). Three rodenticides (Diphacinone 50 Conservation -- 0.005% diphacinone, Brodifacoum 25D Conservation and Brodifacoum 25W Conservation -- 0.0025% brodifacoum) have been registered recently for control or eradication of introduced rodents on islands for conservation purposes. These labels permit bait to be applied by bait stations, in burrows and tree canopies, and by hand or aerial broadcast.

Agriculture

Currently, only Ramik® Mini Bars All-Weather Rat/Mouse Killer (diphacinone based product, SLN HI-980006) and zinc phosphide baits are registered (EPA Registration numbers 12455-17 for Bell Products and 61282 for HACCO Products) for use in agriculture crops in Hawaii. Furthermore, these products are restricted to macadamia nut orchards and sugarcane (zinc phosphide only). No rodenticides are registered for use in many of the emerging agricultural products (e.g., tropical fruits and seed crops). The diversification of Hawaiian agriculture has outpaced the development of new labels for rodenticides. Obviously, additional pesticides should

be evaluated, so if there is a need to label additional pesticides, the required effectiveness and palatability data would be available on rodent species from Hawaii.

Human Health and Safety

Rodents are a primary vector of many diseases worldwide and control of rodent outbreaks with rodenticides is one way to reduce the risk of disease outbreaks. Currently, only Prozap® Zinc Phosphide Oat Bait is registered to control rodent outbreaks for human health in Hawaii (SLN # HI-010001). There is considerable human health risk having only a single product registered because the product may not be available when needed or the product may not be effective.

Objective/Hypotheses

Effective anticoagulant rodenticide baits are needed to control and eradicate rodents. Although many anticoagulant commercial baits are available on the market, there has been little recent testing of efficacy with wild rodents, especially using one standardized protocol. Additionally, rodents often have a choice of food items, so an effective bait must be attractive/palatable, as well as efficacious when presented with an alternative food type. Palatability is a combination of the bait matrix used and in some cases the active ingredient if it can be detected by rodents. We hypothesized that some commercial anticoagulant baits will be consumed and efficacious ($\geq 80\%$ mortality) when presented with an alternative food type (two-choice efficacy trial).

METHODS/PROCEDURES

This study was conducted in 3 tiers. Tier 2 was only conducted depending on the outcome of the Tier 1 trial (Figure 1). Tier 3 was only conducted depending on the outcome of the Tier 2 trial.

The format and rationale of each tier study is:

Tier 1 trials

The Tier 1 trial was a two-choice rodenticide efficacy trial with a 3-day exposure period. Three days is the shortest period of time that rodenticide bait would typically be available to rodents after a single broadcast-bait drop (allowing for weathering and bait consumption by invertebrates such as crabs).

Tier 2 trials

The Tier 2 trial was a two-choice rodenticide efficacy trial with a 7-day exposure period. This study was only conducted if the Tier 1 trial did not achieve a mortality rate of 80% or greater. The Tier 2 trial objective was to determine whether a mortality rate of 80% or greater could be achieved by broadcasting more bait in the initial drop or by using a second bait application.

Tier 3 trial

The Tier 3 trial was a no-choice rodenticide efficacy trial with a 7-day exposure period. This study was only conducted when the Tier 2 trial did not achieve a mortality rate of 80% or greater. The Tier 3 trial objective was to determine if there is a palatability problem with the bait

(and it wouldn't be consumed if an alternative food was available as in the Tier 1 and 2 trials) or if genetic resistance to the anticoagulant occurs in wild rodent population.

Rodenticides are typically distinguished by the mode of action of the active ingredient which consists of two main categories: acutes and anticoagulants. Acute rodenticides work in several ways but typically result in animal death after a single feeding. Acute rodenticides include bromethalin, a neurotoxin, and zinc phosphide which produces phosphine gas after ingestion (Cherry et al. 1982, Johnston et al. 2005). Anticoagulants work by preventing the blood from naturally clotting. Anticoagulants are further distinguished as first or second generation. First generation anticoagulants typically require multiple feedings to result in mortality. First generation anticoagulants include warfarin, diphacinone, and chlorophacinone. Second generation rodenticides were developed in response to rodents becoming resistant to first generation rodenticides after continued use (Thijssen 1995). Second generation rodenticides usually require fewer feedings than do the first generation anticoagulants to be effective and include brodifacoum, bromadiolone, and difethialone.

Two-choice feeding trials (Tier 1 and 2)

Free-ranging rodents, live-trapped near Hilo, were maintained in individual rack cages. The rodents were provided with commercial laboratory rodent chow (5001 Purina Rodent Diet, PMI Nutrition International, LLC, Brentwood, MO) and water ad libitum. The rodents were allowed at least three days to acclimate to the cages before the trial began, although rodents were typically acclimated for longer periods. The acclimation period was kept to a minimum so the

natural behavior of rodents toward novel food items would remain consistent and the rodents would not be acclimated to eating only rodent chow. The rodents were weighed and sexed within one day of the start of the trials.

On day 1 of the 3-day, two-choice feeding trial, 10 caged rodents were randomly assigned to each treatment (Appendix 1; Ramik Green® (0.005% diphacinone); Havoc® 0.0025% brodifacoum; Maki® 0.005% bromadiolone; Rozol® 0.005% chlorophacinone, Generation® 0.0025 % difethialone, Gunslinger® 0.01 % bromethalin, Prozap® Zinc Phosphide Oat Bait (2% zinc phosphide), Prozap® Zinc Phosphide Pellets (2% zinc phosphide), and Adios® Mouse Killer 0.025% warfarin) group; another 5 caged rodents were assigned as the control group. Each chemical was tested on each rodent species. All rodents were at least 2 months of age (i.e., sexually mature). Each group was made up of 2-3 female rodents, with the remainder of the group being males. When insufficient gender numbers were captured these male/female ratios were not used for the control group in order to maintain the consistency of gender ratios in the treatment groups. The control group continued to receive rodent chow and water during the treatment periods. The treatment groups were given the rodent chow supplemented with an assigned rodenticide bait and continued to receive water, ad libitum. About 30 g of the rodenticide bait was added initially to each cage. Rodenticide bait and rodent chow were replenished as needed so that rodents always had both types of food available. The rodenticide bait and rodent chow were placed in separate ceramic food bowls and bowls randomly placed in the cage each day to eliminate position feeding bias of animals. Food consumption was calculated by weighing the food when the trial began, the food when replenished, the accumulation of spillage below the wire cage on the tray, and the food remaining in the cage and

on the tray after the each day. Excessively soiled (urine/feces) bait was replaced. All rodenticide bait was removed at the end of the third day in an effort to simulate the amount of time aerially-broadcast bait might be available to rodents on an island before it is consumed by rodents and other animals (especially crabs and other invertebrates) or weathered and deteriorated.

All rodents were examined at least once daily by the study director or his designee and the condition of the rodents and any mortalities were recorded on a data sheet. The study director was notified and consulted if any symptoms of pain or stress were noted. If an animal was experiencing excessive pain or death was imminent, the study director could choose to euthanize the animal. Dead rodents were placed in a labeled zip-lock bag and refrigerated for later necropsy. The bag was labeled with the QA number, study director's name, date, ID number, and weight. When necropsied, they were examined for signs of anticoagulant poisoning as described by Stone et al. (1999). Rodents were observed for another 10 days after the rodenticide bait was removed before surviving rodents were euthanized and processed as described above. During that 10-day period, all rodents were maintained on rodent chow and water. Any mortalities that occur in the 10 day observation period were recorded and carcasses processed as described above. After necropsy, all carcasses from the study were stored frozen.

If <80% of the rodents of a rodenticide treatment were dead or moribund at the end of the 10-day observation period, the Tier 2 study was conducted with another group of 10 rodents. This trial was performed as described above with the exception that the rodenticide bait was provided for 7 days before being withdrawn and the 10-day observation period commencing.

No-choice feeding trials

This Tier 3 trial was only conducted if <80% of the rodents in a treatment group were not dead or moribund by the end of the trial for the 7-day rodenticide exposure period (Tier 2). This trial was to determine whether the rodenticide was consumed and caused mortality when no alternative food was available and to ensure that the rodents are not genetically resistant to the rodenticide (important because genetic resistance to some anticoagulant rodenticides has been found in some populations [Pelz et al. 1995]). Rodents not used in previous trials were used and the trial was conducted essentially the same as the above two-choice trial, except that no rodent chow was presented until after day 7 of the trial. As before, there was a 5-rodent control group that was fed rodent chow throughout the trial.

Three of the baits used in this study -- Maki® (bromadiolone), Rozol® (chlorophacinone), and Ramik Green® (diphacinone) -- are already U.S. EPA registered and commercially available over the counter. Two of the test active ingredients (diphacinone and brodifacoum) have been registered for controlling introduced rodents for conservation purposes on islands.

Experimental Design and Statistical Analyses

Rodents were randomly assigned to the treatment and control groups using a randomization computer program (random-number generator) RANDSEL (Sugihara 1997). The food consumption by groups was compared with a GLM multiple analysis of variance test and a Students Newman Keuls multiple range test (Zar 1999). All statistical tests were completed with SAS (2004).

RESULTS

We tested the efficacy and palatability of 9 rodenticide formulations on 165 R. exulans, 170 R. rattus, and 140 M. musculus. Rodents spent on average 11.5 days becoming acclimatized to captive conditions, with laboratory rodent chow as food, before being offered alternative baits. Efficacy and palatability varied by rodenticide tested and rodent species of interest. On average, rodenticides were more effective against mice than either of the rat species and mice tended to eat more rodenticide bait than laboratory chow. However, some rodenticides were not effective against mice in the no choice tests.

Tier 1 results

Only 12 of the 27 rodenticide trials resulted in 80% or greater mortality from a 3 day exposure. The highest mortality rates were from second generation anticoagulants (Fig. 2, Tables 1-3). The only rodenticide that had 80% or higher mortality for all three species was Generation® (difethialone). Maki® bromadiolone and Havoc® brodifacoum had 70% or higher mortality rates for all three rodent species. Two rodenticide formulations, Adios® Mouse Killer (warfarin) or Ramik Green® (diphacinone) had mortality rates of 40% or less for the rodent species tested during the three day trial.

Palatability varied greatly between species (Fig. 5) and products (Fig. 6). The Rozol® chlorophacinone bait was more preferred by all rodent species than any other product ($df=8$, $F=10.97$, $p < 0.001$; Table 4). On average, all rodent species ate more than 7 times as much Rozol® (chlorophacinone) bait as they did the laboratory rodent chow during the 3 day exposure

period (Fig 5). Palatability was significantly linked to mortality and rodents that died ate more bait than laboratory chow (Table 4). Adios® Mouse Killer (warfarin), Prozap® (zinc phosphide pellets), Prozap® (zinc phosphide oats), and Gunslinger® (bromethalin) were avoided by the two rat species but eaten by the mice. Overall, mice ate more poison bait over than laboratory chow in every trial (Table 3).

Tier 2 results

For 7 day two choice trials, six of 15 rodenticide products tested resulted in 80% or greater mortality (Fig. 3, Tables 5-7). The Rozol® (chlorophacinone) product was 100% effective on the two species tested. Only Ramik Green® (diphacinone) did not achieve 80% or higher mortality for at least one of the three rodent species in tier 1 or 2 trials. Adios® Mouse Killer (warfarin), Prozap® (zinc phosphide pellets), and Prozap® (zinc phosphide oats) had low mortality rates. The only products that achieved 80% or greater mortality in the two choice tests for all rodent species (Tier 1 and 2) were Rozol® (chlorophacinone), Maki® (bromadiolone), Havoc® (brodifacoum), and Generation® (difethialone).

Palatability was similar to the Tier 1 results (Fig. 7). Adios® Mouse Killer (warfarin), Prozap® (zinc phosphide pellets), Prozap® (zinc phosphide oats), and Gunslinger® (bromethalin) were avoided by the two rat species. The Rozol® (chlorophacinone) bait was preferred.

Tier 3 results

In no choice tests, only three of 9 trials did not achieve 80% mortality: Ramik Green® (diphacinone) on mice, Adios® Mouse Killer (warfarin) on mice, and Prozap® (zinc phosphide

pellets) on *R. exulans* (Fig. 4, Tables 8-10). All products were effective against black rats.

Rodents tended to consume less amounts of Gunslinger® (bromethalin), Adios® Mouse Killer (warfarin), Prozap® (zinc phosphide pellets), and Prozap® (zinc phosphide oats) than animals in the control group consumed of the maintenance laboratory rodent chow.

DISCUSSION

Rodenticide categories

As expected, the second generation anticoagulants performed better on average than the first generation or the acute rodenticides. Nonetheless, the choice of rodenticide for a specific application needs to include potential for secondary hazards and overall safety. Second generation rodenticides are more hazardous to human health and wildlife than other rodenticide products. The Environmental Protection Agency is considering limiting the use of many second generation rodenticides (EPA 2007). The efficacy of any product is a combination of the toxicity of the material and the palatability to the target species. Second generation rodenticides had higher average palatability preference ratios than acute rodenticides, thus, they tended to perform better overall in efficacy tests.

The preference ratios tended to be lower for acutes than the anticoagulants. This was especially evident in the two rat species, as mice tended to prefer any bait over the laboratory chow.

Palatability was low for baits containing zinc phosphide (Prozap® oats and pellets), as has been found previously (Johnston et al. 2005). The low palatability of zinc phosphide products may be the direct result of the active ingredient being detected by rodents. Adios® Mouse Killer (warfarin) and Gunslinger® (bromethalin) also had low preference ratios but it is unclear if this

is the result of the bait matrix or the active ingredient. Because the preference ratios are based on feeding, Polynesian rats and mice may have stopped feeding on the Gunslinger® (bromethalin) after ingesting a lethal dose and still died. However, black rats also had a low preference ratio for Gunslinger® (bromethalin) and had a low mortality rate, 20% in the three day trial.

Specific Products

Difethialone (Generation ®) was the only product that achieved 80% or higher mortality on all three species in the three day two-choice tests. This product also had higher preference ratios than other second generation anticoagulants. The two other second generation products (Havoc ® brodifacoum, and Maki® (bromadiolone) had similar mortality rates and lower preference ratios than Generation® (difethialone). The combination of the high toxicity and high palatability resulted in high efficacy for these three products.

Rozol® (chlorophacinone) performed close to the level of the second generation rodenticides. Although this is a first generation anticoagulant, the high preference ratios resulted in mortality rates that were comparable to the second generation products. After the two choice trials (3 and 7 day), Rozol® (chlorophacinone) achieved results that were indistinguishable from the second generation rodenticides.

Only three of the 9 trials in the no choice test failed to achieve 80 % or greater mortality. In these tests, rodents refused to eat the bait even though no other food was available. Obviously from the low preference ratios, R. exulans preferred not to eat Prozap® (zinc phosphide pellets).

However, the low mortalities of Ramik Green® (diphacinone) or Adios® Mouse Killer (warfarin) on mice are not as clear from the overall totals. For these two products, mice often refused to eat the products for several days, thus they may not have received a lethal dose. Over the course of the test period, mice ate less and less of these two products. Ramik Green® (diphacinone) was the only product that did not have at least 80% mortality for a single rodent species in the two choice tests. Adios® Mouse Killer (warfarin) has similar results but had 80% mortality of black rats in the 7 day two choice test. The low efficacy of these two products was the result of low overall product toxicity and low palatability compared to Rozol® (chlorophacinone) and the second generation anticoagulants.

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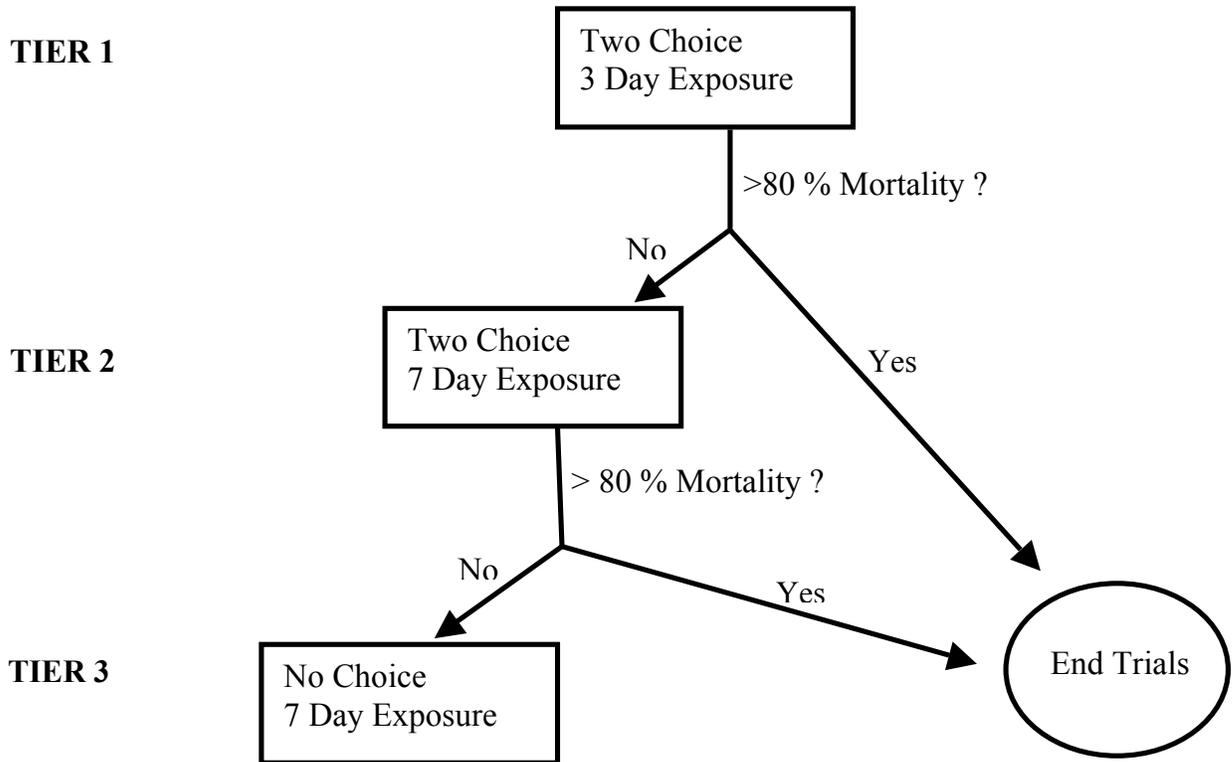


Figure 1. Schematic of the three tier system to evaluate rodenticide efficacy and palatability on rodents.